Infections in Hematology

Modern Challenges and Perspectives

Igor Stoma and Igor Karpov

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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-sulfametoxazole 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/μL	89 (65.9)
100-500 cells/μL	28 (20.7)
>500 cells/μL	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)	*P*
	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 *A. baumannii* or *P. aeruginosa*	6 (6.5%)	17 (40.5%)	23 (17.0%)	7.37 (2.54 21.35)	0.0002
ESBL producing *Enterobacteriaceae spp.*[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)	*P*
Inadequacy of empirical antibacterial therapy	15.04 (95% CI 4.88-46.37)	<0.0001
Isolation of carbapenem-resistant *A. baumannii* or *P. aeruginosa*	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 (%)
Klebsiella pneumoniae	34	25.2
Escherichia coli	25	18.5
Acinetobacter baumannii	16	11.8
Pseudomonas aeruginosa	12	8.9
Stenotrophomonas maltophilia	1	0.7
Staphylococcus epidermidis	11	8.2
Staphylococcus aureus	23	17.0
Staphylococcus hominis	4	3.0
Staphylococcus haemolyticus	3	2.2
Streptococcus pneumoniae	2	1.5
Enterococcus faecium	2	1.5
Enterococcus faecalis	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 $\chi 2$ 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 cells/mm^3 with a slight neutrophilic left shift; the erythrocytes were 3,8 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 (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).

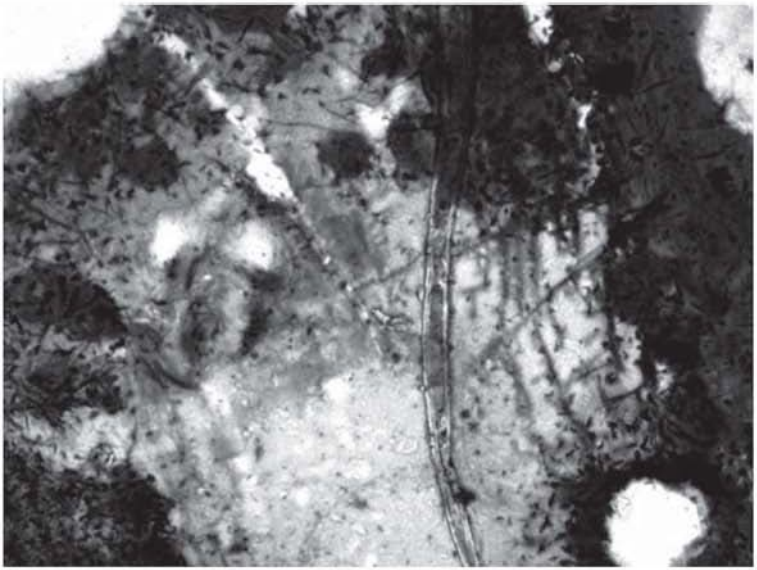

Figure 2.1. Sputum microscopy photography from a patient with a probable invasive aspergillosis

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].

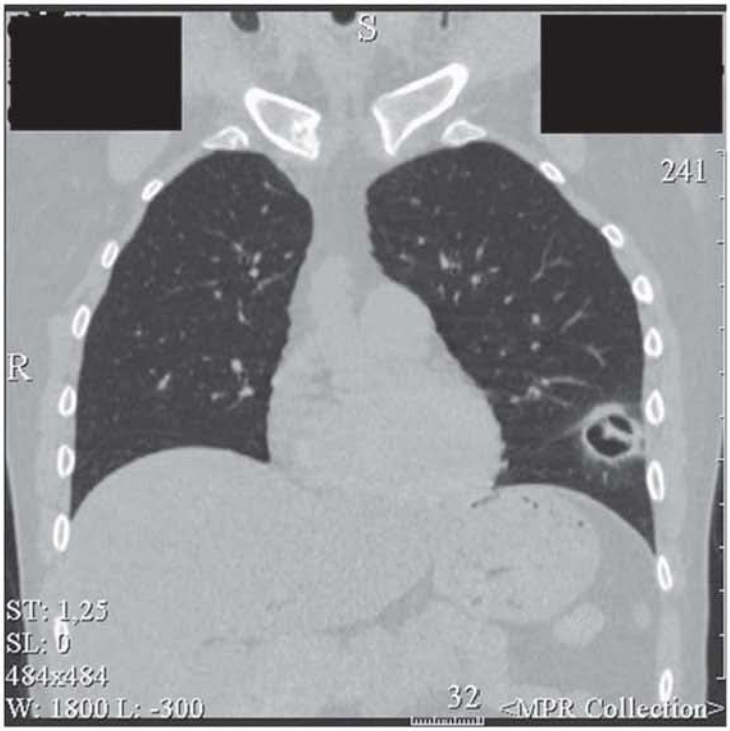

Figure 2.2. Thoracic CT-scan image of a patient with a probable invasive aspergillosis

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).

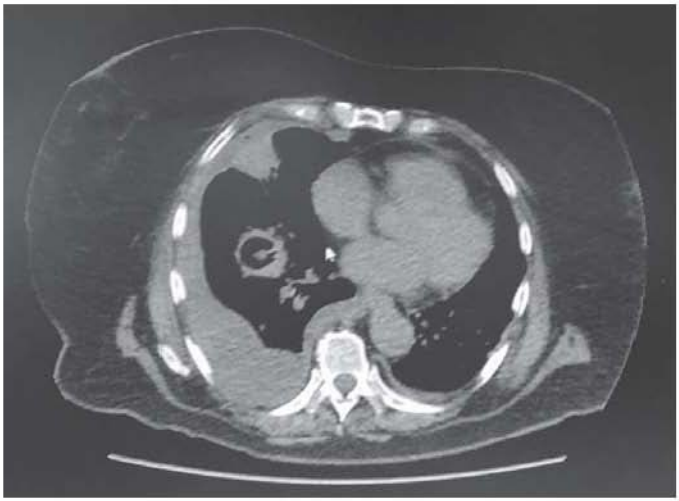

Figure 2.3. Thoracic CT-scan image of a patient with aspergilloma (soft tissue opacity)

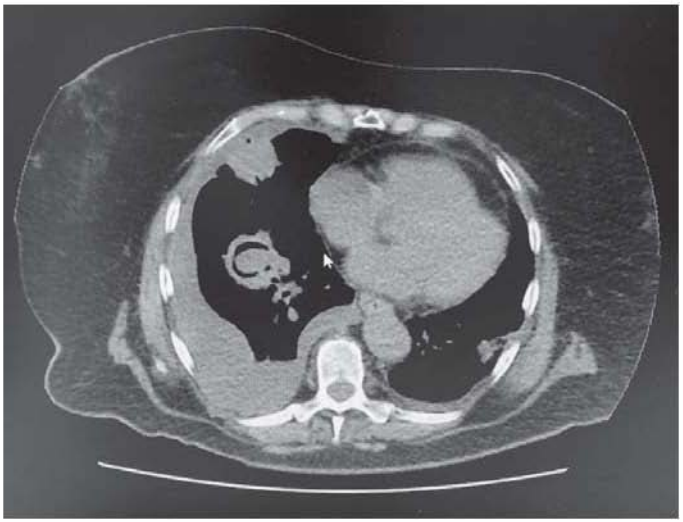

Figure 2.4. Thoracic CT-scan image of a patient with aspergilloma (air-crescent sign)

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.

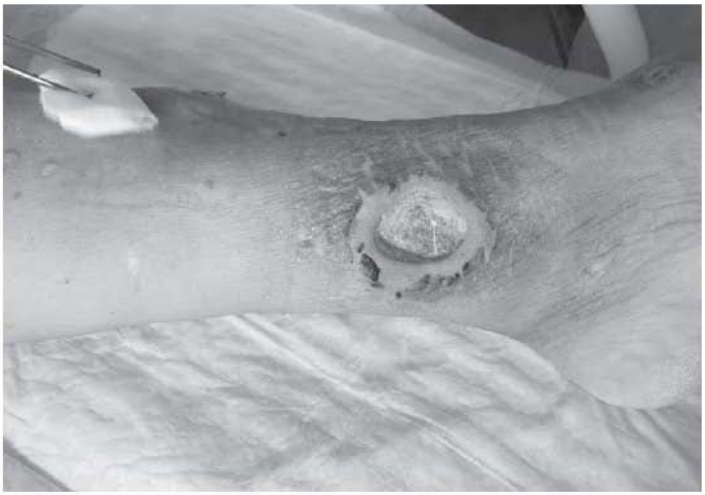

Figure 2.5. Cutaneous aspergillosis in a patient with leukemia, who is having chemotherapy.

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 allogenic 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 allogenic HSCT. Febrile neutropenia was assessed using the definition created by Freifeld et al.: a single oral temperature measurement of >38.3°C or a temperature of>38.0°C sustained over a 1-hour period with an absolute neutrophil count in peripheral blood (ANC) of $\leq$500 cells/mm^3 or an ANC that is expected to decrease to $\leq$500 cells/mm^3 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

patient with febrile neutropenia in a period of 30 days after HSCT, and this was taken as an endpoint in the analysis. In the case of a fatal outcome, blood samples were still included in the analysis. Blood samples for presepsin, PCT, and CRP were obtained in all of the included patients up to 4 hours after the onset of febrile neutropenia. Blood samples (for microbiological analysis and biomarker detection) were taken before the initiation of empiric antibacterial therapy in all patients included in this study. C-reactive protein was measured in the blood using the automatic biochemical analisator, Architect c8000 (Abbott Laboratories, USA), with the reagents from Dialab (Austria). Procalcitonin in blood was measured using an automatic analisator miniVIDAS/Blue with the reagents, VIDAS BRAHMS PCT (BioMerieux, France). Presepsin was measured in EDTA-blood taking into consideration the hematocrit level measured by an automatic analisator PATHFAST and PATHFAST Presepsin reagent (Mitsubishi Chemical Medience Corporation, Japan). The cut-offs for the biomarker levels were determined prior to initiating the test. The isolation of pathogens was performed by standard means using BacT/ALERT Standard Aerobic/Anaerobic bottles and a BacT/ALERT 3D automated microbial detection system. The identification of antibiotic resistance was studied with a VITEK 2 system, E-tests, and disc-diffusion methods.

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 a 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 0.5 g BID orally) starting from the initiation of conditioning regimen until the level of neutrophils in peripheral blood exceeded 500 cells/mm^3. 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 immunologic recovery after HSCT. Prophylaxis of infections caused by herpes viruses was performed by acyclovir. A real time quantitative polymerase chain reaction (PCR) was used to monitor CMV DNA levels in HSCT patients weekly during the pre-

engraftment period, with ganciclovir used as a first line pre-emptive therapy if there is a risk of an active CMV infection and the patient was then excluded from the study. During the period of severe neutropenia (ANC<100 cells/mm^3), 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/mm^3 some of the clinically stable patients were moved to the intensive care department with 2 patients in a room and positive air pressure. The institution's standard protocols for the initial empirical antibiotic therapy for febrile neutropenia include cephalosporins (cefepime or cefoperazone/sulbactam) or carbapenems (imipenem/cilastatin or meropenem) depending on the patient's risk group with the addition of vancomycin if an infection caused by gram-positive pathogens is suspected [19].

Data processing and analysis was performed using MedCalc Statistical Software v.14.10.2 (MedCalc Software bvba, Ostend, Belgium). ROC-analysis was performed with DeLong method [21]. Probabilities <0.05 were considered significant. The classification of quality levels of diagnostic models is shown in Table 3.1.

Table 3.1: Levels of quality of diagnostic models in ROC-analysis.

Area under ROC-curve	Quality of model
0.9 1.0	Excellent
0.8 0.9	Good
0.7 0.8	Average
0.6 0.7	Poor
0.5 0.6	Unsatisfactory

Therefore, there was a total of 52 patients with febrile neutropenia after HSCT was included. Their age was between 18 and79 years with an age median of 41 years (25–75 percentiles; 28–51 years): 28 (53.8%) female and 24 (46.2%) male. The primary diagnoses included acute myeloid leukemia, Hodgkin's lymphoma, multiple myeloma, and non-Hodgkin lymphomas. 8 patients received related allogeneic HSCT, 4 patients received unrelated allogeneic HSCT, and 40 patients received autologous HSCT. Microbiologically Gram-negative bloodstream infection was proved in 30 patients. In 22 patients, the bacterial etiology of febrile episode was excluded by way of multiple blood (or sputum) samples, microbiological analysis, and additional clinical investigations (chest X-ray, urine analysis). After the exclusion of bacterial BSI, the causes of febrile neutropenia in the other 22 patients was discussed individually:

most of them were non-infectious febrile reactions; only 3 of them had CMV reactivation proven by means of quantitative PCR; and 1 patient had candidemia, caused by *Candida albicans.*

Table 3.2: Demographical and clinical baseline characteristics of patients with febrile neutropenia in the pre-engraftment period after HSCT.

Baseline characteristics	*Absolute number (n=52), %*
Age (years, median, interquartile range)	41 (28 51)
Sex (male)	24 (46.2)
Type of HSCT:	
Autologous	40 (76.9)
Allogeneic	12 (23.1)
Conditioning regimen:	
Myeloablative	9 (17.3)
Non-myeloablative/reduced intensity	43 (82.7)
Primary diagnosis:	
Acute myeloid leukemia	12 (23.1)
Hodgkin's lymphoma	6 (11.5)
Multiple myeloma	9 (17.3)
Non-Hodgkin's lymphoma	25 (48.1)

CRP showed a poor level of sensitivity (40%); although it had 91% specificity in the analysis. The positive likelihood ratio of CRP was 4.40 (95% CI 1.1–17.7), while the negative was 0.66 (95% CI 0.5–0.9). The specificity of CRP was 100% at 225.7 mg/l, and the optimal cut-off value in such patients was 165 mg/l. The area under the ROC-curve (AUC) for CRP was 0.707 (95% CI 0.564–0.825; p=0.0049), which can assess the quality of the model as average. The ROC-curve for the C-reactive protein as a biomarker of Gram-negative BSI is shown in Figure 3.1 below.

The optimal cut-off value for procalcitonin as a biomarker of Gram-negative BSI in patients after HSCT was shown to be 1.5ng/ml, while sensitivity was 62% and specificity was 88%. Specificity was shown to be 100% when PCT was 26.7 ng/ml. The AUC for PCT was 0.741 (95% CI 0.573–0.869; p=0.0037), which assesses the quality of this model as average. The positive likelihood ratio for PCT was 5.26 (95% CI 1.4–20.2) with a negative of 0.43 (95% CI 0.2–0.8).

The optimal cut-off value for presepsin as a biomarker of Gram-negative BSI was shown to be 218pg/ml, while its sensitivity was 75% and specificity was 100%. The negative likelihood ratio for presepsin was 0.25 (95% CI 0.08–0.80), while the positive likelihood ratio was not calculated due to its 100% specificity parameter. The AUC for presepsin was shown to be 0,889 (95% CI 0.644–0.987; p<0.0001), which assesses the quality of this model as good. The results of the comparative analysis of the diagnostic parameters of presepsin, CRP, and PCT as biomarkers of Gram-negative BSI in adult patients after HSCT are presented in Table 3.3. The spectrum of pathogens which caused Gram-negative BSI in patients after HSCT is shown in Table 3.4.

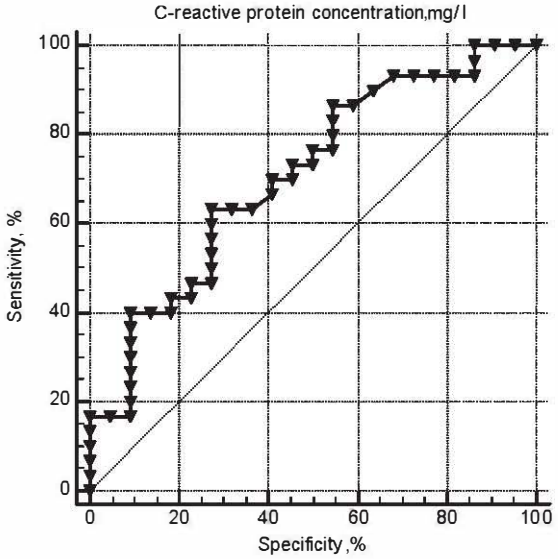

Figure 3.1: The ROC-curve for C-reactive protein.

Table 3.3: Diagnostic parameters of presepsin, CRP, and PCT as biomarkers of Gram-negative BSI in adult patients after HSCT.

Diagnostic parameter	Biomarker		
	C-reactive protein	Procalcitonin	Presepsin
Cut-off value	165 mg/l	1.5 ng/ml	218 pg/ml
Sensitivity, %	40	62	75
Specificity, %	91	88	100
Positive likelihood ratio	4.40 (95% CI 1.1 17.7)	5.26 (95% CI 1.4 20.2)	
Negative likelihood ratio	0.66 (95% CI 0.5 0.9)	0.43 (95% CI 0.2 0.8)	0.25 (95% CI 0.08 0.80)
Area under the ROC-curve	0.707 (95% CI 0.564 0.825)	0.741 (95% CI 0.573 0.869)	0.889 (95% CI 0.644 0.987)
Stand error	0.0735	0.0831	0.085
p	0.0049	0.0037	<0.0001
Quality of model	Average	Average	Good

Table 3.4: Causes of Gram-negative BSI after HSCT in the study.

Pathogen	Absolute number	Frequency of isolation, %
K. pneumoniae	12	40
E. coli	8	26.6
A. baumannii	6	20
P. aeruginosa	4	13.3

Therefore, in the study, among the causes of Gram-negative BSI in adult patients after HSCT were Gram-negative non-fermenting microorganisms at 33.33%, and members of the *Enterobacteriaceae* family at 66.67% in a total etiological spectrum. Procalcitonin was used as a biomarker of systemic bacterial infections in adults and children [22, 23]. There have been non-specific elevations of PCT in certain groups of patients: e.g. elevation was described in neonates during the first 18–30 hours up to 20 ng/ml with a decrease of biomarker to 1.5 ng/ml by 72 hours [24]. Non-specific elevations of PCT have also been seen in patients with severe trauma, burns, massive surgical interventions, and even with chronic kidney failure [25]. There is data published concerning the use of PCT in patients after solid organ transplantation; for example, researchers have

shown that the use of PCT as a biomarker of bacterial infection is possible in patients after liver and heart transplantations [26–27]. Non-specific elevation of PCT and CRP were seen in patients receiving antithymocyte immunoglobulin, T-cell therapy, and certain chemotherapy regimens [28–29]. C-reactive protein is a widely used inflammatory marker and one of the so-called acute phase proteins. It was shown to increase with various conditions: infections, trauma, autoimmune diseases, acute cardiologic diseases, and graft versus host disease [18]. Presepsin is one of the novel sepsis biomarkers that has been implemented in clinical practice in recent years. Shozushima et al. have shown that presepsin is an effective diagnostic marker in bloodstream infections but large enough samples of patients after HSCT have not yet been evaluated as a separate group [30]. In other populations, it was shown that the level of presepsin swiftly decreases after initiating antibacterial therapy, making it important to study this aspect in HSCT recipients [31]. All of the patients included in this study had their blood samples taken up to 4 hours after the onset of a febrile episode, so the results may be used as early diagnostic characteristics of biomarkers in HSCT recipients. As previously demonstrated, time is extremely important when prescribing antibiotics: in septic patients antibiotics should be started within 1 hour of the diagnosis and, if antibacterial therapy is delayed in a patient with septic shock, mortality may increase by 7.6% per hour [32].

The results of the comparative analysis of the diagnostic parameters of sepsis biomarkers in adult patients with Gram-negative BSI after HSCT prove that the best level of quality in these conditions is demonstrated in diagnostic model with presepsin (AUC 0.889; 95% CI 0.644-0.987; p<0.0001), rather than with PCT (AUC 0.741; 95% CI 0.573–0.869; p= 0,0037). CRP does not have enough sensitivity (40%) to be widely recommended as a sepsis biomarker in adult patients with Gram-negative BSI after HSCT and its cut-off value in these conditions should be at a concentration of 165mg/l. It is important to underline that this data concerns the levels of biomarkers only in the first 24 hours after the onset of febrile neutropenia because the outcome of BSI in neutropenic patient significantly depends on adequate empiric antibacterial therapy prescribed in the first 24 hours of a possible infectious complication. The results of the microbiological part of the study confirm that Gram-negative BSI in patients after HSCT is mostly caused by members of the *Enterobacteriaceae* family (66.67%), along with the important influence of non-fermenters (33.33%). The results of recent meta-analysis (including 2159 sepsis cases and conducted by Wu et al.) showed the pooled sensitivity of presepsin for sepsis to be 78% (95% CI 76–80%), while its

pooled specificity was 83 (95% CI 80–85%); the pooled positive likelihood ratio was 4.63 (95% CI 3.27–6.55) and the pooled negative likelihood ratio was 0.22 (95% CI 0.16–0.30); the area under the curve of the summary receiver operating characteristics curve was 0.89 (95% CI 0.84 to 0.94), which is close to the data achieved in our study [31]. Zhang et al. have also conducted a meta-analysis, including 11 published studies, on the overall diagnostic sensitivity of presepsin for sepsis of 83% (95% CI 77–88%), with a specificity of 78% (95% CI 72–83%), and the area under the summary receiver operating a characteristic curve of 88% (95% CI 84–90%) [33]. Still, it is important to state, that the sensitivity of presepsin in HSCT in our study was slightly lower than in the above meta-analyses (75% vs 78%; 75% vs 83%).

A limitation of this study is the relatively small sample, which is too small to build a firm conclusion; however, with regard to the cost of every HSCT procedure and the high risk of fatal outcome in cases of BSI, even such small surveys may be important. The other limitation was that the biomarkers were only measured during the first 4 hours after onset of a febrile episode because it was not possible to perform multiple measurements in all of the patients. Finally, this study was conducted in one clinical center but it is important to mention that it performs HSCT for patients from all parts of the country. The end-point in the study also has some important limitations because it is based only on bloodstream infections and concerns only Gram-negative pathogens; yet, in some regions of the world, gram-positive infections remain the leading cause of sepsis in immunocompromised hosts [1]. Also, we could still have missed active infections without bacteremia because pneumonia or urinary tract infections in neutropenic patients may not have clear manifestations. Although presepsin is the receptor of the lipopolysaccharide-lipopolysaccharide binding protein (LPS-LBP) complexes, which is an important component of Gram-negative bacterial cell wall, previous studies showed no disparity of serum presepsin concentration between infections caused by Gram-negative and gram-positive pathogens [31, 34]. So, there are research questions left which will be important to study in HSCT recipients.

Therefore, in comparison with the rest of biomarkers, presepsin determined in first 4 hours after the onset of a febrile episode has shown a relatively higher diagnostic value as a marker of BSI caused by Gram-negative pathogens in adult patients after HSCT with an optimal cut-off value of 218pg/ml. PCT was also effective in diagnosing BSI with a cut-off value of 1.5ng/ml, but with a relatively low sensitivity (62%) which

may cause a clinically dangerous false-negative results. The use of CRP as a biomarker of Gram-negative BSI should not be routinely recommended in adult patients after HSCT because of its average diagnostic quality and low sensitivity (40%); still, in such cases, an optimal cut-off value for CRP should be 165mg/l.

Common clinical practice in febrile neutropenia management is based on an immediate search for infectious foci and initiating antibiotic therapy rapidly after taking culture samples. Later, the decision to modify or stop antibacterial therapy depends on the workup, which often takes time. This means that the patient would be committed to broad-spectrum antibiotics for some time before the culture results reveal a pathogen, which is a practice that has led to the worldwide anti-microbial resistance catastrophe. Furthemore, cultures may turn out to be negative in 40% of patients with sepsis [35]. Therefore, there is a need for a rapid test that could help to quickly rule out an infectious cause. Hence, a biomarker would be most useful as a screening test: i.e., a negative value confirms the absence of infection. A good screening test is characterized by a great sensitivity. However, the sensitivities found in this study are still not high enough to recommend them as a test for febrile neutropenia in HSCT patients (presepsin sensitivity was 75%). Therefore, presepsin may only be recommended as a possible additional supplementary test in a febrile neutropenic patient after HSCT to rule out sepsis that has been caused by a Gram-negative pathogen when the pre-test probability of sepsis is already borderline and clinicians are hesitant about keeping the patient off antibiotics.

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CHAPTER FOUR

COMBINATION OF SEPSIS BIOMARKERS MAY INDICATE AN INVASIVE FUNGAL INFECTION

Infectious complications in immunocompromised patients with neutropenia or on long-lasting immunosuppressive treatments are a serious issue. Invasive fungal infections (IFI) represent cases in which delayed or inappropriately targeted treatments can have fatal consequences. Today, *Aspergillus spp.* and *Candida spp.* are the two most important genera, accounting for about 95% of all cases of IFIs [1]. The early diagnosis of IFI still remains a complicated issue [2, 3]. In combination with clinical and imaging data, the detection of galactomannan antigen in aspergillosis and mannan antigen in candidemia is broadly used. However, the concentration of these antigens is related to the invasiveness of the infectious agents [4–6]. PCR assays are considered to be promising detection methods but are not yet recommended for routine use in clinical practice due to the lack of conclusive validation for commercially available assays, variety of methodologies in the literature, and questions about the extent to which results assist diagnosis [7]. Due to the diagnostic issues and the complicated detection of fungi, diagnosing IFIs is still expressed on a scale of probability: proven, probable, and possible [7,8].

Based on the current recommendations from the European Conference on Infections in Leukemia and Guidelines from Infectious Diseases Society of America, febrile neutropenia empirical antifungal treatment is prescribed after 72–96 hours of broad-spectrum antibacterial treatment without an observed clinical improvement [9, 10]. This means that in a large number of cases patients with neutropenia and fungal infection do not receive any effective therapy for at least the first 72 hours of a fever episode, which may have the expected risk of a fatal outcome. Due to the previously mentioned practical issues, there is a need to distinguish between bacterial and fungal infections as early as possible in patients with fever and neutropenia. Biomarkers are among the broadly implemented diagnostic methods nowadays, and the most frequently used are C-reactive protein (CRP), presepsin, and procalcitonin (PCT).

While levels of CRP, as an acute phase protein, are elevated in many inflammatory conditions and are used to monitor inflammation in many fields of medicine, an increase in PCT is associated with bacterial infections [11]. Various studies are published on PCT and CRP in febrile neutropenic patients; however, existing studies include only small samples of patients after hematopoietic stem cell transplantation [12–14]. Presepsin (sCD14) is a novel biomarker implemented in clinical practice in 2004. The receptor of lipopolysaccharide-lipopolysaccharide binding protein (LPS-LBP) complexes is generated as the body responds to bacterial infection. Additionally, phagocytosis may play a major role against bacteria other than just as an inflammatory response [15–17]. Furthermore, there is a lack of compelling information concerning the use of presepsin in hematological patients, and there is a practical need to assess the diagnostic characteristics of combinations of biomarkers as an indicator for IFIs in immunocompromised hosts.

As a basis for this study, we have used our clinical experience at our tertiary hematology and bone marrow transplantation center, where we have observed a number of cases of discordant results of increased CRP and low levels of PCT or presepsin in patients with subsequently confirmed IFIs. This prospective observational clinical study was performed during the period from 2013 to 2018. The study was approved by the Institutional Review Board and Ethics Committee. Adult patients hospitalized to receive chemotherapy for hematological malignancies or treatment for graft-versus-host disease after an allogeneic hematopoietic stem cell transplantation, who were having an episode of microbiologically proven bacterial/fungal infection, were included in the study. The criteria for febrile neutropenia was assessed based on definition by the Infectious Diseases Society of America [10]. Patients with febrile episodes were screened for infections using standard hospital protocols, and during the first 48 hours after onset of the fever biomarkers (CRP, PCT, or presepsin) were measured. Additionally, during the course of neutropenia, all of the patients had their CRP measured daily along with their peripheral blood neutrophil count. Either PCT or presepsin was measured in all patients based on their physician's decision; this was obligatory during the first 48 hours after the onset of fever and non-obligatory later, depending on the clinical course of the patient. In all of the patients, the onset of the febrile episode was during the hospitalization period. Blood samples for presepsin, PCT, and CRP were obtained in all of the patients up to 48 hours after the onset of the febrile episode. Blood samples (for microbiological analysis and biomarker detection) were taken before the initiation of empiric antibacterial therapy, and all of the

biomarkers were measured in fresh plasma. CRP was measured in the blood by an automatic biochemical analyzer Architect c8000 (Abbott Laboratories, Abbott Park, Illinois, USA) with the reagents from Dialab (Vienna, Austria). PCT in the blood was measured by an automatic analisator miniVIDAS/Blue with the reagents VIDAS BRAHMS PCT from BioMerieux (Marcy l'Etoile, France). Presepsin was measured in EDTA-blood taking into consideration the hematocrit level given by the automatic analisator, PATHFAST and PATHFAST presepsin reagent (Mitsubishi Chemical Medience Corporation, Tokyo, Japan). Galactomannan antigen was detected using Platelia Aspergillus assay, with a test result considered positive if an index of positivity≥ 0.5 was obtained in two consecutive blood samples or in a single bronchoalveolar lavage specimen. The isolation of pathogens was performed by standard means with BacT/ALERT Standard Aerobic/Anaerobic bottles and the BacT/ALERT three-dimensional automated microbial detection system, Biomerieux (Marcy l'Etoile, France).

Clinical diagnosis of invasive fungal infections (IFI) was based on probability classification: proven/probable/possible [7, 18, 19]. A galactomannan antigen blood test was performed during the first 96 hours of the febrile episode, with blood cultures gathered during the first 24 hours. A rectal colonization screening was conducted on the day of hospitalization and repeated later if the patient was colonized by a highly-resistant pathogen. A blood galactomannan test and CT-scan were performed in all patients with abnormalities on their chest X-ray and with febrile neutropenia. Bronchoscopy was performed only in cases when it was considered clinically safe. Contraindications for bronchoscopy included severe hypoxemia, bleeding, and platelet transfusion-refractory thrombocytopenia. Therefore, the inclusion criteria included a microbiologically proven bacterial bloodstream infection, and a proven/probable invasive fungal infection. The diagnosis of a bloodstream infection was made according to the CDC criteria [20]. Then all patients were divided either into a group with a bacterial or a fungal infection.

The institution's standard protocols for the initial empirical antibiotic therapy for febrile neutropenia include cephalosporins (cefepime or ceftazidime or cefoperazone/sulbactam) or carbapenems (imipenem/cilastatin or meropenem) depending on the risk group of the patient; vancomycin was added in those with a possible infection caused by gram-positive pathogens [9]. The standard antibacterial prophylaxis in the institution is based on fluoroquinolones (mainly ciprofloxacin 0.5g orally twice a day during the neutropenic period). No additional routine antibacterial

prophylaxis against *Streptococcus pneumoniae* is administered. Fluconazole, as the primary antimycotic prophylaxis, is administered routinely, and the secondary antimycotic prophylaxis is chosen according to the patient's history, while the empirical antifungal treatment is based on voriconazole or echinocandins [21]. During profound neutropenia (ANC <100 cells/mm3) patients are isolated in a protective environment, with infection controlled strictly in all patients. The study was conducted in accordance with STARD 2015 guidelines and according to the Helsinki declaration. The study flowchart is shown in Figure 4.1.

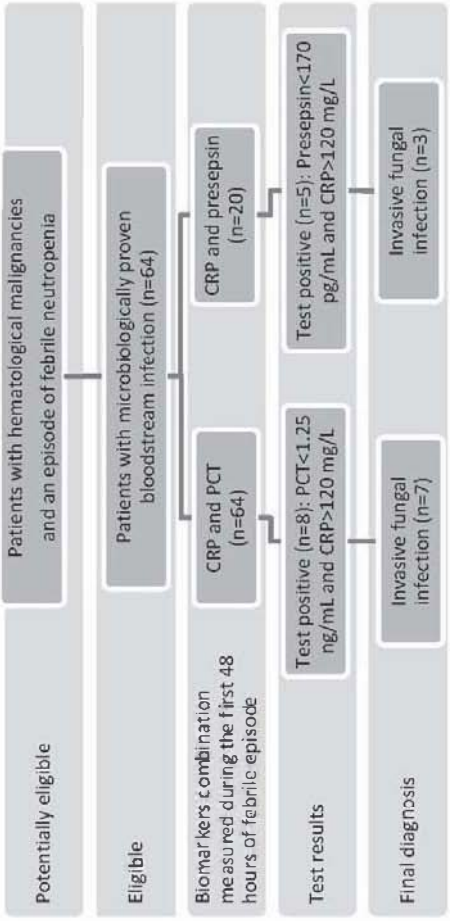

Figure 4.1: Flow diagram of the conducted diagnostic study.

A receiver operating curve (ROC) analysis using the DeLong method was performed to assess the diagnostic parameters of biomarkers [22]. According to Montagna et al., the overall incidence of IFIs in adults with hematological malignancies was estimated to be 5% in ROC-analysis [23]. Youden's J statistic and area under the curve (AUC) were used to summarize the performance of the diagnostic test. A false negative (FN) decision was judged to be twice as costly as a false positive (FP) decision, and no assumptions were made about the costs for true positive and true negative decisions. Analysis of the diagnostic parameters of a combination of biomarkers was performed using a logistic regression model with two biomarkers as the explanatory variables, and the subsequent ROC-analysis of predicted probabilities from that model. Probabilities <0.05 were considered significant. Data processing, analysis, and plotting were performed using R version 3.4.0 (R Development Core Team, Vienna, Austria) and MedCalc Statistical Software version 16.4.3 (MedCalc Software bvba, Ostend, Belgium).

Therefore, between 2013 and 2018, there were 64 patients in total with the inclusion criteria. The patients' demographical and clinical characteristics are shown in Table 4.1 and the detailed causes of infections are shown in Figure 4.2.

Table 4.1: Baseline clinical and demographical characteristics of patients in the study.

Characteristic	Abs. number (%)
Age (median, interquartile interval)	41 (34 51)
Sex (male)	34 (54)
Primary diagnosis:	
Acute myeloid leukemia	42 (66.7)
Acute lymphoblastic leukemia	6 (9.4)
Multiple myeloma	5 (7.8)
Hodgkin lymphoma	4 (6.2)
Non-Hodgkin lymphoma	3 (4.7)
Chronic myeloid leukemia	2 (3.1)
Aplastic anemia	1 (1.6)
Myelodysplastic syndrome	1 (1.6)
Abs. neutrophil count below 500 cells/mm^3	48 (75.0)
Cause of infectious episode:	
Bacteria	53 (82.8)
Fungus	11 (17.2)

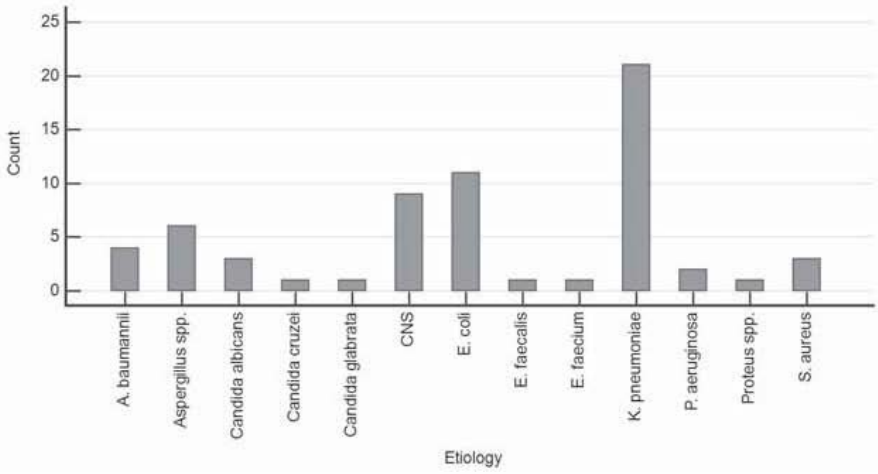

Fig 4.2: Spectrum of infectious episodes among patients in the study.

During the preliminary analysis we have already observed that in most patients with IFIs there are low numbers of PCT or presepsin and high numbers of CRP, which corresponds with our expectations based on our previous clinical experience. As a first step for ROC-analysis we have estimated the optimal cut-off for IFIs in all of studied biomarkers based on the Youden index; this is shown in Table 4.2.

Table 4.2: Results of the cut-off estimation for CRP, PCT, and presepsin in patients with IFIs.

	C-reactive protein	Procalcitonin	Presepsin
Youden index	0.443	0.786	0.600
Optimal cut-off value	>120.4 mg/L	<=1.26 ng/mL	<=173 pg/mL

Based on these findings, we performed the second step of analysis and estimated the diagnostic characteristics of combinations "low PCT and high CRP" and "low presepsin and high CRP" with the above cut-off values. As expected, both combinations showed high quality diagnostic parameters; this is shown in Table 4.3, and Figures 4.3 and 4.4.

Table 4.3: The diagnostic parameters of the different combinations of CRP, PCT, and presepsin as indicators of IFIs in immunocompromised patients.

Diagnostic parameter	Biomarkers combination	
	PCT<1.25 ng/mL and CRP>120 mg/L	Presepsin<170 pg/mL and CRP>120 mg/L
Area under the ROC curve	0.962 (95% CI 0.868 to 0.995)	0.907 (95% CI 0.692 to 0.990)
Sensitivity (%)	90.0 (95% CI 55.5 99.7)	80.0 (95% CI 28.4 99.5)
Specificity (%)	92.9 (95% CI 80.5 98.5)	86.67 (95% CI 59.5 98.3)
Ratio of positive likelihood	12.6 (95% CI 4.2 38.2)	6.0 (95% CI 1.5 23.4)
Ration of negative likelihood	0.11 (95% CI 0.02 0.70)	0.23 (95% CI 0.04 1.3)
Positive predictive value	39.9 (95% CI 17.9 66.8)	24.0 (95% CI 7.5 55.2)
Negative predictive value	99.4 (95% CI 96.5 99.9)	98.8 (95% CI 93.4 99.8)
Standard error of AUC	0.0287	0.0758
P-value of AUC	<0.0001	<0.0001
Cost	0.0779	0.147
Youden index	0.83	0.6667

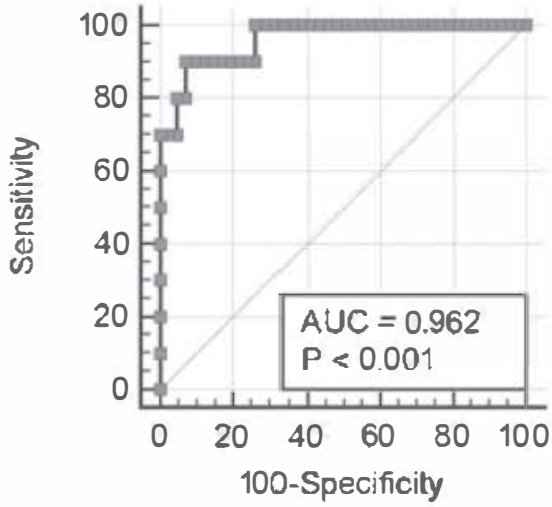

Fig 4.3: ROC-curve for the combination of PCT<1.25 ng/mL and CRP>120 mg/L in the diagnosis of IFIs.

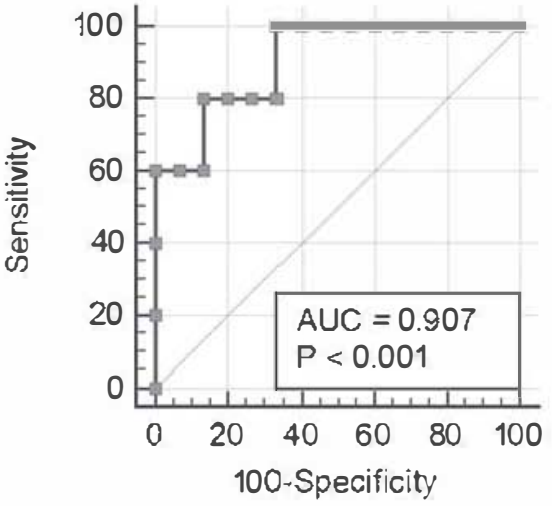

Fig 4.4: ROC-curve for the combination of presepsin<170 pg/mL and CRP>120 mg/L in the diagnosis of IFIs.

Therefore, both of the combinations have shown high quality diagnostic parameters with slightly better characteristics in the "low PCT and high CRP" group, possibly due to the larger number of patients that were tested for PCT compared with presepsin in the study. In the final step of analysis, we performed a pairwise comparison of ROC-curves for the described biomarker combinations ("low PCT and high CRP" vs "low presepsin and high CRP") using the DeLong method and found no significant differences in diagnostic characteristics (difference between AUC 0.0933; st. error 0.0758; 95% CI -0.0552 to 0.242; p=0.2180).

To our knowledge this is the only existing large clinical study focused on the clinical significance of various combinations of presepsin, CRP, and PCT for the early diagnosis of IFI and guided empirical antifungal treatment. There is a published research paper on the combination of biomarkers in diagnostics of fungal infections in patients with chemotherapy-induced immunosuppression. This is where Markova et al., using a smaller cohort of hematological patients, showed the phenomenon of "low PCT and high CRP" in cases of fungal infections [24]. It is important to mention that our findings correspond with the results of Markova et al.; however, it is important to note that we had a larger cohort of patients, and were first to show the diagnostic parameters of combination "presepsin and CRP" in the diagnosis of IFI. There was also data published on the use of CRP and PCT in surgical patients in ICU, where an increase in CRP in addition to the usual amount of PCT showed the shift in predictive effect from bacterial infections to candidemia [25].

It is also interesting to mention, that an elevation of CRP was observed in most of the patients in our study, while the elevation of PCT or presepsin was only found in patients with bacterial BSIs. The low practical value of measuring PCT as a marker of fungal infections was reported earlier by other groups, while there is no data published on presepsin use in patients with fungal infections [26–28]. The possible limitations of the study included the small number of patients with fungal infections although the sample size was sufficient enough to reach statistical significance in the analysis. Future multicenter studies may overcome this research issue in the fungal infections. The other issue we need to mention is that we compared the groups of patients with fungal infections and bacterial infections and did not involve patients with an increase of CRP of a non-infectious origin. This solution was performed because of the low level of positive microbiological results in a large number of patients with febrile neutropenia, which often makes it complicated to distinguish between the "infectious" and "non-infectious" origin of febrile condition in hematology.

Still, our results provide an important practical way to distinguish between bacterial and fungal infections, which will lead to earlier empirical antifungal treatment and have a potential effect on survival.

Therefore, as a result of this study, we may use the combination of increased levels of CRP (over 120mg/L) with a low level PCT (<1.25ng/mL) or low level presepsin (<170pg/mL) as a combined biomarker of invasive fungal infection in immunocompromised patients during the first 48 hours of a febrile episode. One of the practical issues from the observed results is the fact that CRP is an easily accessible and obtainable biomarker to test in most of clinical settings, while specific fungal biomarkers (1, 3-beta-D-glucan, galactomannan) and PCR-based diagnostic methods are less accessible for regular monitoring, even in hematology and cancer centers.

Based on the presented findings, the combination of seriously elevated CRP results with minimal changes in PCT or presepsin levels, in cases of development of febrile neutropenia, may provide a suitable argument for early empirical antifungal treatment. This may lead to the early use of empiric antifungal therapy, which should have an important effect on infection-related outcomes. The results of our study are significant for a number of immunocompromised patients, especially with regard to the low accessibility of fungal antigen and PCR-based tests.

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CHAPTER FIVE

INFECTIONS ORIGINATING FROM THE GUT MICROBIOME AND THEIR ROLE IN MODERN HEMATOLOGY

The earliest mechanism for the development of infectious complications is damage to the mucous membrane of the internal organs during chemotherapy. Cytotoxic therapy has a direct effect on cells with a high mitotic index (epithelium of the oral cavity and the gastrointestinal tract), which is expressed by the clinical manifestations of mucositis. Mucositis of the oral cavity is manifested by functional complaints (pain during swallowing) and morphological changes (edema, erythema, ulceration), while mucositis of the gastrointestinal tract is accompanied by nausea, vomiting, diarrhea, and abdominal pain. At the same time, the bacteria of the gut microbiome is translocated into the bloodstream. Chemotherapy regimens containing melphalan, etoposide, methotrexate, cytarabine, and idarubicin are the most frequent cause of mucositis, which is significantly complicated by the combination of anthracyclines with the total body irradiation and cyclophosphamide as part of the conditioning regimen of HSCT [1, ,2]. Also, one of the important consequences of mucositis is the violation of the absorption of oral forms of drugs. E.J. Johnson et al. established a decrease in ciprofloxacin absorption in patients with mucositis and febrile neutropenia during chemotherapy [3].

Interestingly, the connection between a decrease in the level of neutrophils in peripheral blood and the frequency of infection in 1966 was proved for the first time by Dr. Gerald P. Bodey from Houston, USA [4]. At the same time, bloodstream infections are considered the main clinical form of bacterial infectious complications in adult patients with HSCT [5, 6].

It is important to note that in the cohort of immunocompromised patients with oncological and hematological diseases, HSCT recipients are the most studied in the context of the microbiome. Much fundamental data about the human microbiome and its clinical significance was based on the model of immunosuppression in HSCT. In particular, one of the well-

known works by Y. Taur et al. shows a significant decrease in the biological diversity index of the microbiome (Shannon index) in patients over the course of HSCs transplantation, along with revealing the negative effect of antibiotics and chemotherapy on the diversity of the microbiome [7].

In a significant number of cases, infections of the bloodstream and sepsis, which are often found in immunocompromised patients, are the result of disorders of the intestinal microbiome and damage to the intestinal mucosa under immunosuppression. These phenomena occur due to a combination of chemotherapy, radiation therapy, and the widespread use of antibiotics, which ultimately leads to the translocation and generalization of some intestinal bacteria. The most common bacteria capable of enteric translocation are oxygen-resistant microorganisms, including vancomycin-resistant enterococci; members of the *Enterobacteriaceae* family, such as *E. coli* and *Klebsiella spp.*; and viridans group streptococci [8–12].

Cytotoxic chemotherapy remains one of the most common treatments for various types of cancer. This group includes many drugs that violate the process of cell reproduction (mitosis), thereby mainly affecting the rapidly dividing cells, which include cancer cells. However, cancer cells are not the only group of rapidly dividing cells in the human body. Gastrointestinal tissue cells and bone marrow hematopoietic stem cells are also two important cell populations that quickly divide in a healthy body. Thus, these cells also become susceptible to the antimitotic collateral effects of chemotherapy [13, 14].

Specialized stem cells of the gastrointestinal tract usually compensate for the loss of mucosal epithelial cells in order to maintain the integrity of the barrier. However, cytotoxic chemotherapy, in addition to causing damage to the epithelial cells of the intestinal lining, initiates a microbiome-associated recovery reaction by activating a transcription factor (nuclear factor NF-κB); this is not limited to epithelial cells as it occurs in all surrounding cells and tissues. Next, the production of pro-inflammatory cytokines is initiated, thereby forming a pathophysiological chain with a positive feedback to enhance the inflammatory response at the primary site of damage. The combination of the inflammatory reaction and the apoptosis of the cells in the mucous membrane of the gastrointestinal tract leads to the development of abdominal pain in the patient, impaired absorption of dietary substances, and damage to the mucous barrier. Together, these processes are called mucositis, which is a very common complication of chemotherapy [15].

As we have already noted, chemotherapy simultaneously affects hematopoietic stem cells in the bone marrow. These cells are the source material of all types of blood cells, with the greatest contribution to neutrophils. Neutrophils are the most common, but short-lived white blood cells that act as immediate primary protection against infectious agents [14]. One of the earliest precursors of neutrophils are promyelocytes, which are characterized by a high activity of DNA synthesis and, accordingly, are very vulnerable to the anti-mitotic effects of chemotherapy. Their offspring is composed of myelocytes, which are the most numerous proliferating precursors of neutrophils and, therefore, are the largest population of cells damaged by chemotherapy. All subsequent cells after myelocytes do not divide. Thus, the loss of bone marrow myelocytes has the greatest effect on neutropenia in peripheral blood, and the rate of recovery of cells in this population largely determines the duration of neutropenia. Additionally, patients with deep and/or long-term chemotherapy-associated neutropenia are usually prescribed prophylactic antibiotics to prevent potentially fatal systemic infections. Together, these factors form the cycle of pathogenesis of intestinal infections.

First, neutropenia reduces the patient's ability to limit the local bacterial process. Secondly, the use of antibiotics, as well as chemotherapy, contribute to the disruption of the intestinal microbiome and the selection of antibiotic-resistant colonizing bacteria. Thirdly, mucositis creates a "window"—i.e., a portal for the penetration of intestinal bacteria into the systemic circulation—due to the damage to the complex barrier of the gastrointestinal tract. If these conditions are met, then the colonizing microorganism enters the bloodstream and causes a systemic infection. At the same time, the reservoir of the microorganism stays in the intestine, and allows it to constantly enter the bloodstream against the background of the patient's reduced protective mechanisms.

Another problem associated with gut microbiome and bloodstream infections is the spread of the highly drug resistant Gram-negative bacteria. Infections caused by Gram-negative bacteria (GNB) have become a major healthcare threat in the last decade, despite the implementation of antibiotic stewardship and infection control strategies. GNB, in particular members of *Proteobacteria* phylum, have an increasing role in immunocompromised hematology and cancer patients, including hematopoietic stem cell transplantation (HSCT) settings with high numbers of morbidity and mortality. As previously stated, a gut domination over 30% by *Proteobacteria* is a predictor of a subsequent Gram-negative bloodstream infection in allogeneic HSCT [7].

In this observational cohort study, we characterized the effect of clinical risk factors (age, sex, underlying primary disease, conditioning regimen intensity, hematopoietic stem cell source, and antibiotic use) on gut microbiota composition in allo-HSCT patients. The participants were enrolled in a fecal collection protocol with at least three sequenced fecal samples taken during hospitalization for HSCT; this also included the engraftment day. DNA was extracted and purified from each fecal sample, and the V4 to V5 region of the 16S rRNA gene was polymerase chain reaction (PCR)-amplified using modified universal bacterial primers. Purified PCR products were sequenced using the MiSeq Illumina platform. The Cox proportional hazards time-dependent regression model was used to evaluate the predictive effect of clinical variables on gut domination outcome. A total of 765 patients met the study criteria and were selected for analysis. The independent protective effect of fluoroquinolones against *Proteobacteria* phylum gut domination was observed in a conducted time-dependent multivariate analysis (HR 0.50; CI 0.26-0.97; p=0.041), while other antibiotics (β-lactams, metronidazole, vancomycin and linezolid) showed no statistically significant effect on the risk of *Proteobacteria* gut domination. Therefore, it proved that preventive use of fluoroquinolones in allogeneic HSCT recipients during the engraftment period has a protective effect against *Proteobacteria* phylum gut domination, which may decrease the incidence of Gram-negative bloodstream infections.

Discussions over the clinical and microbiological efficacy of routine antibacterial prophylaxis with fluoroquinolones in patients with chemotherapy for hematological and oncological diseases, as well as organ and tissue transplantation, have been conducted throughout the world. Some centers routinely perform this procedure and some refuse to use it due to its risk of complications. In addition, the question of the importance of fluoroquinolones in the prevention of infectious complications now requires a revision, particularly given the introduction of the new methods used to study the human microbiome. The discussion about the efficacy and safety of routine fluoroquinolone antibiotic prophylaxis in patients with hematology and with HSCT is still ongoing, especially because of the ubiquitous increase in bacterial resistance to fluoroquinolone. Earlier, Bucaneve et al. showed the effectiveness of these types of prophylaxis for conditions with a level of resistance to fluoroquinolones of 20%, which was later taken as a possible threshold, while routine prophylaxis was recommended in centers with lower resistance to these antibiotics [16-17].

Although the majority of European and American hematology and transplantation centers currently report a level of bacterial resistance to fluoroquinolones above 20%, the decision about their routine use as a prophylaxis remains the subject of discussion. The main limitation of a sound clinical decision for physicians is the lack of evidence regarding the effect of prophylaxis on overall mortality in the clinical center, while the protective effect of fluoroquinolones against the development of bloodstream infections and sepsis has been reliably demonstrated in many publications. Interestingly, Chong et al. showed that stopping fluoroquinolone antibiotic prophylaxis in their center led to an increase in the incidence of Gram-negative sepsis in patients after allogeneic HSCT, including infections caused by extended-spectrum beta-lactamase producers [18].

One of the latest meta-analyzes performed by Mikulska et al., on behalf of the European Conference on Infections in Leukemia (ECIL), included the results of two randomized controlled trials and 12 observational studies on the problem of antibacterial prophylaxis with fluoroquinolones in patients with hematopoietic system tumors and neutropenia. Despite the fact that among the included studies there were only 2 studies with an emphasis on allogeneic HSCP, the results of the meta-analysis reliably showed that fluoroquinolone prophylaxis is associated with a lower incidence of bloodstream infections, sepsis, and episodes of febrile neutropenia. It is also important that, according to the results of the meta-analysis, there was no effect on the initial level of resistance of bacteria to fluoroquinolones in the center (i.e., the background of the antibiotic resistance) on the clinical effectiveness of antibacterial prophylaxis [19].

Systemic antibiotic therapy and chemotherapy are known factors that affect the structure of the intestinal microbiome in immunosuppressed patients. As a rule, against the background of these factors, excessive reproduction of normal microorganisms occurs, including the most significant bacteria in the structure of the etiology of sepsis: *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterococcus faecium*. The revealed protective effect of fluoroquinolones against the development of Gram-negative sepsis in hematology makes a significant contribution to the discussion about the clinical efficacy of antibacterial prophylaxis in patients with chemotherapeutic-associated neutropenia.

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CHAPTER SIX

HUMAN HERPESVIRUS 6 INFECTIONS IN HEMATOLOGY: UNEXPECTED CONSEQUENCES

Herpes viruses, due to their potential latency and neurotropism, are considered as possible agents in the pathogenesis of many central nervous system disorders, including mental diseases. Nowadays the number of diseases associated with HHV-6 infection is increasing, with a pathogenic role currently being discussed in neurologic disorders, such as epilepsy; multiple sclerosis; and psychiatric diseases, such as schizophrenia [1].

The human herpes virus 6B (HHV-6B) is a ubiquitous beta-herpesvirus that infects over 90% of people within the first two years of life. Typically, HHV-6B reactivation occurs in approximately 40% of allogeneic HCT recipients (range 13.9%–93.6%). However, the reactivation rate of HHV-6 can vary greatly depending on the level of immunosuppression, the sensitivity of the diagnostic assay, and the stem cell source (the reactivation rate may surpass 90% in T-cell depleted umbilical cord blood transplant recipients). HHV-6B is the most frequent cause of encephalitis in HCT recipients. Other signs and syndromes associated with HHV-6 reactivation include fever, rash, diarrhea, thrombocytopenia, and pneumonitis [2–5]. Several studies have suggested that HHV-6B plays a role in aGVHD following HCT [6].

Further, we will present a rare clinical case of a child who developed HHV-6 encephalitis after an allogeneic hematopoietic stem cell transplant (allo-HSCT), with mental disorders and the late onset of childhood schizophrenia. The male 15-year old patient, who had diagnosed acute myeloid leukemia and a high primary risk in remission 1, was enrolled in non-related allo-HSCT conditioning protocol with treosulfan, endoxan, melphalan, and anti-thymocyte globulin. On day 13, after HSCT, the patient developed clinical signs of encephalitis with abnormalities on a brain MRI scan in the basal ganglia area. The blood PCR test detected HHV-6 in serum, while CSF showed DNA HHV-6 only during the pre-

transplant period. Immediate treatment with ganciclovir was started with a dosing regimen of 5 mg/kg IV q12h for 7 days and a maintenance dose of 5mg/kg IV q24h. Unfortunately, acute renal failure developed soon on the maintenance dosing, leading to a discontinuation of treatment under clinical evaluation with CSF and blood PCR-monitoring. During the late post-transplantation period, the patient developed mental abnormalities, with depressive disorder being main clinical syndrome. Later in the clinical course, the mental disorder developed into childhood schizophrenia, which was confirmed by psychiatrists and persisted until the patient was 18 years. No additional risk factors for the development of schizophrenia were identified, and there was no family history of mental disorders. The discontinuation of the maintenance antiviral treatment might be the explanation for such a complication. In the absence of other risk factors, HHV-6 encephalitis may be considered as an initial trigger of mental disorders in childhood, including schizophrenia. Children with profound immunosuppression, including chemotherapy and HSCT procedures due to hematological neoplasms, are at risk of HHV-6 encephalitis.

It is important to mention that, although umbilical cord blood transplantation (CBT) represents a risk factor for HHV-6 encephalitis [7–8], other risk factors remain unconfirmed. Treatment recommendations have been made based on in vitro data [9, 10] but there is very little data available on its clinical effectiveness.

Finally, HHV-6 encephalitis should be diagnosed if the patient satisfies all of the following criteria:

a) presence of central nervous system (CNS) dysfunction
b) a positive PCR result for HHV-6 DNA in cerebrospinal fluid (CSF)
c) absence of other identified causes of CNS dysfunction, including other infectious agents

The study by M. Ogata et al. clearly showed a poor prognosis for allo-HSCT recipients who developed HHV-6 encephalitis. As well as encephalitis, various other conditions were also associated with patient death, including GVHD. The authors proved that full-dose antiviral therapy was associated with lower incidences of sequelae and death due to HHV-6 encephalitis, and the use of foscarnet, but not ganciclovir, was also associated with a low incidence of early death [11–12]. Previous in vitro studies have shown that foscamet offers better in vitro activity against HHV-6 than ganciclovir [9–10]. It is important to mention that

myelosuppression, due to ganciclovir, might increase the risk of infectious diseases. Combination therapy with foscarnet and ganciclovir seems to be most effective but still needs to be evaluated in more studies.

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CHAPTER SEVEN

PNEUMOCOCCAL VACCINATION IN HEMATOLOGY AND THE EFFECTS OF THEIR IMPLEMENTATION

Currently, the vaccination of immunocompromised patients with hematological malignancies is of particular practical importance, since it is in this population that the frequency and severity of vaccine-preventable infections is much higher [1], while the level of coverage of specific immunoprophylaxis programs unfortunately remains low, especially in adult patients [2–5]. The small percentage of revaccinated adult hematologic patients is often due to the fact that their clinicians are provided with insufficient or inaccurate information on the safety, efficacy, and contraindications. In some cases, there is a lack of the practical knowledge needed to develop an individual vaccination calendar for adult patients with a hematological malignancy.

In general, vaccine-preventable infections in patients with immunosuppression can be classified as follows:

1) more frequent and/or more severe infections: pneumococcal infection, *Haemophilus influenza* type B (HIB), influenza, and varicella-zoster virus (VZV)
2) infections with the same frequency as in the general population but where there is a mandatory vaccination program: tetanus, diphtheria, poliomyelitis, and hepatitis B
3) infections that need to be vaccinated in special situations (travel to endemic areas, and epidemic outbreaks)

Pneumococcal infections remain significant causes of morbidity and mortality in hematologic patients receiving chemotherapy. According to the published data, the immune response to the polysaccharide 23-valent pneumococcal vaccine was significantly reduced in patients with hematological neoplasms, including patients with multiple myeloma and Hodgkin's lymphoma on chemotherapy [6–9]. It should also be

emphasized that, in most cases, the introduction of live (attenuated) vaccines is strictly contraindicated in patients receiving chemotherapy due to the high risk of developing disseminated disease [2].

Multiple myeloma (MM) is a neoplasm of the hematopoietic tissue with a predominant effect based on plasma cells, which affects older patients (mean age 69 years) and has an overall survival rate of 6–7 years [10, 11]. In recent years, new therapeutic agents have been actively introduced into clinical practice, resulting in improved survival rates in patients with MM. The main classes of new agents include proteasome inhibitors, immunomodulating agents, and monoclonal antibodies. These agents are commonly used in double or triple combinations, which include a chemotherapeutic and/or glucocorticosteroid (VRD, VCD, VD, RD, BBD, maintenance therapy with bortezomib). Patients with MM have an extremely high risk of developing life-threatening infectious complications, especially pneumococcal infection, which frequently becomes fatal during the drug-induced immunosuppression period.

In these patients, specific prophylaxis for pneumonia was limited for many years by the fact that polysaccharide pneumococcal vaccines showed a low efficacy in hematological patients, due to a decrease in the level of immunological response of T and B cells [12]. Earlier, Cordonnier et al. proved that a regimen with 3 doses of 13-valent conjugated pneumococcal vaccine after the allogeneic transplantation of hematopoietic stem cells, followed by a booster dose after 6 months, created an adequate immune response [13]. An analysis of the clinical outcomes for reducing the incidence of pneumonia and survival has yet to be done to fully understand the effectiveness of vaccination in certain hematological diseases. It is important to note that the clinical efficacy and safety of conjugated pneumococcal vaccines in patients with multiple myeloma, against the background of therapy with new agents, has not previously been reported.

At the same time, the rates of infection-related mortality in MM patients remain impressive. For example, in a large cohort from the UK, it was shown that 1 in 10 patients die from infectious complications within 10 weeks of a diagnosis of multiple myeloma [14]. Moreover, in a Swedish cohort of 9253 patients with MM it was found that 22% of patients die from infectious complications within the first year of diagnosis [15]. The revolution in MM therapy has now led to the rewriting of the clinical history of this disease. The patients' life now lasts significantly longer due to disease control and this brings with it the promise of a functional cure

for MM, which may be a realistic perspective in the coming years. However, these exciting events may not be useful due to the fact that 1 out of 10 patients still die from infections in the early treatment period and, as the response to MM therapy improves, the attributive effect of early infectious complications on the risk of early mortality increases.

One of the most interesting studies on the prevention of infections in MM patients receiving therapy with novel agents is devoted to routine antibiotic prophylaxis with levofloxacin. The authors of the clinical study TEAMM, which was performed in the UK, evaluated the efficacy of a single dose of 500mg levofloxacin daily for 12 weeks of intensive MM therapy with novel agents. In this study, febrile neutropenia episodes and patient survival were taken as the outcome endpoints in the analysis, with a statistically significant survival difference in favor of the group with antibiotic prophylaxis compared with the control group over the course of 12 weeks [16]. However, vaccination may be the most safe and effective measure for the prevention of pneumonia in hematological patients, especially since pneumococcus plays an important role in the structure of infectious morbidity in hematology [1, 2].

The aim of the discussed study was to examine the clinical efficacy of the 13-valent pneumococcal conjugate vaccine (PCV13) in patients with MM receiving novel agents (proteasome inhibitors and immunomodulating drugs), namely bortezomib, and lenalidomide in combination with steroids. As part of the pilot project to study the efficacy and safety of the introduction of the 13-valent conjugated pneumococcal vaccine in patients with MM, between June 2017 and August 2018, 17 patients with a confirmed diagnosis of MM were treated with novel agents. Vaccination with PCV13 was recommended whenever possible between periods of chemotherapy and was performed 3 times with a minimum interval of 1 month.

All patients gave informed consent and commitment to vaccination, and the study was approved by an official Ethics Committee of the institution. Adverse events during vaccination in patients with MM were not recorded. The control group was recruited using the paired matching method in a 1:1 ratio, with a distribution control for sex and age. The statistical reliability of the differences between the samples was determined by nonparametric statistics (chi-squared test, Fisher exact test) using the R free software environment. The clinical and demographic data of patients (n = 34), as well as the incidence of episodes of febrile neutropenia (FN) and clinically-radiologically confirmed pneumonia are presented in Table 7.1.

Table 7.1: Preliminary clinical effects due to the introduction of pneumococcal vaccinations in patients with multiple myeloma and receiving novel agents.

Parameter	Vaccinated cohort (n=17)	Matched controls (n=17)	p-value
	Abs. number (%)	Abs. number (%)	
Age, median (interq. int)	54 (52 66)	55 (50 61)	0.5002
Sex (male)	14 (82)	11 (73)	0.6761
Febrile neutropenia	3	9	0.0339
Pneumonia	2	6	0.1344

Thus, despite the small sample size, a statistically significant effect of PCV13 vaccination on the incidence of febrile neutropenia episodes (p = 0.0339) has already been observed. Meanwhile, there is a tendency to decrease in incidence of clinically-radiologically confirmed pneumonia in patients with MM after vaccination (p = 0.1344). An increase in the sample size of this ongoing study may give a more confident answer to the question of the clinical efficacy of introducing greater vaccination in adult patients with MM, who are using novel agents.

Therefore, as conclusions we may underline the following points:

1. Vaccination with 13-valent pneumococcal conjugate vaccine may lead to a decrease in the incidence of febrile neutropenia episodes and a trend towards a reduction in the incidence of pneumonia in patients with MM, who are receiving treatment with novel agents.
2. Vaccine-preventable infections, including invasive pneumococcal infections, are characterized by a high risk of fatal outcomes in adult immunocompromised patients with hematological diseases and hematopoietic stem cell transplant recipients.
3. Further implementation of vaccination programs for patients with multiple myeloma should be continued with the participation of a multidisciplinary team of specialists. The development of a vaccination registry may become an important step in reducing the incidence and mortality from infectious complications in adult hematology.

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CHAPTER EIGHT

GRAFT-VERSUS-HOST DISEASE AND INFECTIONS IN TRANSPLANT RECIPIENTS

Acute GVHD is one of the most common serious complications of allogeneic HSCT and remains a leading cause of mortality in HSCT recipients all over the world. Various publications showed the association of acute GVHD with higher risk of infectious complications. There is a lack of data on the risk factors for developing an episode of infection and a spectrum of infections in patients with acute GVHD after allogeneic HSCT.

An ambispective observational study was performed to evaluate the risk factors and characteristics of infectious complications in adult patients having acute GVHD after HSCT. A proven case of infection (bacterial, viral, or fungal) was taken as a primary outcome in the study. There were 68 adult patients with II-IV grades of acute GVHD after allogeneic HSCT included in the study. The following covariates included in the multivariate analysis were taken: procedure of the MCSs transplantation; type of allogeneic HSCT (related/non-related); main disease progression; absolute neutrophil count (ANC) on the first day of the infectious complication; and the GVHD-involved organs.

Microbiological data concerning the causes of infectious complications in patients with acute GVHD has shown the major impact of viral infections (CMV) and invasive pulmonary aspergillosis. There were no bloodstream infections caused by *Candida spp.* registered in the study. Bacterial infections were mostly presented by bloodstream infections (caused *by K. pneumoniae, E. faecalis, E. coli*) and pneumonia. The progression of the main disease (OR 5.52; 95% CI 1.21–25.06; p=0.0270) was found to be an independent risk factor for the development of invasive pulmonary aspergillosis and bacterial infectious complications. Patients with a hematopoietic stem cell transplant from an unrelated donor remain in a high-risk group for bacterial infections in case of developing acute GVHD (OR 3.50; 95% CI 1.12–10.97; p=0.0316). The procedure of MSCs transplantation as a potential treatment of GVHD showed no influence on

risk of invasive aspergillosis (OR 3.25; 95% CI 0.64-16.47; p=0.1536) or CMV-reactivation (reciprocal OR 0.42; 95% CI 0.12-1.49; p=0.1802) in the study.

Therefore, among the most frequent infections in acute GVHD are CMV, invasive aspergillosis, and bacterial infections. Among risk factors for infectious complications in patients with acute GVHD are the progression of the main disease and neutropenia below 500 cells/mm3 (for aspergillosis) and unrelated HSCT in the past history and progression of main disease (for bacterial bloodstream infections and pneumonia). MSCs transplantation as a treatment strategy for acute GVHD showed no statistically significant association with a risk of infectious complications in the performed multivariate analysis.

Studies focusing on the microbiota composition in patients before and after allo-HSCT, report a drastic loss of bacterial diversity after treatment. This loss of diversity was more pronounced in patients who developed gut GVHD [1]. The loss of microbiota diversity is linked to an increased risk of infections and GVHD [2]. The commensals induce the production of antimicrobial peptides (AMPs) by the Paneth cells. These AMPs keep the pathogens at bay. Severe gut GVHD correlates with a decrease in Paneth cells in biopsies [3-4].

The degree of HLA disparity between donor and recipient is a well-known and widely accepted independent risk factor for GVHD development [2]. With the growing understanding of GVHD pathogenesis, there is increasing attraction to non-HLA genotypes as a tool for GVHD prediction in the last ten years [3].

The current understanding of acute GVHD pathogenesis can be summarized as follows:

a) Initial tissue damage induced by the conditioning regimen, which is followed by the denudation of auto- and allo-antigens, and accompanied by massive inflammatory cytokine secretion ("cytokine storm"), thereby activating APCs.
b) Auto- and alloantigen presentation mediated by APCs together with the costimulatory, which signals the prime donor's cytotoxic T lymphocytes and their proliferation.
c) The migration of activated cellular effectors toward GVHD target tissues [5].

The pathogenesis of chronic GVHD is much more complex, thereby reflecting its variable clinical manifestation. Mechanisms involved in chronic GVHD pathogenesis partially overlap with acute GVHD, especially in chronic GVHD developing from pre-existing acute GVHD. The pathogenesis of chronic GVHD is based on alloreactive T-cell and deregulated B-cell interactions as well as innate immunity effectors, such as macrophages, dendritic cells, and neutrophils. The activation of fibrotic processes is a consequence of the aforementioned steps [5]. Three phase-based concepts of cGVHD pathogenesis are currently accepted:

a) First Phase: Pre-Existing Inflammation
b) Second Phase: Deregulation of Adaptive Immunity
c) Third Phase: Excessive Fibrosis [6]

Historically, GVHD prophylactic treatments focused on the use of alkylators and antimetabolites following HCT. The first widely adopted treatment was methotrexate, which is a folate antagonist that has been shown to be effective at reducing GVHD severity and prolonging survival [7].

Extracorporeal photopheresis has recently been intensively studied as a treatment for steroid refractory acute GVHD [8–11]. Overall response rates were variable, but ranged from 65 to 100%. Of the total of 249 patients with reported results for individual organs, complete responses were observed in 85% (176/208) of skin, 57% (54/95) of liver, and 62% (66/106) of gastrointestinal GVHD cases. The number and frequency of extracorporeal photopheresis treatments required to achieve a response have not yet been established but, generally, patients receiving intensive initial therapy (2–3 times/week) until a response was achieved resulted in the best outcomes [7].

Mesenchymal stem cells (MSCs) transplantation, through its immune suppressive properties, can rescue some of the steroid-refractory acute GVHD patients and is frequently applied for this purpose. It is generally assumed that about half of steroid-refractory acute GVHD patients are responsive to MSC therapy [12–17].

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CHAPTER NINE

MESENCHYMAL STEM CELLS TRANSPLANTATION AND INFECTIONS IN HEMATOLOGY

Mesenchymal stem cells (MSCs) are multipotent adult stem cells that are found in multiple tissues, including the umbilical cord, bone marrow, and fat tissue. These cells are well known for repairing tissue, supporting hematopoiesis, and modulating an immune and inflammation response. MSCs are approved and widely used in patients with hematological diseases and hematopoietic stem cell transplant (HSCT) recipients, who have poor graft function and graft-versus-host disease (GVHD). Acute GVHD is one of the most common serious complication of allogeneic HSCT and remains a leading cause of mortality in HSCT recipients all over the world [1–3].

Currently, the role of MSCs in infection prevention and treatment is still discussed in transplant and hematological patients. Some of the recent studies showed that MSC transplantation increased the risk of infectious complications by suppressing T-cell response and secreting VEGF and IL-6 [4–6]. However, other published studies showed that the incidence of infections did not increase after MSC transplantation treatment for acute GVHD and engraftment failure [7–10].

An observational study was performed to estimate the possible modern risk factors for infections in patients receiving MSCs transplant as a treatment for GVHD. The diagnosis of GVHD, as well as its type and grading, was made according to the criteria defined by the Consensus Workshop [11, 12]. Patients were included in the MSCs transplantation list if they developed a GVHD after an allogeneic HSCT during September 2010 to September 2016. Patients were excluded from the study if they had severe organ insufficiency and/or any abnormality in vital signs or if they had a chronic GVHD. The period of observation for patients who had received MSCs transplants was set up to one year in the study. A proven case of infection (bacterial, viral, or fungal) was taken as

a primary outcome in the study. Patients with proven GVHD during an earlier period (2002–2012) were taken as a historic control in the study with matching based on age, gender, grade of GVHD, and a primary diagnosis. The control group was estimated to be equal to the number of cases in the study (n=34). Covariates included in multivariate analysis were taken: procedure of MCSs transplantation; type of allogeneic HSCT (related/non-related); main disease progression; absolute neutrophil count on the first day of infectious complications; GVHD-involved organs. Among concurrent diseases of patients included in the study were diabetes mellitus, chronic gastritis, and chronic cystitis (each registered only in a single patient). Additionally, retrospective overall survival analysis was performed both in the study and historical control groups.

Epidemiological, clinical, and laboratory data were prospectively collected in each adult patient with GVHD undergoing MSCs transplantation from September 2010 to September 2016. Data concerning the characteristics of the control group without MSCs transplantation was taken from internal databases and archived medical documentation retrospectively and ensuring that double-control was used for the sources. Cultures for bacterial and fungal infections were obtained with standard precautions from all patients with febrile neutropenia or other signs of infection (including biomarkers as procalcitonin, presepsin, C-reactive protein, and galactomannan) after MSCs transplantation, with identification and in vitro antibiotic susceptibility testing being performed via standard means. The criteria for febrile neutropenia was a 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 absolute neutrophil count (ANC) of <500 cells/mm^3, or an ANC that was expected to decrease to <500 cells/mm^3 during the next 48 hours [13]. The diagnostic criteria of viral infections (CMV, VZV, EBV, HSV) during the study were defined by published recommendations from the Infectious Diseases working party of the EBMT and guidelines from European Conference on Infections in Leukemia [14, 15]. The diagnostic criteria of infections caused by *Aspergillus spp.* and *Candida spp.* were defined by the Practice Guidelines for the Diagnosis and Management of Aspergillosis [16] and Clinical Practice Guideline for the Management of Candidiasis [17]. HSCT 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). GVHD prophylaxis regimens included cyclosporine, methotrexate, and tacrolimus. All patients received methylprednisolone combined with calcineurin inhibitors as first-line treatments for acute GVHD. Anti-thymocyte globulin was administered in

cases of unrelated donors as a preventive measure. MSCs were isolated and cultivated from bone marrow aspirates and adipose tissue by standard laboratory techniques. Immunologic characteristics of MSCs were evaluated with flow cytometry with the expression of antigens: $CD45^-$ $CD34^-$ $CD90^+$ $CD105^+$ $CD13^+$ (Beckman Coulter).

Standard antibacterial prophylaxis was based on fluoroquinolones (mainly ciprofloxacin 0.5g BID orally) starting from the initiation of conditioning regimen until the level of neutrophils in peripheral blood exceeded 500 cells/mm^3. No routine antibacterial prophylaxis against *Streptococcus pneumoniae* was administered. The institution's standard protocols for the initial empirical antibiotic therapy for febrile neutropenia included cephalosporins (cefepime or cefoperazone/sulbactam) or carbapenems (imipenem/cilastatin or meropenem), depending on the risk group of the patient; there was also an addition of vancomycin in cases of possible infection caused by gram-positive pathogens [18]. Antifungal prophylaxis with fluconazole was prescribed to non-neutropenic patients and micafungin was used as antifungal prophylaxis in patients undergoing allogeneic HSCT with neutropenia. Prophylaxis against *Pneumocystis jirovecii* with trimethoprim-sulfamethoxazole was administered to all patients from engraftment until 6 months post-HSCT; it was also administered beyond 6 months post-HSCT to those receiving immunosuppressive therapy or with remaining GVHD. Prophylaxis of infections caused by herpes simplex viruses was performed by acyclovir. Real time quantitative polymerase chain reaction (PCR) was used to monitor CMV and EBV DNA levels in HSCT patients weekly during the pre-engraftment period and after MSCs transplantation, with ganciclovir used as first line pre-emptive therapy in case of a possible active CMV infection. CMV-DNA and EBV-DNA were monitored twice a week until GVHD was resolved or until the end of the antiviral therapy.

During severe neutropenia (ANC<100 cells/mm^3), 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/mm^3 some of the clinically stable patients were moved to the intensive care department with 2 patients in a room and positive air pressure. Methods of non-parametric statistics for categorical (Chi-squared or Fisher's exact tests) and quantitative (Mann-Whitney U-test, Odds Ratio) were used to provide 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 the previously performed univariate analysis. The 6-months overall

survival (OS) was estimated using the Kaplan-Meier method and compared with the log-rank test. Data processing and analysis was performed using MedCalc Statistical Software v. 17.2 (MedCalc Software bvba, Ostend, Belgium), and the results were regarded as statistically significant when $p<0.05$.

Therefore, during the study period, there were 34 procedures in total of MSCs transplantation performed in patients with acute GVHD. All patients included in the study had II-IV grades of acute GVHD and were steroid-resistant. The median time from HSCT to MSCs transplantation was 91 days (interquartile range 31–131 days). The median number of MSCs transplantations per patient was 2 with an interquartile range from 1 to 3 and a maximum number of 5 transplantation procedures per patient. Tables 9.1 and 9.2 show the baseline demographic and clinical characteristics of the patients and MSCs transplants in the study.

Table 9.1: Demographical and clinical baseline characteristics of patients, transplants, and GVHD from the cases and control groups in the study (n=68).

Baseline characteristics	*Absolute number of MSCs transplants (n=34), %*	*Absolute number of controls with acute GVHD (n=34), %*
Age (years, median, interquartile range)	37.5 (30 43)	36 (29 45)
Sex (female)	23 (67.6)	20 (58.8)
Type of allogeneic HSCT:		
Related	13 (38.2)	16 (47.1)
Unrelated	21 (61.8)	18 (52.9)
Primary disease:		
Acute myeloid leukemia	12 (35.3)	11 (32.4)
Hodgkin's lymphoma	6 (17.6)	10 (29.4)
Chronic myeloid leukemia	9 (26.5)	9 (26.5)
Myelodysplastic syndrome	5 (14.7)	2 (5.9)
Acute lymphocytic leukem.	2 (5.9)	2 (5.9)
Primary disease stage:		
Progression	14 (41.2)	15 (44.1)
Remission	20 (58.8)	19 (55.9)
Level of neutropenia:		
100-500 cells/mm^3	3 (8.8)	9 (26.5)
>500 cells/mm^3	31 (91.2)	25 (73.5)
MSCs donor type:		
Related	10 (29.4)	14 (41.2)
Unrelated	24 (70.6)	20 (58.8)

GVHD involved organs:		
Gastrointestinal tract	12 (35.3)	9 (26.5)
Liver	14 (41.2)	16 (47.1)
Skin	8 (23.5)	9 (26.5)
GVHD grade:		
II	9 (26.5)	11 (32.4)
III	18 (52.9)	17 (50.0)
IV	7 (20.6)	6 (17.6)

Table 9.2: Laboratory characteristics of MSCs transplants in the study (n=34).

MSC transplant characteristics	*Median (interquartile range); abs. number (%)*
Number of cells per infusion (mln/kg)	1.32 (0.87 2.16)
Vitality of MSC (%)	97.0 (95.0 98.0)
MSCs cultivation time (days)	22 (19.0 30.0)
MSCs source (abs. number; %):	
Bone marrow	24 (70.6)
Adipose tissue	10 (29.4)
Passage of MSCs (abs. number; %):	
P1	6 (17.6)
P2	19 (55.9)
P3	6 (17.6)
P4	3 (8.8)
Cryopreservation of MSCs (abs. number; %)	26 (76.5)

Microbiological data concerning the causes of infectious complications in patients with acute GVHD has revealed the major impact of viral infections (mainly CMV) and fungal infections (invasive pulmonary aspergillosis). There were no bloodstream infections caused by *Candida spp*. registered in the study, which may be explained by the broad use of micafungin as a prophylaxis antifungal regimen in allogeneic HSCT recipients. Bacterial infections were mostly presented by bloodstream infections (caused by *K. pneumoniae, E. faecalis, E. coli*) and pneumonia. The period of observation for patients received MSCs transplant was set up to 1 year in the study but all of the infectious episodes occurred during the 100-day period after MSCs transplantation. Not all of the patients in the study had serious prolonged febrile episodes and some of the episodes of infectious diseases (19/33.9%) were associated with a low fever for a short time period. It is interesting to underline that 85% of patients in the study did not have neutropenia at the onset of infectious complications.

Table 9.3 shows the spectrum of infections registered in patients who had received MSCs transplantation to treat GVHD and in the control group of patients with acute GVHD. A comparison of the spectrum of infections in both the case and control groups is shown in Figures 9.1 and 9.2.

Table 9.3: Causes of infections in patients who had received MSCs transplants (n=34) as a treatment for acute GVHD and in the control group with acute GVHD (n=34).

Pathogen	MSCs transplant recipients, absolute number (%)	Patients with acute GVHD (control group), absolute number (%)
Viral infections:		
CMV	5 (14.7)	9 (26.5)
EBV	2 (5.9)	3 (8.8)
Bacterial infections:		
Bloodstream infection	7 (20.6)	6 (17.6)
Pneumonia	5 (14.7)	7 (20.6)
Fungal infections:		
Invasive aspergillosis	7 (20.6)	5 (14.7)

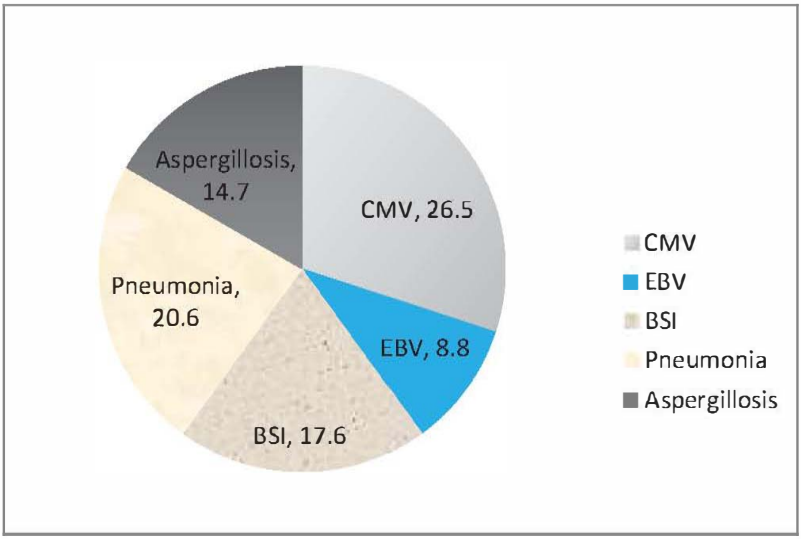

Figure 9.1: Infectious complications in patients with acute GVHD (control group) [%].

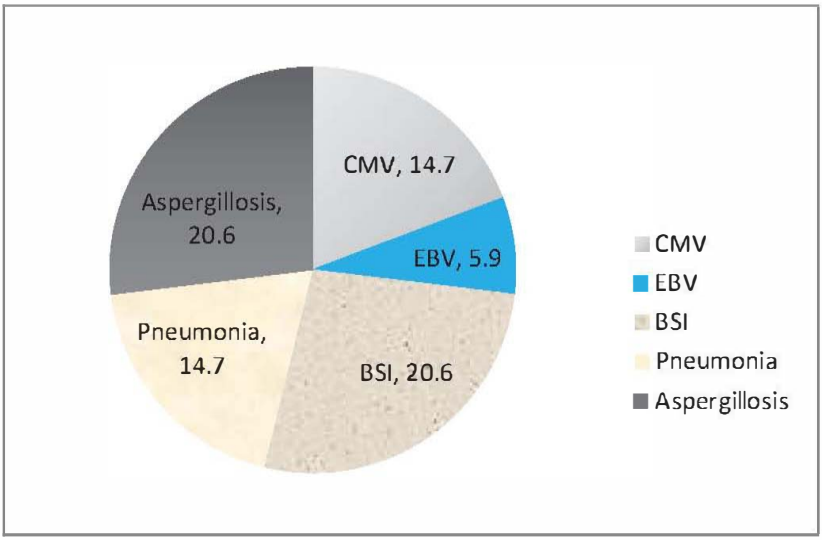

Figure 9.2: Infectious complications after MSCs transplantation in patients with acute GVHD [%].

Therefore, in the conducted study it was observed that after MSCs transplantation the incidence of CMV-disease decreased (26.5% versus 14.7%) while the incidence of invasive pulmonary aspergillosis slightly increased (14.7% versus 20.65%). In the risk factor analysis part of the study all of the covariates were included in the univariate analysis. Risk factors that showed statistical significance ($p<0.2$) in univariate analysis were subsequently checked for independency in multivariate analysis using logistic regression. The results of multivariate analysis are shown in Table 9.4.

Table 9.4: Independent risk factors for infectious complications in patients with acute GVHD from the conducted multivariate analysis (patients with and without MSCs transplantation, n=68).

Risk factor	*Odds ratio*	*95% confidence interval*	*p*
Aspergillosis:			
ANC < 500 cells/mm^3	7.16	1.31 39.04	0.0229
Progression of main disease	5.52	1.21 25.06	0.0270
Bacterial infections:			
HSCT from unrelated donor	3.50	1.12 10.97	0.0316
Progression of main disease	3.13	1.04 9.37	0.0417

No statistically significant differences were noted in the 6-months overall survival in the both groups (58.82% in MSC group versus 38.24% in historic control group; $p=0.0678$ in the log-rank test); although a trend towards the clinical efficacy of MSC transplantation was observed (Figure 9.3). This may be explained by the fact that both the study and control groups contained partial responders to MSC treatment, as well as patients with complete response and non-responders to MSC treatment, which could lead to the potential selection bias.

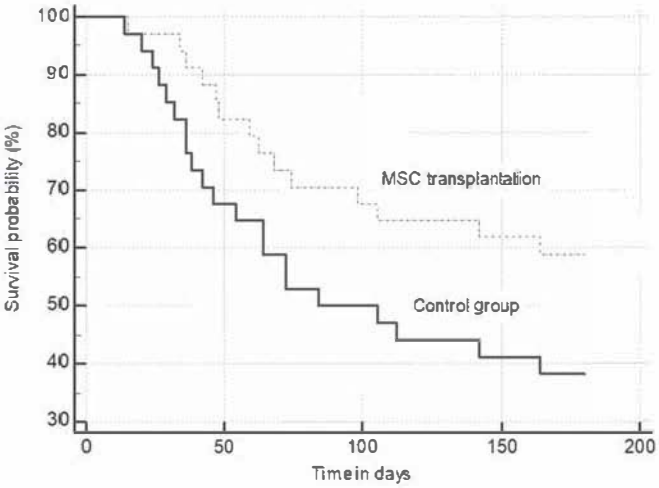

Figure 9.3. Overall survival rate for patients treated with MSC and the historical control group with aGVHD. The statistics were estimated using the Kaplan-Meier method and log-rank test (p=0.0678).

A result of multivariate analysis progression of main diseases was found to be an independent risk factor for the development of invasive pulmonary aspergillosis (OR 5.52; 95% CI 1.21-25.06; p=0.0270). Another independent risk factor for invasive aspergillosis was neutropenia below 500 cells/mm^3, which should be considered even in patients who receive MSCs to treat acute GVHD. It was also proven in published studies on risk factors in HSCT [19] that patients with hematopoietic stem cell transplant from an unrelated donor remain at high risk for bacterial infections even after MSCs transplants (OR 3.50; 95% CI 1.12-10.97; p=0.0316). The progression of the main disease was also an independent risk factor for the development of bacterial infection in patients with acute GVHD, with or without MSCs transplantation. MSCs transplants showed no influence on the risk of bacterial infections in the study. The MSCs transplantation procedure did have a statistically significant association with the risk of invasive aspergillosis in the conducted multivariate analysis (OR 3.25; 95% CI 0.64 16.47; p=0.1536). In the analysis of risks of CMV-disease in patients with acute GVHD MSCs transplantation also showed no statistically significant protective effect (reciprocal OR 0.42; 95% CI 0.12-1.49; p=0.1802), but the trend towards protection should be investigated in larger studies. It is also interesting to note that younger

patients in the study also had a higher chance of CMV reactivation (reciprocal OR 0.94; 95% CI 0.87-1.01; p=0.1061).

MSCs transplantation has been included in medical practice worldwide as a possible treatment for GVHD since the first published article by Le Blanc et al. in 2004 [20]. The relation between MSCs transplantation and the risk of infections because of MSCs immunosuppression qualities have been controversial [21–23]. There were studies published recently that discussed the effects of MSCs transplantation on the host inflammatory immune response and the possible direct antimicrobial activity of MSCs by means of secreting soluble factors [24]. Meisel et al. have discussed the possible mechanisms of antimicrobial activity of MSCs in studies with indoleamine 2,3-dioxygenase [4]. However, this data is mainly based on in vitro experiments and there is a lack of information on spectrum and possible risk factors for infections in MSCs transplantation. In this study, we observed the spectrum of infectious complications and evaluated possible risk factors for developing infections after MSCs transplantation in patients with acute GVHD. The results of conducted analysis show that MSCs transplantation has no statistically significant impact on risk of bacterial infections, CMV-disease, and invasive aspergillosis. The possible impact of age on the risk of CMV reactivation needs further study. The clinical efficacy of MSC transplantation should be evaluated in randomized controlled trials with a stratification of patients based on the type of response.

The association between the progression of the main hematological disease, neutropenia with ANC below 500 cells/mm^3, and the risk of aspergillosis in GVHD patients after MSCs transplantation shown in the study should be taken into account when choosing the regimen of antifungal prophylaxis, with an additional importance placed on prophylaxis with voriconazole in acute GVHD patients with or without MSCs transplantation. Finally, both unrelated allogeneic HSCT and main disease progression should be defined as the most important risk factors for developing bloodstream infections and bacterial pneumonia in patients with acute GVHD. The main limitation of our study was the relatively small sample of MSCs transplantation cases, but concerning the cost of GVHD treatment, as well as every MSCs transplantation and HSCT procedure, such numbers of observations may be significant. It is important to mention that we have used historical controls for patients with acute GVHD in a survival analysis and a risk factor analysis with matching based on age, gender, grade of GVHD, and their primary diagnosis. Also, this study was conducted in one clinical center but we

need to state that they perform MSCs transplantation for patients from all parts of the country.

In conclusion, MSCs transplantation has shown no statistically significant association with risk of infectious complications in patients with acute GVHD in a performed multivariate analysis. Among the most frequent infections in acute GVHD are CMV, invasive aspergillosis, and bacterial infections (bloodstream infections or pneumonia). Among the risk factors for infectious complications in patients with acute GVHD with/without MSCs transplantation are the progression of the main disease and neutropenia below 500 cells/mm^3 (for aspergillosis) and unrelated HSCT in the past history and progression of main disease (for bacterial bloodstream infections and pneumonia).

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CHAPTER TEN

REAPPRAISAL OF SELECTIVE ORAL DECONTAMINATION IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

MDR/XDR (multidrug-resistant/extensively drug-resistant) Gram-negative bacteria have emerged as an important cause of bloodstream infection (BSI) in hospitalized patients, especially in immunocompromised hosts. It was shown earlier that intestinal colonization with extended-spectrum β-lactamases (ESBL)-producing or carbapenem-resistant *Enterobacteriaceae* spp., carbapenem-resistant *A. baumannii*, and *P. aeruginosa* may be a prolonged condition in certain populations of patients [1, 2]. It is especially dangerous in patients with hematological malignancies and HSCT during chemotherapy-induced neutropenia, which is when a mucosal colonization by MDR/XDR pathogens is considered to be a risk factor for subsequent infectious complications [3–6]. It was also demonstrated that among the risk factors for mortality in adult patients with BSI in the pre-engraftment period after hematopoietic stem cells transplantation (HSCT) was the inadequacy of empirical antibacterial therapy and the isolation of carbapenem-resistant *A. baumannii* or *P. aeruginosa* [7].

There were numerous studies published on decolonization strategies in patients with different primary conditions [8–12] but, due to the broad preventive use of antibiotics and profound neutropenia, the problem of choice of strategy of intestinal decolonization of MDR/XDR Gram-negative bacteria is primarily important in hematology. Earlier decolonization regimens have been studied for *Staphylococcus aureus*, but there is a certain lack of data on the regimens to decolonize Gram-negative carriage [13, 14]. To the investigators' knowledge no randomized clinical trial has been performed to study the efficacy and safety of selective intestinal decolonization using Colistimethate sodium (colistin) in high-risk adult patients with hematological malignancies. It is important to mention that, with the high incidence of carbapenem-resistant Gram-negative bacteria, colistin remains a single therapeutic option in a number

of cases. Being a non-absorbable antibiotic, colistin may have a certain importance as a decolonizing agent, especially in the case of carbapenem-resistant Gram-negative colonization. We have estimated that the possible decolonization of MDR/XDR Gram-negative bacteria in hematological patients could be important for patients as it reduces the risk of infection and for the community as it also reduces the risk of transmission. The aim of the proposed study is to assess the efficacy and safety of selective intestinal decolonization of MDR/XDR Gram-negative bacteria with the oral administration of Colistimethate sodium in adult patients with hematological malignancies.

This was a non-blind parallel assignment controlled trial with balanced (1:1) randomization. The primary purpose of this Phase 4 trial was the prevention of BSI caused by XDR/MDR Gram-negative bacteria in patients with hematological malignancies by means of a decrease in intestinal colonization levels through selective intestinal decolonization. The trial protocol was approved by the local institutional review board (IRB) and the Ethical Committee (Protocol №1 1) of the Republican center for hematology and bone marrow transplantation (Minsk, Belarus) and has been registered with the US National Institute of Health (NIH) and the National Library of Medicine (NLM): A Study of Decolonization in Patients with Hematological Malignancies (DEHAM); ClinicalTrials.gov Identifier: NCT02966457.

Participants were enrolled in the study from November 2016 to October 2017. Patients with hematological malignancies aged ≥18 years with a positive rectal swab for MDR/XDR Gram-negative microorganism and the ability to provide an informed consent were eligible. MDR/XDR classification of Gram-negative bacteria was performed according to Magiorakos et al. [15].

Active screening of patients admitted to hospital with hematological malignancies, primarily to receive a course of chemotherapy, was performed by the way of rectal swabs during the study period. Patients with signs or symptoms of active bacterial, viral, fungal, or protozoal infection were excluded from the study. Among other exclusion criteria were pregnant or nursing women; the use of antibacterial therapy in previous 10 days; contraindication to the use of the study drug (including known hypersensitivity); enrollment in another study, or in the present study for a previous episode; psychiatric disorder or no ability to understand or follow the protocol directions; and resistance to the primarily isolated colonizing microorganism to polymyxin antibiotics

(MIC ≥ 2 mg/L). No standard antibacterial prophylaxis was used during the study period in the included patients. In all cases, measures of contact precautions were established to prevent the spread of XDR/MDR microorganisms. Prophylaxis against Pneumocystis jirovecii with trimethoprim-sulfamethoxazole was administered to all patients in the study with an absolute neutrophil count (ANC) of <100 cells/mm3. The prophylaxis of infections caused by herpes simplex viruses (HSV) was performed by acyclovir only in patients with a high clinical risk of HSV reactivation. Real time quantitative polymerase chain reaction (PCR) was used to monitor CMV and EBV DNA levels in high-risk patients weekly.

Patients randomized to the treatment arm received selective intestinal decolonization with colistin in dose of 2mln I.U. 4 times per day PO for 14 days. Patients in the control group were observed during the study period without any interventions while they received their standard treatment for hematological malignancies (a "watch and wait" strategy).

Patients were assessed on the last day of treatment (day 14) and on day 21 after the end of treatment. At each visit, rectal swabs were performed and immediately inserted in culture media. The pre-defined primary outcome of the study was the detection of intestinal MDR/XDR Gram-negative bacteria carriage using a rectal swab during day 21 post-treatment (rate of eradication of MDR/XDR Gram-negative bacteria at day 21 post-treatment). Secondary outcomes were the safety of the study regimen (incidence and intensity of possible adverse effects) and the change in colistin MICs between the baseline and the final visit.

Microbial cultures were isolated and grown on different manufactured culture media. Identification and antimicrobial susceptibility testing were performed using a bioMerieux VITEK 2 automatic system and commercial panels, and the ESBL-phenotype was determined using a VITEK 2 ESBL Test System. Additional antimicrobial susceptibility in carbapenem-resistant isolates (resistance to imipenem, meropenem, and doripenem) was confirmed by E-tests and disc-diffusion assays. The minimum inhibitory concentration (MIC) breakpoints used for susceptibility testing were current Clinical and Laboratory Standards Institute (CLSI) [16] and EUCAST guidelines [17]. According to previously published studies, the colistin resistance of Gram-negative bacteria may be underestimated by Phoenix100/Vitek2 systems, potentially leading to inappropriate colistin administration. It is also recommended to retest the isolates with MIC to determine the colistin susceptibility breakpoint (2 mg/L) [18]. Keeping these arguments in mind,

we have decided to estimate the susceptibility to colistin isolates as MIC < 0.5 mg/l.

Based on our clinical experience, we assumed that 25% of patients would clear the MDR/XDR Gram-negative intestinal colonization spontaneously within the time period of the study and hypothesized that a decolonization regimen would be clinically effective if it was able to clear colonization in a 60% of patients. Using a two-sided Alpha of 0.05 and a power of 80%, with an enrollment ratio of 1 and a dichotomous endpoint, we calculated a minimal sample size of 60 patients. Randomization was performed by a computerized randomization program (ALEA) in the proof assistant Coq v. 8.3., which is validated for use in randomized clinical trials. The block size randomly varies between 4, 6, and 8. Due to the decision of IRB and Ethical Committee, blinding in the planned study was not considered appropriate from an ethical standpoint, so the study protocol did not include it.

Based on the study design, the intention-to-treat analysis was performed, while no patients were excluded in the process of the trial and the study characteristics were analyzed according to the randomization scheme. Due to the ongoing chemotherapy treatment for primary hematological disease, there were no cases of data missing or exclusion of the patients in the process of the trial due to a loss of follow-ups. All of the patients in the study were included in the monitoring of adverse effects of the decolonization regimen. The distribution of the variables was determined by the Shapiro-Wilk test. Differences in MDR/XDR Gram-negatives carriage between the study groups were analyzed by methods of non-parametric statistics for categorical variables (Chi-squared or Fisher's exact tests). Univariate logistic regression was used to determine the odds ratio for the presence of MDR/XDR Gram-negative intestinal colonization in both the treatment and the control group. The probability of development of BSI after decolonization was estimated using the Kaplan–Meier method and compared with the log-rank test. The day count used the Kaplan-Meier probability test and it started from day 21 post-treatment and included 90 days of observation. Data processing and analysis was performed using MedCalc Statistical Software v. 18 (MedCalc Software bvba, Ostend, Belgium) and SPSS v. 21.0 (IBM Co., Armonk, NY, USA), and results were regarded statistically significant when $p<0.05$.

The study flowchart according to the CONSORT Statement [19] is shown in Figure 10.1.

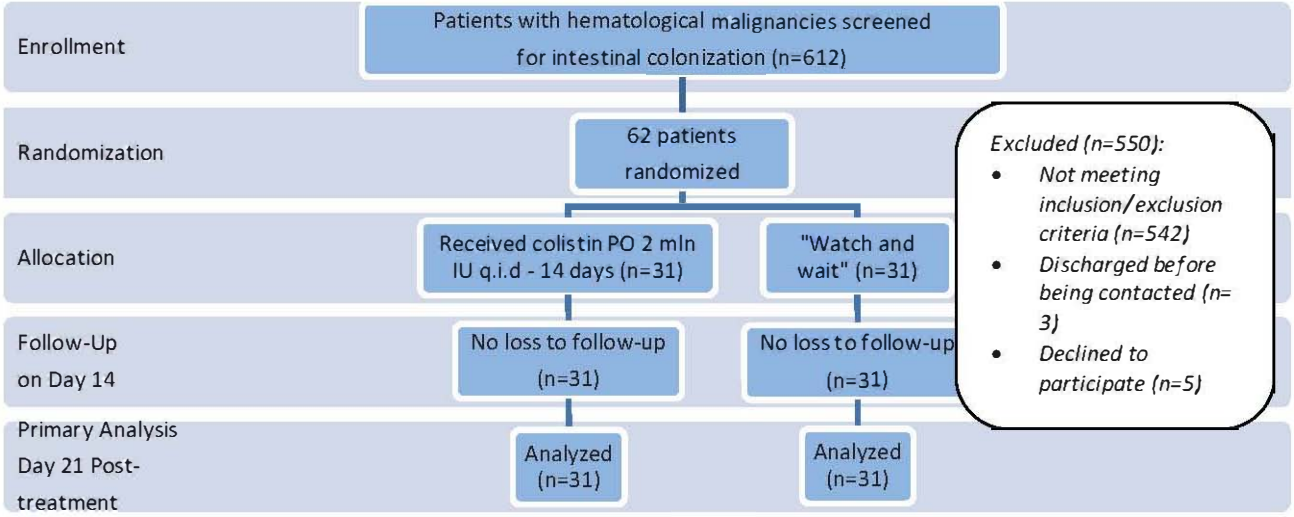

Figure 10.1: Flow diagram of the randomized controlled trial

Therefore, among the main causes of exclusion from the study before a randomization procedure were the absence of MDR/XDR Gram-negative intestinal colonization on baseline screening and the use of antibacterial therapy in the previous 10 days. After the baseline assessment there were a total of 62 patients included in the parallel allocated groups at a ratio of 1:1. After the randomization procedure two study groups showed similar baseline clinical and demographic characteristics (Table 10.1).

Table 10.1: Demographical and clinical baseline characteristics of patients by group (randomized patients).

Baseline characteristics	*Decolonization group (n=31), %*	*Observation group (n=31), %*
Age (years, median, interquartile range)	49 (39 63)	49 (35 63)
Sex (female)	15 (48.4)	16 (51.6)
BMI (kg/m^2, median, interquartile range)	25.5 (24.1 28.4)	25.8 (24.2 27.6)
Primary disease:		
Acute myeloid leukemia	17 (54.8)	16 (51.6)
Multiple myeloma	7 (22.6)	9 (29.0)
Hodgkin's lymphoma	1 (3.2)	
Chronic lymphocytic leukemia	3 (9.7)	4 (12.9)
Myelodysplastic syndrome	1 (3.2)	1 (3.2)
Acute lymphocytic leukemia	2 (6.5)	1 (3.2)
Primary disease stage:		
Progression	19 (61.3)	17 (54.8)
Remission	12 (38.7)	14 (45.2)
Level of neutropenia on day 1:		
<100 cells/mm^3	15 (48.4)	18 (58.1)
100-500 cells/mm^3	5 (16.2)	3 (9.7)
>500 cells/mm^3	11 (35.5)	10 (32.3)
MDR/XDR resistant species at baseline rectal swab:		
K. pneumoniae	16 (51.6)	13 (41.9)
E. coli	4 (13.0)	8 (25.8)
A. baumannii	5 (16.1)	5 (16.1)
P. aeruginosa	6 (19.4)	5 (16.1)
Infections caused by colonizing microorganism in the previous 6 months	4 (12.9)	3 (9.7)

The median age of all of participants in the study was 49 years (interquartile interval 36–63 years); 31/62 (50%) were female. More patients in the control group were colonized by MDR/XDR E. coli (8/31 versus 4/31), while the decolonization group had more patients with MDR/XDR *K. pneumoniae* colonization at the baseline (16/31 vs 13/31). Overall, *K. pneumoniae* was the most frequent intestinal colonizer in adult hematological patients in the study, detected in 29/62 (46.8%) patients. All of the selected microorganisms at the baseline showed susceptibility to colistin (with MIC<0.5 mg/l).

In the primary outcome analysis, 19 out of the 31 patients (61.3%) in the treatment group and 10 out of the 31 (32.3%) in the control group showed a negative rectal swab for MDR/XDR Gram-negative bacteria on the last day of oral decolonization regimen (day 14). Although, on day 21 post-treatment the numbers of intestinal colonization by the same pathogens remained similar to some extent, with 13 of 31 patients (41.9%) showing a decolonization effect in the treatment group and 12 of 31 (38.7%) in the control group. The observed changes may indicate that this procedure of selective oral decolonization by colistin had only a limited effect, with no long-lasting microbiological benefits.

Based on the results of univariate statistical analysis using the Chi-squared test and logistic regression, there was a positive microbiological effect of oral decolonization using colistin on intestinal MDR/XDR Gram-negative bacteria in the conducted study (OR 3.32; 95% CI 1.17–9.44; p=0.0241) on the last day of treatment (day 14). Although, on day 21 post-treatment there was no statistical significance shown in the treatment or control groups (OR 1.14; 95% CI 0.41–3.16; p=0.7958). As an additional characteristic of the efficacy of oral decolonization of MDR/XDR Gram-negative bacteria in patients with hematological malignancies, the number needed to treat (NNT) was analyzed on the last day of treatment (NNT 3.44; 95% CI 1.89–18.99; p=0.0241) and this showed the short-term effects of treatment. Figure 10.2 displays the evolution of the MDR/XDR carriage over time in the colistin oral decolonization group and the observation control; it also shows the short-term effect of decolonization.

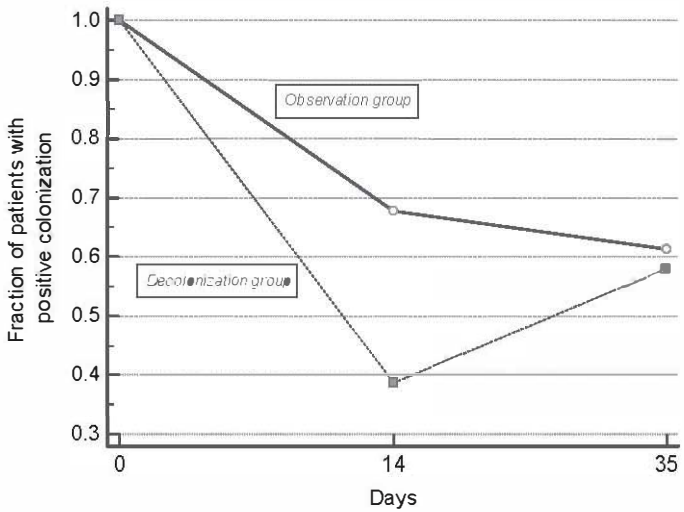

Fig. 10.2: The evolution of the rectal carriage of XDR/MDR Gram-negative bacteria over time with regard to decolonization with oral colistin.

Additionally, to get an understanding of the possible clinical effect of decolonization in MDR/XDR colonized hematological patients, the incidence of bloodstream infections (BSI) was monitored for 90 days in both decolonized and control groups. All of the patients included in the study were continuing to receive chemotherapy and follow-up visits for their primary hematological disease, while being monitored clinically and microbiologically for possible infectious complications. In total, there were 5/31 (16.13%) cases of BSI observed in the decolonization group and 7/31 (22.58%) cases in the control group. Due to the adequately prescribed empiric antibiotic treatment, no adverse clinical outcomes in the study groups were reported. The incidence of BSI in the decolonization group was lower in the first 30 days after the intervention (3.2% vs 12.9%), but overall in the 90-day observation period it did not show any advantages in comparison with the control group (log-rank test; p=0.4721). A probability graph for subsequent bloodstream infections in patients with intestinal colonization due to MDR/XDR Gram-negative bacteria with regard to decolonization by oral colistin is shown in Figure 10.3.

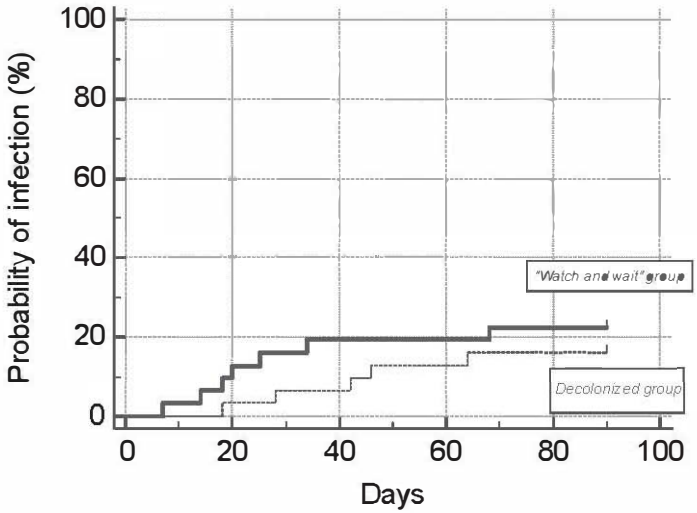

Figure 10.3: The probability of the development of bloodstream infections in patients with intestinal colonization due to MDR/XDR Gram-negative bacteria with decolonization by oral colistin in the 90-day period after the intervention.

No increase in resistance to colistin above an MIC of 0.5 g/l was observed in any of the isolates during the study and follow-up period. Among the registered events, there were only 6 cases of liquid stool without any systemic effects or signs of infection occurring in 4 patients in the decolonization arm and 2 patients in the control arm of the study (Fisher's exact test; 12.9% vs 6.45%; P = 0.06713). None of the patients in the study had to stop treatment prematurely due to serious adverse effects. This may be explained by the low intestinal absorption of colistin, leading to potentially minimal systemic effects of the drug.

This randomized, controlled trial on an oral colistin decolonization regimen using MDR/XDR Gram-negative bacteria in adult patients with hematological malignancies demonstrated a significant temporary suppression of rectal colonization rate on the last day of treatment (day 14), with no sustained effect at 21 days after the treatment. Observation of the incidence of BSI in the studied groups during a 90-days period has additionally shown the limited time of the protective effect of decolonization on the risk of BSI up to the first 30 days after the treatment. This may be explained by the quantitative decrease of MDR/XDR colonizing bacteria in the gut, which may have some protective effect

during chemotherapy-induced mucositis. To our knowledge, we have reported the first randomized, controlled trial examining a decolonization strategy with colistin for carriers of MDR/XDR Gram-negative bacteria in a group of adult patients with hematological malignancies, including patients with chemotherapy-induced neutropenia.

The possibilities for the eradication of colonizing microorganisms in various groups of patients have been previously studied in different settings. For example, Huttner et al. have shown the temporary decolonization effect of oral colistin on ESBL-producing *Enterobacteriaceae spp.* rectal carriage in patients with various comorbidities, which may correspond with the results of our study [9]. Additionally, Saidel-Odes et al. have demonstrated in their study, that a colistin-based regimen could be a suitable decolonization therapy for selected patients who have been colonized with carbapenem-resistant *K. pneumoniae*, such as transplant recipients or immunocompromised patients pending chemotherapy [8]. Oral gentamicin was also reported to be a possible decolonizing agent in an HSCT setting [12]. It is important to mention, that one of the most effective directions of research in the studied area should be based on investigation of changes in intestinal microbiota composition, leading to the expansion and domination of certain bacteria, with a future possibility of establishing the risk factors for the domination of MDR/XDR microorganisms and potential preventive strategies, including decolonization regimens [20, 21]. In future studies, it may be suggested that not only rectal swabs should be studied in hematological and HSCT patients populations, but also the pharyngeal carriage and skin colonization by MDR/XDR Gram-negatives, which may lead to important practical recommendations [22].

One of the most important limitations of the conducted study is an absence of a blinding procedure due to ethical reasons, especially in high-risk patients with hematological malignancies. The other important issue is that rectal swabs may be inadequate to detect resistant pathogens present in small amounts and stool cultures may be an inappropriate way to monitor gut colonization [23]. In some cases, we were not able to differentiate between the exogenous and endogenous rebound of colonization, which may have been controlled by genotyping techniques. Finally, this study was conducted in one clinical center, meaning that the external validity of this trial may be limited.

Due to the fact that intestinal colonization by MDR/XDR Gram-negatives is an independent risk factor for adverse clinical outcomes in hematological patients with neutropenia, even a temporary suppression of

MDR/XDR Gram-negatives intestinal carriage may result in a clinical benefit during profound chemotherapy-induced neutropenia. Thus, a strategy of early detection and selective suppression of highly-resistant microorganisms in such patients during prolonged periods of immunosuppression could result in a reduction in the incidence of subsequent bloodstream infections in a short-time period. A large multicenter trial is needed to test this hypothesis.

Therefore, we observed a temporary suppression of MDR/XDR Gram-negative bacteria carriage during oral antibiotic treatment using colistin at the end of the decolonization regimen. The study, however, did not demonstrate the effect of the decolonization regimen on rectal MDR/XDR Gram-negative bacteria carriage 21 days after the end of treatment. Therefore, in high risk hematological patients with chemotherapy-induced neutropenia, the strategy of selective intestinal decolonization with colistin may be beneficial to decrease the short-term probability of developing bloodstream infections up to 30 days from the end of treatment with a low incidence of adverse effects and a minimal risk of increase in colistin drug resistance in Gram-negative colonizing bacteria.

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CHAPTER ELEVEN

VACCINATION IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

The vaccination of immunocompromised patients in hematology, transplantation, and oncology is of particular importance, since it is in this population where the risk and severity of vaccine-preventable infections are significantly increased [1], while the level of vaccination coverage remains low [2–4]. The small percentage of vaccinated immunocompromised patients is often due to the fact that clinicians have insufficient or inaccurate information regarding safety, efficacy, and contraindications for the vaccination of such patients. In some cases, there is a lack of the necessary infrastructure to develop an individual vaccination calendar for groups of patients with an increased risk of developing infectious complications.

The Infectious Diseases of America (IDSA) classification was introduced in order to understand the levels of immunosuppression in various diseases and conditions. In particular, patients with the most profound acquired immunodeficiency include:

- Patients with hematological or oncological diseases, receiving chemotherapy
- Patients within 2 months after the transplantation of a solid organ
- Patients with HIV infection and a CD4 cell count of less than <200 cells per mm^3 for adults and adolescents
- Patients receiving biological agents, such as a tumor necrosis factor-alpha or rituximab
- Patients receiving prolonged corticosteroid therapy [5]

Many patients have a time period before the start of a course of chemotherapy, during which the patient can be considered immunocompetent, as well as having no specific contraindications to vaccination. However, it is important to emphasize that the treatment of the underlying disease should not be postponed to achieve the goals of vaccination. It is shown that the effectiveness of vaccination is higher before the start of

immunosuppression. It is also believed that after the introduction of live attenuated viral vaccines, the replication of the virus and the development of the immune response usually starts before the 3-week period. Therefore, vaccination with live vaccines should not be less than 4 weeks before the planned immunosuppression [6–7]. The use of live attenuated vaccines less than 2 weeks before chemotherapy is accompanied by a high risk of complications and is strictly contraindicated. In contrast to live vaccines, the numerous evidence available indicates that inactivated vaccines usually have the same safety profile in immunocompromised patients as immunocompetent patients [5].

Persons aged older than 6 months who live with an immunocompromised patient should be vaccinated annually with an inactivated influenza vaccine, and must also be vaccinated according to a standard vaccination calendar. In the absence of immunity in the varicella-zoster virus, family members are also recommended to have vaccinations. The oral polio vaccine (OPV) should not be given to individuals who live with an immunocompromised patient [5].

Treatment of oncological and hematological diseases is currently experiencing a rebirth: chemotherapy regimens have become more intense and, additionally, monoclonal antibodies and target therapy are widely used. Since many studies in the field of vaccinology were conducted in an older era of immunosuppressants, the results of such studies, unfortunately, may not always accurately reflect the current risks and benefits of vaccination against the background of individual chemotherapy regimens. As a rule, inactivated vaccines are safe in patients with oncological and hematological diseases. It is important to note that vaccines should not be routinely administered during the induction and consolidation phases of chemotherapy due to a weak immune response during these periods [8]. Although vaccines administered during the less intensive stages of chemotherapy are less immunogenic compared with cases when chemotherapy was completely stopped at the time of vaccination [9], they are not harmful and, apparently, provide protection for some pathogens [10–13].

The tactics of individual revaccination with single doses in the adult population is necessary in some cases [14, 19]; however, the current data available on the clinical significance of this approach is not sufficient. The most appropriate solution in adult patients after chemotherapy may be serological testing for vaccine-preventable diseases with proven serological correlations (for example, diphtheria toxoid, Hib, HepA,

HepB, IPV, rubella, measles, tetanus toxoid, chickenpox vaccine) and making the decision to vaccinate those with an insufficient concentration of protective antibodies in the serum.

According to current international guidelines, annual vaccination with seasonal inactivated influenza vaccine is indicated for patients ≥6 months of age with hematologic malignancies or solid tumors, with the exception of the period of anti-B-cell antibodies treatment or intensive chemotherapy induction/consolidation period for acute leukemia, or the conditioning and the early period after hematopoietic stem cell transplantation [20, 21].

The results of vaccination studies in hematological patients are still controversial, and are dependent on the type of main disease and the chemotherapy regimen used. For example, in a published study by Robertson et al. in patients with multiple myeloma, the immune response to a single dose of inactivated influenza vaccine was only 19% [22]. Higher immune responses to influenza vaccination were later shown in patients with lymphomas [23]. The strategy of double vaccination against influenza has been tested in several studies. Adult patients with lymphomas who received two consecutive doses of inactivated influenza vaccine demonstrated an immune response in 30% and 45% of cases after the first and second vaccinations, respectively [24]. Parkman and colleagues showed that two doses of influenza A (H1N1) vaccine in patients with chronic myeloid leukemia and B-cell hematological tumors resulted in higher seroconversion than a single dose; however, seroconversion levels were still lower than after a single vaccination in immunocompetent patients in the control group [25]. It is important to note that none of the patients who received rituximab had an immune response when vaccinated against influenza. Similarly, in the work of Yri et al. it was demonstrated that none of the 67 patients with lymphomas had an immune response to the influenza A (H1N1) vaccine for 6 months after rituximab therapy [26].

According to current international guidelines, a conjugated 13-valent pneumococcal vaccine should be recommended for patients with newly diagnosed hematologic malignancy [5]. Polysaccharide 23-valent pneumococcal vaccine can be used in patients older than 2 years at least 8 weeks after the administered dose of the conjugated 13-valent pneumococcal vaccine [5]. Data on the use of conjugated 13-valent pneumococcal vaccine in patients with hematological tumors is currently still limited [6, 45], but the recommendations of the Center for Disease Control and Prevention (CDC) set the routine use of such vaccine in

immunocompromised patients [6, 46]. The administration of live (attenuated) vaccines is strictly contraindicated in patients receiving chemotherapy [5] due to the risk of developing a disseminated disease. Some studies have shown the safety of using live vaccines 3–6 months after completion of immunosuppressive therapy [10, 13].

Vaccination against pertussis, diphtheria, and tetanus remains an important issue for patients after chemotherapy. Hammarström et al. showed that 41% of patients with acute leukemia did not have sufficient immunity against tetanus [47]. At the same time, other researchers have published evidence that radiation therapy in patients with non-Hodgkin's lymphomas did not affect their immunity to tetanus [48]. Therefore, immune response and the efficacy of vaccination against diphtheria and tetanus in adult patients with hematological malignancies have not previously been studied systemically.

It was shown that patients with hematological malignancies (in particular with B-cell lymphomas) having treatment with anti-CD20 monoclonal antibodies, are highly susceptible to the reactivation of chronic hepatitis B infection [49]. At the same time, the level of immune response to hepatitis B vaccination in this category of patients is reduced [50–51]. Thus, it is worthwhile to vaccinate these patients with the hepatitis B vaccine whenever possible, either before the scheduled course of chemotherapy or after its completion (between the courses). The immune response to the hepatitis B vaccine has been significantly reduced for at least 6 months in patients who received monoclonal antibodies [52–53].

All vaccine-preventable infections in HSCT patients can be classified as follows:

1) More frequent and/or more severe infections in HSCT recipients: pneumococcal infection, *Haemophilus influenzae* type B (HIB), influenza, varicella zoster virus (VZV)
2) Infections with the same frequency as in the general population but for which there is a mandatory vaccination program: tetanus, diphtheria, poliomyelitis, hepatitis B
3) Infections requiring vaccination in special situations (traveling to endemic areas, etc.)

It has been repeatedly shown that vaccines containing inactivated microorganisms, just antigens, or subunit vaccines are safe and do not have any special adverse reactions in patients after HSCT compared with

the general population. Live vaccines have a potential risk of developing an active infection in HSCT recipients and should not be used at all (oral polio vaccine, BCG) or used with caution in special situations (measles, VZV, rubella). It is important to remember that the use of oral polio vaccine (OPV) is strictly contraindicated not only among the patients themselves but also among family members, as well as employees of transplant departments. As a rule, the immune response to vaccination is significantly decreased within the 6 months following HSCT; however, in the case of the conjugated pneumococcal vaccine, HIB vaccine, and hepatitis B vaccine, there is evidence of the effectiveness of vaccinating earlier (starting 3 months after THSC) [62–64]. The strategy of the preliminary vaccination of a HSCs donor with the presence of a certain immunological effect in the recipient has been described; however, the implementation of this strategy has been limited, due to various organizational and ethical aspects.

Pneumococcal infections remain significant causes of morbidity and mortality in patients with HSCT, while the development of severe pneumonia has been described in both the early and late post-transplantation periods. According to a study by the European Society for Blood and Marrow Transplantation, the frequency of invasive pneumococcal infections is 8.23 per 1000 transplants. This number increases dramatically in patients with chronic GVHD, reaching 20.8 per 1000 transplantations. The use of polysaccharide pneumococcal vaccine in patients with HSCT was limited for a long time to low immunogenicity, especially during the first year after transplantation, as well as in patients with chronic GVHD [69–73]. It is important to note that during the first year after HSCT a significant number of invasive pneumococcal infections are registered, which indicates the need for the introduction of early vaccination. So, according to the recommendations of the IDSA, three doses of a 13-valent conjugated pneumococcal vaccine, starting 3–6 months after HSCT, should be recommended. At 12 months after HSCT vaccination with a 4th dose of a 13-valent conjugated pneumococcal vaccine or a 23-valent polysaccharide pneumococcal vaccine could be recommended as a booster dose [5].

Patients with chronic GVHD may have a reduced response to protein-based vaccines. The risk of exacerbation of GVHD with vaccination is low and is based only on isolated observations [72, 75,7 9]. Vaccination with polysaccharide vaccines in GVHD is often not effective and conjugated vaccines are the prophylaxis measures of choice [73, 80]. Despite the absence of serious research, it makes sense to postpone vaccination in

patients receiving high doses of corticosteroids or monoclonal antibodies (especially rituximab or alemtuzumab), since the immune response to the vaccine in such situations can be very low.

Therefore, vaccine-preventable infections, including influenza and invasive pneumococcal infections, are characterized by a high risk of unfavorable outcomes in immunocompromised patients with hematological malignancies and HSCT recipients. The safety of inactivated vaccines in this group is clearly shown while, in most cases, live vaccines are strictly contraindicated. When hematological malignancy is diagnosed, it is recommended to plan an individual vaccination schedule that allows for possible time differences between intensive chemotherapy and vaccination.

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CHAPTER TWELVE

CYTOMEGALOVIRUS INFECTION IN HEMATOLOGY: NEW AGENTS ARE COMING

CMV is a DNA-virus from the *Herpesviridae* family and its main biological feature is lifelong persistence and possible reactivation. Currently CMV seropositivity ranges from about 40–50% in highly developed countries to almost 95% in developing countries [1]. The following information has been provided by the CMV Drug Development Forum:

a) A CMV infection is defined as a virus isolation or the detection of viral proteins (antigens) or nucleic acid in any body fluid or tissue specimen
b) Primary CMV infection is defined as the first detection of CMV infection in an individual who has no evidence of CMV exposure.
c) Recurrent CMV infection is defined as a new CMV infection in a patient with previous evidence of CMV infection but where the virus has not been detected for at least 4 weeks during active surveillance. Recurrent infection may result from reactivation of latent virus (endogenous) or reinfection (exogenous).
d) Symptomatic CMV infection is diagnosed in patients developing symptoms (fever with or without bone marrow suppression) and who have CMV virions, antigens, or nucleic acid detectable but with no sign of CMV organ disease.
e) CMV disease is diagnosed in a patient with symptoms and/or signs from the affected organ together with detection of CMV by a test with an appropriate sensitivity and specificity.
f) CMV prophylaxis means that antiviral agents are given to a patient to prevent a primary, reactivated, or recurrent CMV infection.
g) Preemptive therapy means antiviral agents are given for an asymptomatic CMV infection detected by a screening assay [2–4].

The risk of CMV recurrence is dependent on the level of incompetency in the immune system, mainly with regard to T-cell immunity, including the presence and function of CMV-specific cytotoxic T lymphocytes. According to the data from J. Styczynski, the median rate of CMV recurrence was estimated to be 37% after allo-HSCT, 12% after auto-HSCT, 5% in patients with hematological malignancies, 14% in patients on anti-CD52 therapy, 30% in solid organ transplant recipients, 21% in patients with primary immunodeficiencies, 20% during active replication in PLHIV (people living with HIV), 3.3% during antiretroviral therapy, and 7% in patients with chronic kidney disease. The highest risk of CMV recurrence and CMV disease is reported for HSCT CMV-seropositive recipients, regardless of donor serostatus. The odds ratio (OR) for CMV recurrence in allo-HSCT is higher for recipient-positive versus recipient-negative CMV serostatus (OR 8.0), donor-negative/recipient-positive versus donor-positive/recipient-positive CMV serostatus (OR 1.2), unrelated/mismatched versus matched-family donor transplantations (OR 1.6), and acute graft-versus-host-disease versus other diseases (OR 3.2) [4].

Therapeutic antiviral options (ganciclovir, valganciclovir, foscarnet, and cidofovir) are still limited due to their high levels of toxicity. Moreover, prolonged antiviral drug exposure may lead to the development of antiviral drug resistance. After many years of few treatment choices, we are now in the process of completing the clinical assessment of three new agents: maribavir, brincidofovir, and letermovir. Maribavir is a competitive inhibitor of ATP binding to the UL97 protein kinase. It is orally administered and has specific antiviral activity against CMV [5-6]. Maribavir also has an in vitro activity against some CMV strains that are resistant to ganciclovir or cidofovir. Brincidofovir is a new broad-spectrum antiviral agent with an in vitro activity against herpes viruses, polyomaviruses, adenoviruses, papillomaviruses, and the smallpox virus [5]. Letermovir inhibits the terminal phase of the virus life cycle by targeting the subunit UL56 of the terminase enzyme complex. Its antiviral activity is highly specific to CMV. Letermovir can be orally or intravenously administered. In vitro, letermovir is currently the most active drug against CMV, with a very low median effective concentration and a preserved activity against viruses resistant to currently available agents. The main benefits of letermovir are its good clinical tolerability and its virological efficacy when treating CMV-resistant infections because of its specific action mechanism. Its main limitation is as a specific action against CMV (without cross-activity against other viruses,

including other herpes viruses). The development of these three new antivirals may soon change the management strategies of CMV infections.

When it comes to CMV prophylaxis in allo-HSCT, there is data available which shows that antiviral prophylaxis significantly reduces CMV disease and infection, while ganciclovir and letermovir appear to be the best options for CMV outcomes [9]. However, there is still a need for randomized controlled trials to work out the best prophylaxis regimen.

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CHAPTER THIRTEEN

TICK-BORNE ENCEPHALITIS AS AN ENDEMIC INFECTION IN IMMUNOCOMPROMISED HOSTS

Changes in modern treatment regimens for hematology, cancer, and autoimmune diseases have changed the survival rates for numerous diseases. Modern approaches include hematopoietic stem cell transplantation (HSCT), solid organ transplantation, monoclonal antibodies and target therapy are becoming more accessible; however, the number of people living with immunosuppression continues to grow. Therefore, there is a higher risk for this population to be infected by tick-borne diseases, including tick-borne encephalitis (TBE).

Currently, there are only few published cases of TBE in immunocompromised hosts that show common aspects of TBE. For example, only two fatal cases of patients treated with anti-CD20 monoclonal antibody rituximab have been reported. The disease course in both cases was extremely fulminant with severe neurological symptoms and damage, and delayed antibody formation was observed in both cases [1]. Two other cases of rituximab treated patients who developed severe tick-borne encephalitis were published by Steininger et al., where the authors indicated that TBE is a previously unrecognized severe infectious complication from rituximab therapy. Unsurprisingly, the inability to generate new antibody responses renders rituximab-treated patients susceptible to TBE and impedes laboratory diagnosis. In patients treated with rituximab, it has been reported that the antibody response is deficient for up to 6 months, thereby making vaccinations a challenge. Therefore, for patients receiving rituximab, information should be given on the importance of protecting themselves from tick bites. There are no general recommendations to vaccinate patients against TBE before rituximab therapy. Repeated vaccinations over time are needed to obtain protection, and this may not be possible in a clinical situation. An accelerated schedule, with three doses on days 0, 7, and 21, has been used in some centers for patients with rheumatic diseases before initiating rituximab and this can be recommended if the clinical situation permits [2].

Recently, there was a reported case of persistent TBE virus infection of an immunosuppressed patient in Italy. The patient had a persistent viremia associated with the erythrocyte fraction due to shedding the virus in the urine for more than 6 weeks, while the underlying factor was chemotherapy for a relapsing blastic plasmacytoid dendritic cell neoplasm [3]. A dramatic case of TBE in a 12-year-old patient was published by Chmelik et al., where an immunosuppressive treatment regimen containing dexamethazone and etoposide contributed to the viral replication and a fatal outcome [4]. It has also been reported that thymectomized patients have shown a delayed humoral immune response to the TBE virus [5].

There has been a description of TBE virus transmission in solid organ transplantation published recently by Lipowski et al. In this case, there were 3 patients who received solid organ transplants from a single donor (2 received a kidney and 1 received a liver) and then developed encephalitis 17–49 days after transplantation with fatal outcomes [6]. The presence of the TBE virus was confirmed by real time PCR in all recipients and in the donor, and the direct sequencing of amplification products showed the presence of the same viral strain. In another published study, which involved 31 heart transplant recipients, the seroconversion rate and the geometric mean of post-vaccinal antibody titers were markedly reduced in comparison to the control group, which served as a basis for other protective measures against TBE virus infection (clothing, avoiding high-risk areas for travel) [7]. This study also reported the safety of TBE-vaccination in the above-mentioned cohort of immunosuppressed patients.

Vaccination against TBE for HSCT patients at risk—e.g., patients living in or travelling to endemic areas—can be performed 6–12 months after transplantation. However, due to the lack of data in HSCT recipients, it cannot be recommended as a routine procedure [8]. The assessment of the immunogenicity of TBE-vaccine in patients with rheumatoid arthritis treated with tumor necrosis factor-inhibitors (TNFi) and/or methotrexate (MTX) was recently carried out by Hertzell et al. In this study, individuals <60 years of age were given three doses of vaccine at month 0, 1, and 12, while individuals ≥60 years old received an additional priming dose at month 3 (a total of 4 doses), then the TBE neutralizing antibodies were assessed by a rapid fluorescent focus inhibition test. The results revealed an insufficient antibody response one month after a complete schedule of 3 or 4 doses, compared to healthy age and gender matched controls [9].

Therefore, based on the published clinical cases, TBE has been proved to be more severe in immunocompromised patients, with a prolonged viral shedding and a higher risk of fatal outcomes, while the standard vaccination schedules seem to be less effective in this population.

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CHAPTER FOURTEEN

EPSTEIN-BARR VIRUS POST-TRANSPLANT LYMPHOPROLIFERATIVE DISEASE

The Epstein–Barr virus (EBV) is a globally spread γ-herpesvirus with a tropism to B lymphocytes, which may stay as asymptomatic infection for the patient's entire life. More than 90% of the population worldwide are estimated to be infected by EBV [1]. EB viremia and EBV-PTLD (post-transplant lymphoproliferative disease) are significant life-threatening complications after HSCT. Before the introduction of rituximab into clinical practice, traditional treatments for PTLD included tapering of immunosuppressive agents, as well as administering anti-viral agents, chemotherapies, and unselected donor lymphocytes infusion, which are still being used in particular cases [2]. The clinical presentation of PTLD usually consists of lymph node enlargement, tonsillitis, prolonged fever, and hemogram abnormalities [2–3]. In addition to the lymph nodes and hematopoietic system, it often leads to the damage of internal organs including hepatitis, colitis, pneumonia, nephritis, and so on.

With regard to the general diagnostic work-up, the progressive increasing of EBV-DNA copies in peripheral blood and lactate dehydrogenase (LDH) levels in serum, along with organ dysfunction, may be signs of PTLD [2, 4]. It is interesting that published studies suggest that a high level of EBV-DNA load after HSCT is a strong predictor of poor survival. Additionally, it was considered that a moderate EBV-DNA load acted as a protective factor of survival [5–6]. It was identified that patients with both high and very low levels of EBV-DNA loads had a shorter overall survival rate than those patients with moderate intermediate EBV-DNA loads. The authors supposed that very low levels of EBV-DNA load, soon after HSCT and during the immune reconstitution period, might lead to an unbalanced control between virus infection and the essential immune system, so the very low EBV-DNA load is too weak to initiate an efficient EBV immune control. Then the low EBV viral load may sneak through the weakened EBV immune control [2, 5–6].

In order to monitor EBV infection and facilitate the early prediction of PTLD, quantitative real-time polymerase chain reaction should be carried out [7]. The criteria of initiating rituximab as a pre-emptive therapy has not yet been well established and most published papers recommend focusing on the EBV-DNA viral load. EBV-specific CTL therapy (Cytotoxic T Lymphocyte Therapy) is a targeted treatment strategy of EBV-PTLD to selectively restore the function of immune system against EBV. For example, it was reported in one study that 114 patients received CTL infusions to prevent or treat EBV-PTLD after HSCT, and none of those treated developed EBV-PTLD [8]. Still the complexity, duration, and technical issues of the method limit its further implementation.

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