Short thesis for the degree of Doctor of Philosophy (PhD)

Infectious complications, engraftment and hemostatic activity following autologous hematopoietic stem cell transplantation

by Zita Brigitta Radnay, MD

Supervisor: Prof. Attila Kiss, MD, PhD

UNIVERSITY OF DEBRECEN

DOCTORAL SCHOOL OF LAKI KÁLMÁN

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Head of the **Examination Committee**: Prof. Dr. Kiss Csongor, MD, PhD, DSc
Members of the Examination Committee: Dr. Szerafin László, MD, candidate
Prof. Dr. Takács István, MD, PhD

The Examination takes place at the Lecture room of Department of Pediatric Hematology and Oncology, Faculty of Medicine, University of Debrecen, 10th of July, 2023. 12:00 am.

Head of the **Defense Committee**: Prof. Dr. Kiss Csongor, MD, PhD, DSc
Reviewers: Dr. Földeák Dóra Melinda, MD, PhD
Dr. Nagy Béla, MD, PhD
Members of the Defense Committee: Dr. Szerafin László, MD, candidate
Prof. Dr. Takács István, MD, PhD

The PhD Defense takes place at Lecture Hall of Building A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, 10th of July, 2023. 14:00 pm.

1. Introduction

The primary goal of hematopoietic stem cell transplantation (HSCT) is the eradication of the malignant hematologic disease. Conditioning chemotherapy and in some cases irradiation were administered and stem cells were infused during the process of HSCT, and the new cell lines repopulate the bone marrow microenvironment as a result of the engraftment. The immunosuppressed state connected to the HSCT carries a higher risk of infectious complications. The mannose-binding lectin (MBL) is an acute phase protein, a part of innate immunity. The MBL level in sera is genetically determined and quite stable. According to the literature, lower serum MBL concentration is associated with serious infections. Endothelial injury, caused by multiple factors during the HSCT course, can result in the development of thrombotic complications. The thrombotic complications are principally a consequence of the imbalance among platelets, von Willebrand factor (VWF), and its specific protease, ADAMTS13 in the circulation. VWF regulates the adhesion and aggregation of platelets to the damaged endothelial surface following an injury at blood vessels with higher relative flow rate (microvessels) and can result in further lesions and clot formation. Intense researches are ongoing about the associations and complex interactions of endothelial injury joined to HSCT, impaired function of the coagulation system and inflammation.

We analyzed in our study the relationship between MBL level and occurrence, frequency, and severity of infectious complications in immunosuppressed state following HSCT in case of autologous HSCT patients, in connection with other laboratory parameters of infections. Lymphoma and multiple myeloma patient groups undergoing autologous HSCT were compared, the changes and associations of platelet count, VWF level, ADAMTS13 activity, and C-reactive protein (CRP) values in consequence of the therapy were prospectively examined, and also their relation with the remission state at 100. day after HSCT, at the time of bone marrow regeneration were investigated. We examined the alteration and clinical significance of hemostatic parameters and soluble markers eligible for the follow-up of diffuse endothelial damage during the course of HSCT and engraftment.

2. Literature review

2.1. The hematopoietic stem cell transplantation

Hematopoietic stem cell transplantation (HSCT) is part of the treatment and can potentially result in healing from life-threatening malignant and non-malignant diseases. The patients' own hemopoietic progenitor cells (autologous HSCT) or cells from healthy donors (allogeneic HSCT) were applied in the course of HSCT. The first HSCT was performed in 1957 by E. Donnell Thomas. The selection of eligible patients for HSCT and the remarkable improvement of transplantation technology, prevention and supportive therapy result in the notable amelioration of the early and long-term outcome. The number of stem cell transplantations per year is increasing gradually as a result of the rapid improvement, such as reduced intensity conditioning (RIC) in allogeneic HSCT of elderly patients, respect for emergency indications of HSCT, and more type of graft source available because of widespread donor registry.

2.2. The course of stem cell collection

The stem cell collection is performed before high-dose conditioning because of its stem cell toxic effect, and the infusion of adequate stem cells following conditioning therapy is essential for engraftment in optimal time. CD34 expression is perceptible on the surface of hematopoietic stem cells and facilitates cell separation. Stem cell harvesting was done previously by multiple aspirations from the cristal bone, but later the less painful collection method from peripheral blood became prevalent and results in a more favorable engraftment time. Stem cell mobilization to peripheral blood can happen following chemotherapy or with granulocyte-colony stimulating factor (G-CSF) administered alone. The mobilization is started after cyclophosphamide administration in case of multiple myeloma, and after disease-specific salvage treatment in lymphoma. In case of allogeneic HSCT, stem cells are collected from the healthy donor's peripheral blood after G-CSF injections. Stem cells are stored until HSCT at -70 C degree frozen with added dimethyl sulfoxide (DMSO). The minimal amount of CD34+ stem cell count needed for HSCT is 2x10^6 cells/kg, but for better engraftment

time the aim is 4-6x10^6 cells/kg quantity. Stem cell harvesting is started optimally at the time ≥20 CD34+ cells/µl is reached. In case of inadequate mobilization, plerixafor is also added to G-CSF for collection. Stem cells are mobilized rapidly from the bone marrow as a result of inhibition of the CXCR4 receptor by plerixafor.

2.3. Conditioning treatment

Conditioning is administered before stem cell graft infusion during the HSCT procedure. The goals of conditioning therapy are the eradication of malignant cells in hematologic malignant diseases, the insurance of adequate immunosuppression for engraftment and protection from rejection or GVHD, and the formation of niches in bone marrow for the new stem cells. Conditioning protocol is selected according to diagnosis, stage of disease, type of HSCT, stem cell donor, and the patient's condition, comorbidities, and age. BEAM protocol is the most often used therapy in lymphoma, which consists of BCNU, etoposide, Ara-C, and melphalan. In follicular lymphoma patients, a radioactive isotope labeled anti-CD20 monoclonal antibody, yttrium-90-ibritumomab tiuxetan (Zevalin) agent was also a part of conditioning in previous years, but it went out of practice because it did not prove overall survival. In multiple myeloma patients, high-dose melphalan is administered as conditioning. Total body irradiation (TBI) also belongs to conditioning in some cases.

2.4. The engraftment

The infusion of an adequate amount of CD34+ stem cells following conditioning treatment is very important for the recovery of the hematopoietic system in optimal time and for the engraftment process of cell lines, in case of autologous and allogeneic HSCT. The healthy stem cells insert into the bone marrow niches during HSCT, there they repopulate, and hematopoietic cells are produced. The early and relevant marker of the successful process is the elevation of white blood cell and platelet count. Stable and long-lasting engraftment is the most important goal during the course of HSCT, to ensure the recovery of hemopoiesis and immune system function. The European Bone Marrow Transplantation Committee (EBMT) determined leukocyte

engraftment definition as the third day of three consecutive days when the absolute neutrophil count (ANC) is more than 0.5 G/l. Platelet engraftment is the third day of three consecutive days when the platelet count (Plt) is more than 20 G/l. In our study according to criteria used in our clinical practice, the neutrophil engraftment time is the day when ANC is more than 0.5 G/l, and Plt engraftment is the day when Plt is more than 20 G/l, and platelet transfusion was not indicated in the previous two days, or three consecutive days with Plt count >20 G/l.

2.5. Transplantation Unit at the University of Debrecen

In Debrecen, the 5th Hematopoietic Stem Cell Transplantation Center in Hungary started functioning in September 2003, as the 648th subcenter of EBMT. Autologous HSCT was performed mostly in cases of multiple myeloma (MM) and lymphoma (non-Hodgkin lymphoma, NHL and Hodgkin lymphoma, HL) patients in continuously increasing numbers during the last decades. Induction treatment, favoring patients to get through HSCT, appropriate treatment and supportive therapy during transplantation, and aftercare following HSCT are well organized. Exhaustive therapeutic and diagnostic decisions are needed, and the teamwork of specialists in hematology, transplantation therapy, and infectious diseases is crucial. The first allogeneic HSCT was performed in 2016. Initially, allogeneic HSCT was done with human leukocyte antigen (HLA) -identical donor, and then in the following years, we gained more experience with matched unrelated donor (MUD) and haploidentical allogeneic HSCT also.

2.6. Prevention of infections following HSCT

Isolation and sterilization protocols and rules in the transplantation unit, hygienic hand disinfection and personal protective equipment, protective isolation, positive air pressure difference between the room and corridor have great importance. Prevention of infections that come from invasive devices and central venous catheters is ultimately required. Filtered and irradiated blood products, neutropenic foods, and antiseptic chlorhexidine bathing are necessary.

In autologous HSCT, G-CSF administration after HSCT is recommended to shorten the neutropenic interval. Antibacterial, antiviral, and antifungal prophylaxis is indicated during the course of HSCT. Fluoroquinolones were used widely previously for antibiotic prophylaxis, but later other sufficient prophylaxis methods are recommended according to local protocols and antibiotic resistance details. If the antimicrobial agents have toxic effects, we can give pre-emptive therapy instead of prophylaxis (for example, regular PCR examination to recognize early cytomegalovirus infection). Trimethoprim-sulphamethoxazole is necessary for Pneumocystis carinii pneumonia (PCP) prophylaxis in immunosuppressed state. Intravenous immunoglobulin (IVIG) routine administration is not recommended, it did not contribute to the improvement of survival results, prevention of infections, and other complications.

Following the recovery of the immune system, mainly in allogeneic HSCT, vaccination is important to produce effective protection, because the recipient lost the previous immunization and defense against infections as a consequence of conditioning therapy and HSCT. The mRNA-based COVID-19 vaccine accommodation is recommended after immune reconstitution and tixagevimab-cilgavimab injection for the prevention of serious infection during immunosuppressive therapy.

2.7. Infectious complications following HSCT

The risk of infection depends on the interaction of multiple factors: the virulence of microorganisms and the degree of exposition, the patient's specific immune response and immune system function, the grade of immunosuppression and tissue and organ damage, presence of invasive devices. The infection rate decreases following the reconstitution of the hematopoietic system, mainly through the recovery of innate and cellular immunity. Higher probability of infections is caused by older age, more comorbidities, more cycles of high toxicity treatment of the hematologic disease, previous HSCT, former infections, and iron overload. HSCT-related risk factors are myeloablative conditioning, haploidentical transplantation, T-cell depletion, and immunosuppressive therapy. Immunogenetic factors and longer and deeper neutropenia

also heighten the rate of infections. The duration of neutropenia depends on the diagnosis, previous treatments, amount of stem cells, conditioning treatment, and quality of the graft. Graft failure, acute and chronic GVHD, and immunomodulating viruses (CMV) can cause higher infection probability after allogeneic HSCT.

The post-HSCT interval is divided into three phases according to characteristic infections: the pre-engraftment phase is 20-30 days between HSCT and neutrophil cell line recovery, the early post-engraftment phase (from engraftment to the 100. day), and the late post-engraftment phase (after the 100. day). In allogeneic HSCT all of the three phases, in autologous HSCT the first two phases are featured by higher rate of infections. More infectious complications are experienced not just during the neutropenic period, but also during the first 6-12 months in autologous HSCT, and 12-24 months in allogeneic HSCT, until the B- and T-cell immune response recovery.

In the pre-engraftment phase, the cutaneous and mucosal barrier damage, the neutropenia, and the decreased phagocytic function are important risk factors for infections. Infection source in the background of neutropenic fever was detected only in 20-30% of the cases. Bacteriaemia occurred in 10-25% of the blood cultures taken during neutropenic fever, mainly aerobic Gram-positive and Gram-negative bacteria species were detected. Pneumonia and gastrointestinal tract infections (neutropenic enterocolitis) can develop. Infiltrates in the lungs are nodular or diffuse, and the engraftment syndrome, a cytokine-controlled inflammatory process can cause also infiltrates. The use of antibiotic prophylaxis during the neutropenic period can protect from serious infectious complications. The cause of persistent fever can be a resistant bacteria species, fungal infection, or inflammatory reaction by tissue damage. In central venous catheter-associated infections, blood samples taken from the catheter and samples from the skin at the entry site are necessary, mainly Staphylococcus species are detected, but often microorganisms can't be verified. Bloodstream infections are related to central venous catheters or mucosal injuries, Gram-positive, and Gram-negative bacteria and Candida infection can be proven.

Mainly Gram-positive cocci are responsible for post-HSCT bloodstream infections, the most relevant are coagulase-negative Staphylococcus, Streptococcus

viridans, Staphylococcus aureus (methicillin-resistant, MRSA), Enterococcus and Staphylococcus epidermidis. Fluoroquinolone prophylaxis decreased the rate of Gram-negative infections, but it is less effective against Gram-positive microorganisms. The incidence of resistant Gram-negative bacteriaemia increase as a result of intravascular devices and wider use of fluoroquinolone prophylaxis. The occurrence of PCP is reduced due to trimethoprim-sulphamethoxazole prophylaxis. PCP causes diffuse pulmonary infiltrates, interstitial pneumonitis, and serious hypoxia, high fever, dry cough. Risk factors for PCP are long-lasting immunosuppressive therapy, steroid, chronic GVHD, previous administration of rituximab, lower T-lymphocyte count, and relapse.

Risk factors for infections in the early post-engraftment phase are still ongoing mucositis, mucosal barrier damage, neutropenia, and in allogeneic HSCT the acute GVHD and immunosuppressive therapy. The prevalence of viral infections is typical between 31-100. days post-HSCT, the most frequent is cytomegalovirus (CMV) pneumonia and gastrointestinal involvement. CMV can produce diffuse pulmonary infiltrate. The risk factors for reactivation are GVHD and immunosuppressive therapy, previous CMV viremia. Regular serologic and PCR examination for sufficient CMV prophylaxis or pre-emptive therapy, and in case of CMV infection, the promptly started treatment and viral copy number measuring to monitor the effectiveness of therapy are crucial. The most common early viral infection is the herpes simplex virus (HSV) causes gingivostomatitis. HSV reactivation contributes to oral mucositis formation and worsening. Common airway viruses cause upper and lower respiratory tract infections. Adenovirus reactivation rarely results in severe disease. EBV reactivation and lower T-lymphocyte count take part in post-transplant lymphoproliferative disease, the symptoms are fever, lymph node enlargement, and extranodal lymphoma proliferation in different organs (liver, lungs, bone marrow, and central nervous system). Hemorrhagic cystitis caused by BK polyomavirus can occur in the early post-engraftment phase after allogeneic HSCT. BK virus damages permanently the renal tubular epithelial cells and urothelial cells. Myeloablative conditioning, haploidentical

HSCT, and post-transplant cyclophosphamide treatment have a role in the onset of infection.

Fluconazole prophylaxis decreased the incidence of fungal infections following HSCT. The two most common, clinically relevant microorganisms are Candida and Aspergillus. Invasive fungal infections after the treatment of malignant hematologic diseases, mainly allogeneic HSCT, can result in life-threatening complications. Rarely, but potentially lethal fungal infections can come from mucormycosis, Fusarium, and other opportunists. The most common causes of infective diarrhea are Clostridium difficile, CMV, adenovirus, and enteral viruses (for example rotavirus), susceptibility factor is lymphopenia.

Infection risks are enhanced by myeloablative conditioning, CMV seronegative donor and seropositive recipient, severe chronic GVHD, and late reconstitution of the immune system in the late post-engraftment phase mainly following allogeneic HSCT. Encapsulated bacteria can occur in the background of sinusitis, upper respiratory tract infections, pneumonia, and meningitis. Staphylococcus and Gram-negative bacteria species can result in bacteriemia. CMV, Pneumocystis carinii, and Aspergillus are seen along with bacterial infections. Varicella-zoster virus (VZV) reactivation is also characteristic of the late phase.

2.8. The mucositis

Oral mucositis is a common toxic side effect in patients following high-dose chemotherapy and HSCT. Conditioning causes inflammation and injury of the gastrointestinal tract mucosal layer. High-grade mucositis evolves in two third part of the patients following myeloablative conditioning. High-dose chemotherapy can change the structural integrity of microvessels and the blood supply of the mucosal layer become irregular by the decrease of submucosal capillary perfusion. The time course of oral mucositis is characterized by a peak between 6. and 12. days after HSCT, and slow gradual improvement in the next 7-14 days. Local treatments, such as local cryotherapy, photobiomodulation, and palifermin are applied to reduce symptoms and in severe cases, painkillers are also indicated. The sufficient amount of fluid and calory intake is

inhibited by the strong pain due to mucositis. The high-grade mucositis can worsen the outcome of therapy by increasing the infection risk, enteral or parenteral nutrition is needed and hospital stay is prolonged.

2.9. Innate immunity and mannose-binding lectin

The innate immune response means immediate defense against infections and activates the specific immune system. The innate immune system is the first line of protection when the specific immune response is immature or compromised. Mannose-binding lectin (MBL) is a C-type serum lectin that has a central role in innate immunity. MBL is synthesized in the liver and functions as an acute phase protein. During the acute phase reaction, the MBL level increases slowly (1-2 weeks after the inducing event) and moderately (at the most, threefold elevation).

MBL is associated with serine proteases in the plasma (MBL-associated serine proteases, MASPs). MASP-2 in the MBL/MASP complex is the enzyme needed for the activation of C4 complement factor. MBL binds to microbial surface carbohydrate patterns and mediates the opsonophagocytic process directly and through activation of the lectin complement pathway. MBL is able to bind to Staphylococcus aureus and species of β-hemolytic Streptococcus A group, but in case of several bacterial types only a part of them (E. coli, Klebsiella species, Haemophilus influenzae) showed significant binding, and the encapsulated microorganisms can inhibit MBL switching. MBL allows opsonization in case of main members of invasive fungal infections, Aspergillus fumigatus, Candida albicans, and Cryptococcus neoformans. MBL takes part in recognizing apoptotic, necrotic cells, and endothelial cells under the effect of oxidative stress. Malignant diseases often cause altered surface glycosylation patterns, so MBL can realize pathologic cells. MBL reactive carbohydrate epitopes occur on the surface of several neoplastic cell lines, and because of surface binding, MBL deficiency can be detected in more cases of malignant hematologic diseases.

The reason for lower MBL serum levels is lower actual MBL concentration or decreased functional activity. The serum MBL concentration is between 5 and 5000 ng/ml, as a consequence of genetic mutations in the gene or the promoter region. More

than 10% of the general population is MBL deficient. The majority of MBL-deficients are healthy, without higher susceptibility to infections, but after the impairment of the immune system increased risk of infections is seen. There is a tight association between MBL concentration and genotype, but among persons with the same genotype 10-fold differences in the MBL level can be observed. The capacity of MBL concentration elevation during neutropenic fever depends on the MBL2 genotype. The normal MBL haplotype results in increasing MBL concentration during acute phase reaction, while most of the patients with a mutation in exon 1 are not able to synthesize functioning MBL, and serum MBL levels do not increase in acute phase response. Insufficient polymerization causes low MBL level and impaired MBL function.

The not adequate MBL function leads to high susceptibility to infections, it is a risk factor for infections in patients receiving myelosuppressive chemotherapy. The microbiologically proven systemic and disseminated infections occur more often during high-dose chemotherapy and autologous HSCT in patients, who are MBL deficient. The duration and deepness of neutropenia influence the frequency and severity of infections. MBL-deficient patients experience longer periods of neutropenic fever. The MBL effector function is severely compromised during neutropenia because, after MBL-induced complement activation, neutrophils would be necessary for enhanced phagocytosis. According to some publications, association was not observed between MBL deficiency and fever or duration of fever, and there was no connection between MBL level and infections related to chemotherapy, or between MBL2 gene mutations and the onset of the first infection after HSCT.

2.10. Thrombotic complications following HSCT

Autologous HSCT can be associated with early thrombotic complications, which are related to endothelial cell (EC) activation and injury during the course of HSCT. Endothelial cell activation is important in the development of post-HSCT thrombotic complications, such as veno-occlusive disease (VOD) in sinusoids of the liver, microangiopathic hemolysis, and thrombotic microangiopathy (TMA).

VOD is a clinical syndrome characterized by painful hepatomegaly, jaundice, ascites, fluid gain, and increased body weight. Mostly occur before the +35. day post-HSCT. The pathophysiology of VOD consists of coagulation, immunology, cytotoxic, and inflammatory factors. The reason for VOD is the primary damage of endothelial cells in the sinusoids and liver cells, and as a consequence the injury of central venules. Cytokine and VWF release stimulate platelet adhesion and aggregation. The platelet concentrate refractory thrombocytopenia is often seen in VOD, because of enhanced platelet consumption due to sequestration in the spleen and endothelial cell damage.

Risk factors of VOD are previous liver damage, conditioning treatment and cytotoxic therapy (high-intensity conditioning, unrelated donor HSCT, cyclophosphamide, orally busulphan), TBI, previous irradiation of the abdominal region, allogeneic HSCT, hepato-nephrotoxic agents (vancomycin, acyclovir, amphotericin) during conditioning, other drugs during HSCT process (norethisterone, cyclosporin and methotrexate combined for GVHD prophylaxis, ketoconazole), and genetic factors.

The prophylaxis of hepatic VOD is indicated from the start of conditioning through the early phase following HSCT till neutrophil engraftment or 1-3 months after HSCT. Low molecular weight heparin (LMWH) or low dose heparin, and prophylactic ursodeoxycholic acid are beneficial. In VOD therapy, defibrotide is an agent which modulates endothelial damage without increasing the risk of hemorrhagic complications and does not worsen the antitumor effect of cytotoxic therapy.

The incidence of TMA related to HSCT is variable, between 0.5% and 70%. TMA occurs in the first 100 days after HSCT, and it is less common following autologous HSCT (at most 2.6%) than in allogeneic HSCT (almost 10%). The mortality rate is high (estimated 60-90%), and the treatment effectivity is modest. The endothelial cell injury starts processes of thrombus formation and fibrin accumulation in microcirculation, thrombocytopenia related to consumption, and the high shear stress cause fragmentation of red blood cells.

2.11. The causes of endothelial cell damage

The causes of endothelial cell dysfunction are multifactorial. ECs can be damaged by chemo-irradiation in conditioning treatment, and later by the cytokines coming from injured tissue during the HSCT procedure. Later endothelial damage is caused by bacterial endotoxin translocation through the injured wall of the gastrointestinal tract. Chemotherapy, growth factors, intravenous catheters, endothelial injury because of GVHD, and profound thrombocytopenia are important in the development of thrombotic complications. The degree of cell injury can be characterized well by the amount of some soluble markers, such as plasma thrombomodulin (TM), von Willebrand factor (VWF), and adhesion molecules. The alterations in capillary permeability and endothelial barrier integrity during systemic infections also take part in the onset of a procoagulant state and thrombotic complications.

According to the literature, significantly higher VWF expression is seen on the 14. day after HSCT compared to the day before conditioning, and later there is a decreasing tendency. The engraftment and G-CSF administration also contribute to the endothelial injury detected on the +14. day. Chemotherapy greatly influences the state and function of the endothelium and platelets and thereby increases the risk of bacterial infections, immune reactions, and thrombotic complications.

2.12. Laboratory markers of HSCT-related thrombotic complications

Platelets and VWF sense the shear forces and become activated, the inflammation increases, and local reactions between platelets and the damaged endothelial cells through VWF result in further vessel injuries and thrombosis. The VWF multimer glycoprotein is formed in megakaryocytes and endothelial cells and is stored in alfa granules of platelets and Weibel-Palade bodies of endothelial cells. The stimulation from endothelial cells and platelets induces the release of active, large VWF multimers. After secretion, ADAMTS13 (a disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13) proteolytically cleaves the large VWF multimers.

The half-life of VWF in circulation is 12.4+/-2.5 hours. VWF regulates the platelet adhesion and aggregation at the injured vessels with high shear stress. The balance of ADAMTS13 and VWF activity is absolutely needed to prevent the accumulation of large VWF multimers and consequential platelet aggregation and thrombus formation.

The *de novo* synthesis of C-reactive protein (CRP) in the liver increases after an acute phase stimulus and can reach even a 1000-fold elevation in the plasma, compared to the normal, less than 5 mg/l value. The synthesis rate determines the CRP level in the circulation, so directly reacts to the intensity of pathologic processes. The CRP concentration in blood circulation rapidly decreases after the cessation of the stimulus. The CRP half-life is 19 hours.

The unusually/ultra-large VWF multimers (UL-VWFM) are cleaved to smaller multimers due to the proteolytic cleavage by the ADAMTS13 enzyme. The insufficient physiologic processing of UL-VWF multimers favors platelet adhesion to highly thrombogenic multimers, which results in platelet aggregation and the occlusion of small vessels. According to the literature, the ADAMTS13 activity is significantly decreased in VOD cases, even before conditioning therapy. The UL-VWF multimers can be presented in the plasma in VOD and without VOD also, together with increased VWF antigen level and activity. UL-VWFM is not presented in normal plasma. Serious ADAMTS13 deficiency is not detected in post-HSCT TMA cases.

3. Aims

The infections and thrombotic events are threatening risks following HSCT. The aim of our study was to identify markers for these complications.

It would be important in case of higher susceptibility to infections, to find relevant markers, that allow earlier administration of antimicrobial therapy. Measurement of MBL concentration may be helpful in the planning of antibiotic therapy, earlier and more intensive treatment may be indicated in MBL deficients. Our goal was the examination of the relationship between serum MBL level and infections following HSCT to answer the questions (1) whether it is likely to observe more severe infection in the MBL deficient group, during the immunosuppressed, neutropenic period, and (2) to complete routinely measured CRP levels with MBL determination is reasonable or not.

The markers of thrombotic events caused by the changes of primary hemostasis (thrombocyte count, VWF, ADAMTS13) are the subject of several publications, but the combined analysis of them is not part of the guidelines. The reason can be that a lot of discrepancies occurred in the previous literature. Our other goal was to detect the cause of the discrepancy. The primary hemostasis parameters of lymphoma and multiple myeloma groups were measured at 5 representative time points during autologous HSCT and the data were compared to answer the following questions: (1) to determine how the platelet count, the VWF level along with the ADAMTS13 activity, and CRP level change as a consequence of conditioning therapy, (2) to reveal whether these parameters correlate with each other and (3) with the recovery at the time of bone marrow regeneration. Although numerous studies have already explored the role of these values, none of them examined their relationship at multiple time points of therapy course, and neither separated nor compared lymphoma and multiple myeloma groups receiving autologous HSCT specifically.

4. Patients and methods

4.1. Analysis of MBL levels and infectious complications following autologous HSCT

The association between serum MBL level and frequency, severity, and occurrence of infections has been studied in 186 patients following autologous HSCT with a license of the local Ethics Committee (DE OEC RKEB/IKEB 3633-2012), who were treated at the Transplantation Unit at the University of Debrecen. CRP was measured several times according to clinical decision, and the maximal CRP level during the first 14 days following HSCT was taken in account.

Subgroups, MM, NHL, and HL were formed and infectious complications have been compared. Among the examined patients, the number of persons with NHL was 63 (female/male: 25/38, age: 52±11), 27 HL patients (female/male: 12/15, age: 34±9), and 94 MM patients (female/male: 55/39, age: 56±8). Two patients with other diagnoses were also involved in the trial. The control group consisted of 296 age- and gender-matched healthy individuals (female/male: 156/140, age: 50±16 yrs). Control ones did not have any hematological or liver diseases. MBL serum levels and the occurrence of MBL deficiency in healthy ones and patients with hematological diseases were compared. We examined the distribution of microbiological results according to MBL level, and the hypothesis that the progression and relapse following HSCT are related to MBL level and susceptibility to infections, among other parameters, or not.

The range of MBL levels in healthy population varies between 5 and 5000 ng/ml, <100 ng/ml means MBL deficiency. MBL antigen levels were measured around 100 days after HSCT, in a period without active infection. MBL level is genetically determined and quite stable. There is a small increase during acute phase responses. In a few cases, MBL concentrations were also measured before and around 100 days after HSCT and were almost equal. Informed consent was signed by the examined patients. After blood samples were taken, native tubes were centrifuged for 15 minutes at 3000 RPM, then sera samples were stored at -70 °C in small aliquots until measuring. We

used a double monoclonal antibody sandwich ELISA system adopted from Minchinton et al to determine MBL levels, at the Clinical Research Centre of Debrecen University.

Continuous variables were summarized as means and standard deviation (mean, SD) or as medians and interquartile range (median, IQR) and were compared with Mann-Whitney U-test or Student T-test. Kolmogorov-Smirnov and Chi-square tests were used to find out the distribution of variations, and Kruskal-Wallis ANOVA by Ranks to compare data from more than two groups. The correlation of variables was analyzed with the Spearman Rank order correlation test. $P<0.05$ was considered as significant alteration. ROC curve analysis was performed to determine the cut-off level of MBL. GraphPad Prism 5 and MedCalc were used for statistical analysis.

4.2. Analysis of hemostasis parameters and HSCT-related complications

One hundred and four patients (45 patients with lymphoma, 59 patients with multiple myeloma) treated with autologous HSCT were selected for this study at the Transplantation Unit CIC 648 of the University of Debrecen, between 2010 and 2013. The protocol was approved by the local Ethics Committee (DE OEC RKEB/IKEB 3633-2012). Written informed consent was obtained from all patients. The HSCT procedure was adapted from the guidelines of the EBMT. Stem cells were collected from peripheral blood and were stored at -70°C. Conditioning was carried out according to the BEAM protocol for lymphoma patients. The protocol contains a single 300 mg/m^2 i.v. dose of BCNU (carmustine) on the second day, 200 mg/m^2 each of etoposide and Ara-C twice a day iv. for 4 consecutive days, and a single 140 mg/m^2 dose of melphalan on the sixth day. Follicular lymphoma patients received, in addition, ibritumomab tiuxetan (n=8; Zevalin, Z-BEAM). The conditioning of multiple myeloma patients was a single 200 mg/m^2 dose of melphalan (n=53), which was reduced to 140 mg/m^2 in renal insufficiency (n=6). All myeloma patients received anti-angiogenetic treatment before conditioning.

Prophylactic low-molecular-weight heparin (LMWH) was administered to all patients at a dose that depended on kidney function and daily platelet count. Once platelet count decreased to 40 G/l, LMWH was replaced by continuous iv. Na-heparin

until engraftment. Na-heparin was stopped when the platelet count decreased below 20 G/l and bleeding or fever appeared, or if the platelet count dropped to 10 G/l when platelet transfusion was indicated. Ursodeoxycholic acid was added continuously from the day following HSCT till engraftment. Prophylactic antibiotics, antiviral and antifungal treatment were administered to all patients during the neutropenic period. Patients were discharged from the hospital after engraftment following HSCT (median and IQR: 12 and 11-14 days). In the following three weeks, the patients returned for monitoring. One hundred days after HSCT, a complete disease state evaluation was carried out which was based on PET/CT results in lymphoma patients (Cheson criteria), bone marrow examination, and electrophoretic analysis of serum proteins in myeloma patients (IMWG criteria) to decide complete or partial remission (Cr/Pr) state, and tests of laboratory markers of bone marrow regeneration (blood count, CRP) in both groups. For statistical comparison, we combined the very good partial remission and partial remission cases into the partial remission group in multiple myeloma.

Blood samples were drawn into 3.2% trisodium citrate, K_2EDTA, and native tubes. Following centrifugation (1500 g, room temperature, 15 minutes) plasma and serum were stored in aliquots at -70°C until measurements. The time points of sampling were: starting day of conditioning (preconditioning; Dpre, which corresponds to D-7 for lymphoma and D-2 for multiple myeloma); day of stem cell transplantation (HSCT, D0); and days 5±1 (D5), 11±1 (D11 or engraftment) and 100±5 (D100 or bone marrow regeneration). Blood counts, hemostatic parameters, kidney- and liver enzymes were measured in the Department of Laboratory Medicine, University of Debrecen.

VWF level and collagen-binding activity (VWF:Ag and VWF:CB) were determined with ELISA, using rabbit primary and secondary antibodies. Human Collagen Type III was used for coating in VWF:CB ELISA. The optical density was measured with a Tecan Infinite 200M photometer, and Magellan software analysis and four parametric Marquardt curve fitting were used to count the VWF:Ag concentration and VWF:CB activity.

ADAMTS13 activity was measured with a fluorescence resonance energy transfer (FRET) assay according to Kokame et al. by using a synthetic 73-residue

peptide (FRETS-VWF73), at III. Internal Medicine Clinic, Clinical Research Laboratory, Semmelweis University, Budapest, from the Dpre and D11 samples. Both VWF:CB and ADAMTS13 activity were normalized to VWF:Ag, and indicated as VWF:CB/Ag and AD13/VWF.

For statistical analysis and graphical presentation Microsoft Excel 16.16.24 and GraphPad Prism 9.0.0. programs were used. Variables were tested for normality and lognormality and analyzed accordingly. The outliers were not removed, because reflected the disease state of patients. Variables were analyzed with one-way ANOVA and Bonferroni correction for differences; Pearson correlation and Benjamini and Hochberg method for an association between variables; linear and logistic regressions for the functional relationships and predictive roles.

5. Results

5.1. Associations between infections following HSCT and MBL level results

Among the examined 186 patients with malignant hematological diseases, 21 patients were proved to be MBL deficient. 51 infectious episodes (elevated CRP level, fever, other clinical symptoms of infection) were found among MBL deficients, and 372 events were in MBL competent group during the first 360 days after HSCT. The median time of onset of the first infection post-HSCT was day +7 [3;8] in MBL-deficient and day +6 [4;8] among non-MBL deficient patients.

With the Spearman Rank order correlation test, there was a strong correlation between the logarithmically transformed (log) MBL/CRP ratio and the time of onset of the first infection ($p=0.04$, and after taking into account the occurrence of infection as a censoring variation, $p=0.0001$), and between log CRP and the time of first infection following HSCT ($p<0.05$). The time of first infection correlated neither with MBL level nor with log MBL ($p=0.35$). The correlation between log MBL and log CRP was almost significant ($p=0.052$), and the correlation between log MBL and log MBL/CRP ratio was significant ($p=0.001$) certainly.

The occurrence of infections was similar among MBL deficient and MBL competent ones (2.429 [1.478;3.379] vs 2.248 [1.993;2.516] infectious

episodes/patient). The number of infections after HSCT correlated with CRP and MBL/CRP ratio but not with MBL level (Spearman Rank order correlation test, r=0.37, -0.17 and 0.07; p=0.02 and 0.34, respectively). Mann-Whitney U-test showed not significant relationship in case of MBL level and occurrence of the first infection following transplantation (p=0.37), and MBL level and first infection in 14 days and 100 days after HSCT. Connections of occurrence of infection in 14 and 100 days and before reaching ANC more than 1.5 G/L and log MBL were not significant with unpaired T-tests. But the relation of occurrence of the first infection in 14 and 100 days and before neutrophil engraftment with log CRP and log MBL/CRP ratio were significant.

The cut-off level of MBL according to the occurrence of severe infections in the posttransplant period, determined by ROC curve analysis was 823 ng/ml. The AUC value of the ROC curve analysis was 0.51, and the p-value was 0.815. Variables of the two patient groups separated by MBL cut-off level were compared with the Spearman Rank order correlation test. The number of infectious episodes (p=0.0611) and time of onset of the first infection after HSCT (p=0.0905) were almost significantly different. The occurrence of infections after HSCT (p=0.0480) and occurrence of infections after the pre-engraftment period in the first posttransplant year (during the period from day +14 until day 360) (p=0.0389) were significantly different in the patient groups separated by MBL cut-off level.

Interestingly, MBL serum level was significantly higher in the examined patients with hematological diseases compared to the healthy control population (MBL median, 1479 [380.8;2849] vs 1067 [253.5;2121], unpaired t-test, p= 0.005, significantly different). The occurrence of absolute MBL deficiency was not significantly different between hematology patients and healthy controls (11.4% vs 13.9%). The proportion of MBL deficiencies was the highest among HL patients. MBL concentration of the control population and the examined patients according to diagnosis (NHL, HL, MM) were compared. The median MBL level was the highest among patients with NHL. The onset of the first infection was the earliest among patients with HL.

The most common infections after HSCT were respiratory tract infections and infections with high CRP, fever, and severe mucositis. The time of neutrophil engraftment is related to the MBL level significantly in the MM group (Spearman Rank order correlation, $p=0.024$). Strong association was shown between platelet engraftment time and MBL/CRP ratio among HL patients ($p=0.003$). Stem cell count and time to engraftment correlated well ($p<0.001$). The distribution of Gram-positive and negative bacteria species in culture from the patients' central venous catheter and blood were analyzed. Positive results of central venous catheter culture ($n=25$) depend on log MBL and MBL/CRP ratio, but the relationship was not significant (t-test, $p=0.23$ and 0.15).

We examined whether the progression and relapse following transplantation are related to the patients' MBL levels or not. The association between the occurrence of relapse and log MBL or log MBL/CRP was not significant (t-test, $p=0.9$ and 0.76). Among the examined patients, 23 patients relapsed during the first year following HSCT, and another 45 patients later. Time to relapse was not related to MBL and MBL/CRP ratio.

5.2. Results of hemostasis examinations and HSCT-related complications

Among the 104 patients, 55 females and 49 males were diagnosed with hematological malignancies: 34 NHL, 11 HL, and 59 MM. Blood-group distribution and basic laboratory results (hematology, kidney and liver functions, coagulation, CRP) were statistically indistinguishable between the two groups. The duration of neutropenia and severe thrombocytopenia was longer in lymphoma patients. The time to platelet engraftment was longer in lymphoma patients than in myeloma patients, even though the times necessary for neutrophil engraftment were equal in both groups. Serious bacterial infections developed in ten lymphoma and five myeloma patients which were fatal in two lymphoma and one myeloma patients. Diagnostic results did not prove thrombotic complications. Complete or partial remission was reached 100 days following HSCT in both patient groups, and enlarged lymph nodes completely or partially disappeared in all lymphoma patients.

Platelet count and ADAMTS13 activity decreased whereas VWF:Ag, VWF:CB, and CRP increased following HSCT. Before conditioning, the medians of VWF:Ag and VWF:CB were at the upper limit of the respective reference intervals. By contrast, the median of the AD13/VWF ratio fell below the lower limit of the reference interval. The decrease in platelet count and increase in VWF and CRP levels were substantial from the first day of conditioning, but all returned within the limits of the reference intervals by D100. VWF:CB activity increased in parallel with VWF:Ag, hence their ratio (VWF:CB/Ag) remained more or less constant throughout.

To reveal the temporal dynamics of the variables, we normalized them to their preconditioning values. In lymphoma patients, the platelet count was reduced to 50% by the time of HSCT and decreased below the critical value of 20 G/l between D4 and D13 after HSCT. By contrast, in multiple myeloma patients, platelet count was reduced to 80% by the time of HSCT and dropped below the critical count 6 days after HSCT.

VWF increased gradually and doubled on D11 in both patient groups while AD13/VWF was reduced to 30-40% during the same period. CRP increased 26-fold on D5 and 13-fold on D11 after HSCT in the lymphoma and myeloma groups, respectively, which equates to 12-13 days in each group from the start of conditioning indicating the effect of chemotherapy on CRP level.

Comparison of lymphoma and MM groups with one-way ANOVA following Bonferroni correction revealed that the Plt_0, Plt_5, and Plt_{11} counts decreased and the CRP levels increased in parallel, but the changes were significantly more relevant in the lymphoma than in the myeloma group. VWF increased and the AD13 activity decreased equally in the two patient groups.

To reveal associations between the evolution in time of the different variables and the remission status at bone marrow recovery, platelet count, VWF, ADAMTS13 activities, and CRP level were correlated with each other, with the critical cell counts and the remission states. The correlation is significant if Pearson's correlation coefficient (r) is above +0.4 or below –0.4, and q is less than the threshold P at Q=5%, as calculated by using the Benjamini and Hochberg method. Multivariable regression analysis between different combinations of continuous variables resulted in the best

between univariable pairs. VWF_{pre} showed a negative correlation with Plt_{100} (R=-0.627), Hgb_{100} (R=-0.512), and a positive one with CRP_{100} (R=0.497) in lymphoma patients. VWF_{pre} is associated with the remission state at the time of bone marrow regeneration. The correlation of ADAMTS13 activity with these parameters was not statistically significant. A quantitative relationship of Plt_{pre} with WBC_{100} was found, but not between $ADAMTS13_{pre}$, CRP_{pre}, or any other variables. In myeloma patients, the relation of Plt_{pre} was significant with Plt_{100} (R=0.564) and WBC_{100} (R=0.388). Lower platelet count at HSCT is related to a longer thrombocytopenic interval.

Correlations between variables from HSCT till engraftment were only significant in the case of critical cell counts. Statistically significant correlation was observed between the D11 platelet count and thrombocytopenic period in the MM group. In lymphoma group, the D11 platelet count is related to the duration of the thrombocytopenic period and the time to reach critical platelet count. The latter is associated with the length of the neutropenic interval and the time to reach the critical ANC. The critical cell counts did not predict the remission state at bone marrow regeneration in both groups.

The number of stem cells correlated significantly with the duration of thrombocytopenic period, the time to critical platelet count, and platelet count at engraftment, in both lymphoma and myeloma patients. On the other hand, no significant relationship was found between stem cell numbers and neutropenia and remission status at the time of bone marrow regeneration.

None of the variables showed statistically significant regressions for the occurrence of infection. Also, no correlation was found between liver function parameters and the routine coagulation variables at preconditioning with any of the laboratory markers following HSCT and the status of bone marrow regeneration.

6. Discussion

6.1. Discussion about infectious complications following HSCT

The engraftment process and the recovery of hemopoiesis following HSCT were influenced by variables depending on patients, disease, and treatment. The pre-

engraftment phase was characterized by neutropenia, damage on the muco-cutaneous barrier, and the use of invasive devices, while the post-engraftment phase by inadequate cell-mediated immunity.

Initiation of the complement system may occur via classical, alternative, and lectin pathways. MBL recognizes carbohydrate patterns. Bacterial infections and autoimmune diseases are frequently associated with complement deficiencies. MBL is a serum lectin, the carbohydrate-binding sites allow interaction with the saccharide repeats on microbial surfaces but are rarely associated with mammalian high-mannose structures. MBL deficiency is a result of impaired assembly or stability of multimers. MBL functions as a TLR co-receptor that enables the molecule to coordinate and synchronize the innate immune system. The serum levels of functional MBL correlate with MBL2 coding genotypes and polymorphisms in the promoter region and in exon 1 of the gene.

According to the literature, MBL deficiency is associated with increased susceptibility to infections, mainly when adaptive immunity is compromised (in early childhood, or following chemotherapy). A significant association was shown between low MBL concentrations and serious infections related to chemotherapy. MBL deficients have more severe infections and the first severe infection is earlier, compared to non-deficients. The association between low MBL and infections was independent of whether patients received prophylactic antibiotics or GM-CSF or not.

The range of MBL level is between 5 and 5000 ng/ml, <100 ng/ml is defined as MBL deficiency. Serum MBL concentration is quite stable and shows small increase during acute phase responses. Among the examined 186 patients 21 ones were MBL deficient. There was a strong correlation between log MBL/CRP ratio and the time of first infection following HSCT, but the onset of the first infection was not correlated significantly with log MBL. The number of infections after HSCT correlated with MBL/CRP ratio but not with the MBL level. We could not find a strong association between MBL level and incidence, frequency, and time of infections. An explanation can be that the effector functions of MBL are severely compromised during neutropenia, because neutrophils are required for enhanced phagocytosis after MBL-induced

complement activation. According to ROC analysis, the number of infections and time of first infection after HSCT were almost significantly different in groups separated by MBL cut-off level. The occurrence of infections after the pre-engraftment period in the first posttransplant year was significantly different in groups separated by MBL cut-off value. The cut-off level of MBL according to the occurrence of severe infections in the posttransplant period, determined by ROC curve analysis was 823 ng/ml. MBL serum level was significantly higher in the examined patients compared to the healthy control population.

Infections might lead to delay or reduction in chemotherapy and might compromise the effectiveness of therapy, and also the engraftment of stem cells, so prevention and therapy at the appropriate time are very important. MBL measurement may be helpful in antibiotic treatment, in MBL deficients earlier and more intensive treatment may be indicated. The most common infections after HSCT are respiratory tract infections and infections with high CRP, fever, and severe mucositis. Most sepsis episodes are associated with infection of the CVC-entry site. Mostly Gram-positive bacteria species were isolated in culture from central venous catheter and blood. The relationship between the positive results of central venous catheter culture and log MBL or MBL/CRP ratio was not significant. Infections are cured with appropriate antimicrobial therapy and in some cases with central venous catheter removal. Among the examined patients, relapse and log MBL or log MBL/CRP were not associated significantly.

Oral mucositis grade did not differ significantly between MBL deficient and MBL competent patients. Mucositis can affect the whole gastrointestinal tract, and according to literature, MBL deficients would be less able to prevent the passage of bacteria from the gut to the circulation as compared to MBL competents.

MBL2 genotypes were not determined, as individuals with the same genotypes may differ by 10-fold in MBL levels. Measurement of MBL serum levels by ELISA allows reliable quantification of the functional activity of MBL pathway in vivo. CRP level was used regularly to monitor infectious complications in previous years in our department, and procalcitonin (PCT) level determination became part of tracking sepsis

and severe infections later. Measurement of MBL concentration would be recommended for planning adequate antimicrobial therapy in case of patients with higher susceptibility to infections.

6.2. Discussion of observations about HSCT-related endothelial damage and thrombotic complications

The association between hemostasis parameters before HSCT, cellular and soluble markers, and factors linked to conditioning or HSCT procedure and HSCT-related thrombotic complications is an intensive research area. VOD, TMA, or venous thrombosis occurring mainly at central venous lines in the early interval following HSCT is the topic of many publications. Conditioning therapy, in some cases with TBI, can cause endothelial damage and convert the surface of endothelial cells to procoagulant. TMA is the result of tissue damage following endothelial injury in small vessels. The infections and irregular function of the complement system can also take part in triggering or worsening TMA, because of the changed capillary permeability during inflammation. According to literature, the higher VWF antigen and activity post-HSCT take part in thrombotic complications in microvessels and VOD.

The aim of our work was to explore quantitative relationships between platelet count, VWF level, ADAMTS13 activity, CRP levels, and remission state of lymphoma patients compared to multiple myeloma patients during the course of autologous HSCT, from preconditioning until the time of bone marrow regeneration. New in our work is the analysis of measured parameters only in autologous HSCT and separation of patient groups, compared to previous publications.

The differences between the changes in Plt count, VWF, and CRP of the lymphoma and myeloma patient groups might be due to differences in neutropenic and thrombocytopenic periods, which were longer in the lymphoma patients by three and five days, respectively. The time to neutrophil engraftment in the lymphoma group was similar to that found in the Karolinska study. By contrast, the time to platelet engraftment was shorter in patients in this study as compared to patients in Ungerstedt et al. publication, but in their report, the critical Plt count was 50 G/l without transfusion.

Platelet- and WBC counts at the time of bone marrow regeneration were linked to preconditioning Plt counts in myeloma patients, while the remission state was not associated with any variables. Platelet count before conditioning is a determining factor for the quality of stem cell graft and microenvironment. The substantial Plt count reduction elicited by different conditioning therapies corroborates the results published earlier.

Importantly, in lymphoma patients, preconditioning VWF levels correlated negatively with platelet count and hemoglobin level and positively with CRP level at the time of bone marrow regeneration. The VWF level before conditioning was linked with the remission state at D100 also. VWF, the co-factor of platelet adhesion, is a well-known marker of endothelial injury. The high preconditioning VWF levels, which increased further following HSCT in lymphoma and myeloma patients, have been observed before and are related to diffuse endothelial injury. However, the results were not analyzed according to separated hematologic malignancies and transplantation types in these studies.

Endothelial damage is caused by chemotherapy, serious thrombocytopenia, severe infections in the cytopenic phase, and intravenous devices together. The regulation of vessel tone and permeability, platelet activation, coagulation cascade, thrombotic and inflammatory processes, and angiogenesis are connected to each other and result in lower platelet count and elevated VWF levels, which lead to HSCT-related thrombotic complications. An inverse correlation between ADAMTS13 activity and VWF level during autologous HSCT supports earlier findings and is explained by endothelial dysfunction associated with chemotherapy-induced toxicity. The ADAMTS13 activity before HSCT was in the normal range and decreased to less than half of the initial activity in the first weeks. Post-HSCT TMA is not associated with serious ADAMTS13 deficiency, higher VWF level is related to diffuse endothelial injury. ADAMTS13 activity is not correlated with the development, severity of HSCT-related TMA, and response to treatment, so measurement is not necessary.

The kinetics of VWF increase were similar during HSCT in the lymphoma and myeloma patients, while the platelet count decreased with different kinetics during this

period. Thus, our results indirectly imply that platelet-derived VWF, accounting for about 25% of the total VWF in circulation, did not contribute to the changed plasma VWF levels, which therefore must essentially be from endothelial origin.

Induction treatment of myeloma patients contains anti-angiogenic compounds that might cause long-term modulation of endothelial cells, hence VWF synthesis. The treatment to reach remission in transplant candidates leads to normal hemopoiesis prior to launching the HSCT protocol, but the remission state at preconditioning could be influenced by these therapeutic interventions. Endothelial dysfunction, angiogenesis, and platelet function are associated, therefore, the production of platelets and VWF is likely to be affected due to specific bone marrow niches and the commitment of HSCs to their lineage. The difference between lymphoma and multiple myeloma groups, which showed that the preconditioning VWF level in lymphoma, while the platelet count before conditioning therapy in myeloma patients was in association with parameters at bone marrow regeneration, can be explained by distinct conditioning treatment according to diagnosis. BEAM and Z-BEAM in lymphoma and melphalan conditioning in multiple myeloma were administered. Lymphoma is a very heterogeneous group, mainly the lymphatic system is involved, but malignant cells can occur in the bone marrow also. In contrast, the bone marrow is highly affected in multiple myeloma cases. The bone marrow microenvironment, cytokines, and regulation of immune mechanisms are different between the two groups.

Infections were the most common HSCT-related morbidity in our study, similar to other studies, but the allogeneic and autologous HSCT patients were not separated in those reports. The kinetic of VWF level changing did not correlate with the kinetic of CRP, indicating that VWF increase was not only an acute phase response, even though the half-life of VWF is somewhat shorter than that of CRP. CRP levels increased and peaked 11 days from the start of the conditioning, in both groups. These results imply a linkage between CRP elevation and the conditioning therapy, but there was no linkage to patient groups regarding the kinetics of CRP elevation after the HSCT, in accordance with earlier observations. CRP is produced by hepatocytes under

the control of proinflammatory cytokine IL-6 which is sensed and responded to by ECs in the course of endothelial dysfunction.

Higher amount of transplanted stem cells is connected with shorter thrombocytopenic period and less time to reach critical platelet count in both groups. Our observations verified the commitment of stem cells to the platelet cell line. Prolonging the apheresis time is recommended to reach the optimal CD34+ stem cell/kg dose to ensure adequate platelet engraftment. In contrast, neither length of neutropenia, nor the remission state was predicted by stem cell count in the examined patients. Sufficient quantity and quality of stem cells are aimed to improve engraftment time and decrease infectious complications. Survival following HSCT depends on recovery from the impacts of cytoreductive therapy, sufficient engraftment, prevention of infectious and thrombotic complications, and the successful eradication of the malignant disease. The impaired liver function has direct effect on bowel integrity, allows bacterial products passage to portal circulation, and results in inflammation. The damaged liver function causes not effective clearance of bacteria, bacterial products, and cytokines leading to a procoagulant state.

The limitations of our study are, that the number of samples differed somewhat because we could not reach all patients at appropriate time points and diagnostic results did not prove thrombotic complications, although abdominal pain due to hepatomegaly, edema, and weight gain caused by fluid retention occurred, these cases were not analyzed separately.

7. New findings

The median time to onset of the first infection and the number of infections during the first year following autologous HSCT were not different between MBL deficients and patients with normal/increased MBL levels. The occurrence and number of HSCT-related infections were correlated well with the MBL/CRP rate, which could be a useful biomarker in the prediction of infectious complications.

The occurrence of infections after the pre-engraftment period in the first posttransplant year was significantly different in groups separated based on the 823 ng/ml MBL cut-off level, so the determination of MBL level before HSCT, in a period without infections, might be helpful in antimicrobial treatment planning.

The platelet engraftment was associated with increased VWF level and decreased ADAMTS13 activity at the same time in both lymphoma and multiple myeloma patient groups, and marked the engraftment processes following autologous HSCT.

In lymphoma patients, the preconditioning VWF level was in a significant inverse relation with platelet count and hemoglobin concentration at the time of bone marrow regeneration, while in positive correlation with CRP level and remission state at 100. day. On the other hand, in multiple myeloma group, the preconditioning platelet count correlated with platelet and white blood cell count at the time of bone marrow regeneration.

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9. References

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List of publications related to the dissertation

1. **Radnay, Z.**, Illés, Á., Udvardy, M., Prohászka, Z., Sinkovits, G., Csányi, M. C., Kellermayer, M., Kiss, A., Hársfalvi, J.: Von Willebrand Factor and Platelet Levels before Conditioning Chemotherapy Indicate Bone Marrow Regeneration following Autologous Hematopoietic Stem Cell Transplantation.
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