Evaluation of Thrombotic Microangiopathy after Allogeneic Hematopoietic Stem Cell Transplantation

Ph.D. Dissertation

Orsolya Horváth MD

Semmelweis University
Doctoral School of Clinical Sciences

Supervisor: Gergely Kriván, MD, PhD

Official Reviewers: Henriette Farkas, MD, DSc
Hajnalka Andrikovics, MD, PhD

President of the Final Examination Board:
Csaba Horváth MD, DSc
Members of the Final Examination Board:
Miklós Garami MD, PhD
András Fogarasi MD, DSc

Budapest
2018

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

aHUS:	atypical hemolytic-uremic syndrome
ADAMTS13:	a disintegrin and metalloprotease with thrombospondin repeats 13
ALL:	acute lymphoblastic leukemia
alloHSCT:	allogeneic hematopoietic stem cell transplantation
AML:	acute myeloid leukemia
BM:	bone marrow
BMT CTN:	Blood and Marrow Transplant Clinical Trials Network
CBU:	cord blood unit
CH50:	total complement activity
CMV:	cytomegalovirus
CNI:	calcineurin inhibitor
DNA:	deoxyribonucleic acid
EBV:	Epstein-Barr virus
GVHD:	graft-versus-host disease
HELLP:	hemolysis, elevated liver enzymes, and low platelet count syndrome
HLA:	human leukocyte antigen
HSCT:	hematopoietic stem cell transplantation
HUS:	hemolytic-uremic syndrome
IWG:	International Working Group
LDH:	lactate dehydrogenase
MDS:	myelodysplastic syndrome
MOF:	multiple organ failure
MUD:	matched unrelated donor
NET:	neutrophil extracellular trap
ns:	not significant
OS:	overall survival
O-TMA:	Overall Thrombotic Microangiopathy Grouping
PBSC:	peripheral blood stem cell
PNH:	paroxysmal nocturnal hemoglobinuria
PTLD:	post-transplantation lymphoproliferative disorder

sC5b-9:	solubile terminal pathway activation marker
RIC:	reduced intensity conditioning
TA-TMA:	transplantation-associated thrombotic microangiopathy
TMA:	thrombotic microangiopathy
TRM:	transplantation-related mortality
TTP:	thrombotic thrombocytopenic purpura
UPN:	unique patient number
VOD:	veno-occlusive disease

1. INTRODUCTION

1.1. Overview of Hematopoietic Stem Cell Transplantation

Hematopoietic stem cell transplantation (HSCT) means the transplantation of blood stem cells derived either from the bone marrow, peripheral blood, or umbilical cord blood into patients with malignant and non-malignant diseases. Since HSCT is associated with high treatment- related complications, including mortality, the procedure is limited to patients with life-threatening medical conditions. (1) Allogeneic HSCT has been available as a therapeutic option for pediatric patients since 1992 in Budapest.

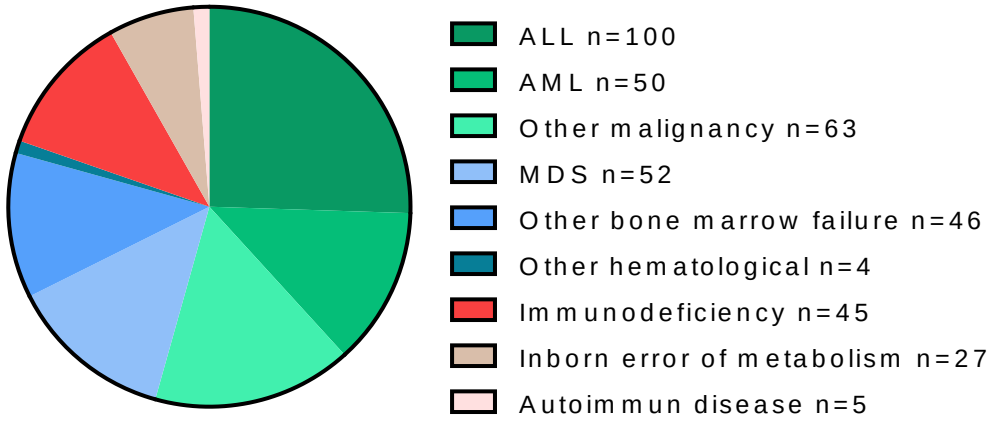


Figure 1

Indications of Pediatric Allogeneic Stem Cell Transplantation in Budapest from 1/1/1992 to 10/31/2017 (n=392)

ALL indicates acute lymphoblastic leukemia; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome.

1.2. Indications of Allogeneic Stem Cell Transplantation in Childhood

Initially, the use of HSCT was restricted to acute leukemias, severe aplastic anemia (SAA), and severe combined immmunodeficiency. (2, 3) Allogeneic stem cell cell transplantation is currently used in different indications during childhood. The indications have been extended to include other primary immunodeficiencies (4), myelodysplastic syndromes (5), and certain forms of inherited metabolic disorders (6) and hematological indications, such as sickle cell anemia (7) or beta thalassemia major.

(8) In severe autoimmune disorders clinical studies also have shown encouraging results. (9, 10) Indications of HSCT in the pediatric population from 1992 to 2017 are shown on Figure 1.

1.2.1. Treatment of Malignancies

The success of transplant for hematologic malignancies derives both from the ability to treat patients with intensive chemoradiotherapy and from potent graft-versus-leukemia effects mediated by donor immunity. (11) Exciting progress is being made in the use of donor lymphocyte infusions to reduce relapse rate. (12) Acute lymphoblastic leukemia (ALL) is the most frequent malignancy in childhood. (13) Despite of the good long-term survival data with conventional therapy in pediatric ALL, HSCT is still required as a curative therapeutic option for the high-risk or relapsed ALL cases. (14, 15) In contrast, acute myeloid leukemia (AML) is still a challenging malignancy in pediatrics, and treatment recommendations for the subgroups of AML are yet to be defined. (16) HSCT can improve the outcome in relapsed or therapy refractory patients. (17)

The indication of allogegenic HSCT in Hodgkin (HL) and non-Hodgkin lymphoma (NHL) is limited to the cases of relapsed lymphoblastic lymphoma, or relapsed HL or NHL. For patients who relapsed after autologous HSCT, allogeneic HSCT could be a promising treatment option. (18) In summary, given the many novel targeted and immunomodulation therapies currently under development, it is important to identify specific patient subpopulations that may benefit from a HSCT compared with those who better suited to new approaches. (19)

1.2.2. Bone Marrow Failure Syndromes and Immunodeficiences

Bone marrow failure syndromes and immunodeficiencies are a heterogeneous groups of pediatric patients, for whom during life-threatening conditions stem cell transplantation can improve the lacking or dysregulated hematopoiesis or immune functions.

Inherited bone marrow failure syndromes represent a heterogeneous group of rare hematological disorders characterized by the impairment of hematopoiesis. Most prominent examples are the failures of deoxyribonucleic acid (DNA) repair mechanism,

such in Fanconi anemia, or as a rarer condition, the failure of ribosomal apparatus in Blackfan Diamond anemia, and failure of telomerase elongation in dyskeratosis congenita. In these congenital disorders, stem cell transplantation can improve the bone marrow failure, but can not correct the co-existing extramedullar organ defects. (20)

Immune destruction of hemopoietic stem cells plays an important role in pathogenesis of severe aplastic anemia. (21) Standard first-line therapy is bone marrow transplantation, if a human leukocyte antigen (HLA) identical sibling donor is available. (22) Other treatment options include first-line immunosuppressive therapy or HSCT with an alternative donor (see in Chapter Graft and Donor Types). (22)

Pediatric myelodysplastic syndromes (MDS) are a heterogeneous group of clonal disorders with an annual incidence of 1 to 4 cases per million, accounting for less than 5% of childhood hematologic malignancies. (23) HSCT is the treatment of choice for many children with myelodysplastic syndromes and is routinely offered to all MDS patients with excess of blasts, to those with MDS secondary to previously administered chemoradiotherapy, and to those with refractory cytopenia in childhood associated with monosomy 7, complex karyotype, severe neutropenia, or transfusion dependence. (5)

Primary immunodeficiencies are genetic immune disorders causing increased predisposition to infections and autoimmunity. (24) Allogeneic HSCT is an extremely effective way of restoring immunity in these individuals. (25) In patients with primary immunodeficiencies, persistent infection and autoimmunity with end-organ damage cause particular problems in the course of transplantation. (4)

1.2.3. Inborn Errors of Metabolism

The inborn errors of metabolism are a diverse group of diseases arising from genetic defects, including deficiences in the production of lysosomal enzymes and abnormalities of the peroxisomal function. (26) Insufficient expression of the affected gene product can be corrected by hematopoietic cells transplanted from a donor with normal gene expression. (6) HSCT allows donor-derived, enzyme producing cells to migrate into the brain and other organs, providing a permanent form of enzyme replacement. Allogeneic HSCT was investigated in numerous lysosomal diseases, such as in Hurler-syndrome, Hunter-syndrome, inherited lysosomal leukodystrophies and Krabbe disease. (26-28)

1.3. Graft and Donor Types

Stem cells can be obtained from different sources: mobilized peripheral blood stem cells, bone marrow, and umbilical cord blood. (29) Choice of a stem cell donor depends on donor availability, donor compatibility and health, recipient disease type, and recipient condition. (30) Currently, accepted stem cell donors for HSCT are the matched sibling donors, matched unrelated donors (MUDs), haploidentical donors, and umbilical cord blood units. (30) Historically, preferred donors for HSCT have been HLA-matched sibling donors. (31) However, the possibility of finding a matched unrelated donor is now more than 70% due to continuous expansion of unrelated donor registries around the world. (32) The outcome of unrelated stem cell transplants in terms of transplant-related mortality (TRM), disease-free survival, and overall survival (OS) is comparable to sibling donors. (32) All types of alternative donors have some advantages and limitations. (33)

1.4. Conditioning Therapy

The term conditioning refers to the preparative treatment before the infusion of the hematopoietic stem cells. Patients can be prepared with chemotherapy alone or combined with radiotherapy with three aims: to reduce the tumor burden -in neoplastic diseases, to create space for the new bone marrow, and to suppress the recipient's immune system, in order to allow engraftment of the stem cells. (34) Conditioning regimens are classified as myeloablative (such as busulphan- or treosulfan-based, total body irradiation), non-myeloablative or reduced intensity conditioning (RIC). (35) Assignment to these categories is based on the duration of cytopenia and on the requirement for stem cell support: myeloablative regimens cause irreversible cytopenia and stem cell support is mandatory. Non-myeloablative regimens cause minimal cytopenia, and can be given also without stem cell support. RIC regimens do not fit criteria for myeloablative or non-myeloablative regimens: they cause cytopenia of variable duration, and should be given with stem cell support, although cytopenia may not be irreversible. (36)

In conclusion, the current aims with conditioning regimens are to reduce the early regimen-related toxicity and mortality, to find the optimal indications for

myeloablative or RIC conditioning regimens, and to provide better long term overall survival and higher quality of life. (37-39)

1.5. Immunosuppression

Maintenance immunosuppressive therapy is administered to almost all recipients in order to prevent graft rejection or failure, and graft-versus host disease (GVHD). (40) Graft rejection/failure is a life-threatening complication following allo-HSCT that is most commonly caused by the reactivity of recipient T cells, natural killer cells or antibodies against donor hematopoietic cells. (41) GVHD is the manifestation of an undesirable immunological reaction between transplanted donor lymphocytes and host tissues. (42) The aim of immunsuppressive therapy during HSCT is to optimize an adequate level of immunosuppression to avoid both graft failure and severe GVHD, and to reduce the incidence of adverse events. (43) The major immunosuppressive agents applied in various combination, are glucocorticoids, methotrexate, anti-thymocyte globulin, alemtuzumab, calcineurin inhibitors (cyclosporin A, tacrolimus), rapamycin (sirolimus), mycophenolate mofetil and post-transplant cyclophosphamide. (43-46) Cyclosporine A is the most widely used immunosuppressive agent after HSCT. (47) Conventional maintenance regimens consist of immunosuppressive agents, that differ by mechanism of action. This strategy minimizes toxicity, and aims to increase optimal dosing. (48)

1.6. Immune Reconstitution

The recovery of the host immune system after allogeneic hematopoietic stem cell transplantation is pivotal to prevent infections, relapse, and secondary malignancies. (49) After conditioning therapy, patients undergo an “aplastic phase” (severe neutropenia or pre-engraftment phase) until neutrophils recover. The reconstitution of innate immunity occurs rapidly within 20–30 days after allogeneic HSCT while reconstitution of adaptive immunity is delayed following HSCT, and can require up to 1 year. (50) Reconstitution of adaptive immunity after alloHSCT is a complex and slow process that can be influenced by several factors, such as recipient age, type of donor (related/unrelated, HLA-matched/HLA-mismatched), type of conditioning (myeloablative/reduced intensity; including or not radiation therapy), ex vivo or in vivo

T-cell depletion of the graft, type of graft-versus-host disease prophylaxis, as well as occurrence and treatments of GVHD. (50, 51)

Several tools have been developed for monitoring the recovery of the adaptive immune system after alloHSCT. Some of them are currently used routinely in clinical laboratories, including measurements of absolute counts and frequencies of main lymphocyte subsets (CD3+CD4+ and CD3+CD8+ T cells, CD20+ or CD19+ B cells) as well as quantification of serum immunoglobulin levels. (52) From Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome patients, it is known that numerical and functional CD4 defects increase the risk of opportunistic infections. (49, 53)

Table 1

Immune Reconstitution after Allogeneic HSCT. BM indicates bone marrow; CBU, cord blood unit; PBSC, peripheral blood stem cell. (50)

Immune cells	Duration after allogeneic HSCT
Neutrophils >0.5 x 10_9 G/L	~14 days for PBSC; ~21 days for BM; ~ 30 days for CBU
NK cells	30-100 days
CD4+ T cells	100-200 days
CD19+ B cells	1-2 years

1.7. Transplantation-Related Complications

1.7.1. Infectious Complications

The time after HSCT is characterized by a state of profound immunodeficiency, during which the patients are at considerable risk of opportunistic infections. (54) Susceptibility to microbial pathogens is generally most pronounced during the first weeks, and risk is decreasing as different parts of the immune system regain their functionality. (51)

Three different periods can be distinguished based on the incidence of certain infections after HSCT. The predominance of the different types of pathogens in each phase is a reflection of different types of immunodeficiencies. (50, 55) Figure 2 illustrates the most common complications in each phase.

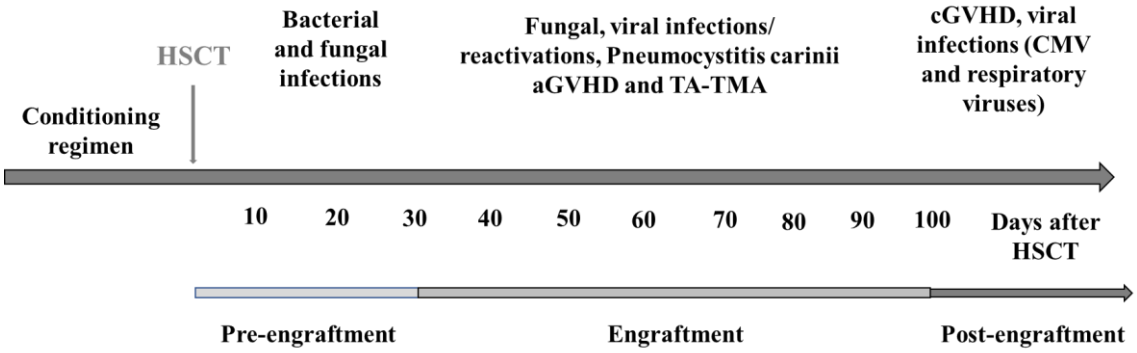


Figure 2

Time Line of the Most Prevalent Infections and Most Significant Complications after Allogeneic HSCT.

The figure shows the most prevalent complications according to the three phases of engraftment. Concomitant infectious complications of bacterial, fungal and viral pathogens are shown accoring to their occurence with acute and chronic GVHD.
CMV indicates cytomegalovirus; aGVHD, acute graft-versus-host disease; cGHVD, chronic graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; TA-TMA, transplantation-associated thrombotic microangiopathy.

The presence of neutropenia and mucosal damage are the leading risk factors in the early pre-engraftment phase. (54) Patients have an increased risk for fungal

infections, especially during the early neutropenic phase or severe GVHD. (56) Bacterial infections remain a common complication, especially also in the pre-engraftment phase. The risk of bacterial infections is mainly related to neutropenia, mucositis, and the presence of vascular lines. (55) Most parts of the world have witnessed a shift in epidemiology toward Gram-negative bacteria. (57)

Despite of the post-transplant screening and pre-emptive therapies, viral reactivations (cytomegalovirus, Epstein-Barr virus, adenovirus) or newly aquired viral infections remain significant causes of morbidity and mortality after HSCT due to the severe immunodeficiency. (58, 59) In spite of early introduced pre-emptive antiviral management, CMV disease occurs in a significant proportion of patients. (59) Routine Epstein-Barr virus (EBV) DNA monitoring allows to start early pe-emptive rituximab therapy. Therefore, EBV related post-transplant lymphoproliferative disorders (EBV-PTLDs) are rare but potentially fatal complications, characterized by uncontrolled proliferation of EBV-infected lymphocytes. (60) EBV-PTLDs commonly manifest as fever and lymphadenopathy and may rapidly progress to multi-organ failure and even death. (61) Intestinal human adenovirus shedding before HSCT confers a greatly increased risk for invasive infection and disseminated disease post-transplant. (62) Moreover, pediatric patients are at an increased risk for adenovirus reactivation, compared to adults. (63)

1.7.2. Graft-versus-host Disease

Acute graft-versus-host disease (GVHD) is a significant cause of morbidity, and represents a major reason for non-relapse mortality, thus is a major determinant of long-term survival after HSCT. (64) This condition is the consequence of an undesirable immunological reaction between transplanted donor lymphocytes and host tissues. (65)

GVHD was first observed in animal models as a combination of symptoms that often occured after allogeneic HSCT and were referred to as „secondary disease” (66). The biology of these graft-versus-host (GVH) reactions were first descriped in 1966, the required conditions for the developement of the GVH reaction were (1) transfer of immunocompetent cells between two individuals, (2) the individuals must differ immunologically from each other, and (3) the host must be immunosuppressed around the time of cell transfer to avoid rejection. (67)

GVHD occurs in acute and chronic forms, each with characteristic symptoms and distinct pathophysiological mechanisms. (65) Acute GVHD is staged and graded according to severity of symptoms in the three target organs: skin, liver and gastrointestinal tract. (42, 68) Figure 3 illustrates skin acute GVHD. The past decade has brought impressive advances in our understanding of the role of stimulatory and suppressive elements of the adaptive and innate immune systems from both the donor and the host side in GVHD pathogenesis. New insights from basic immunology, preclinical models and clinical studies have led to novel approaches for prevention and treatment. (65)

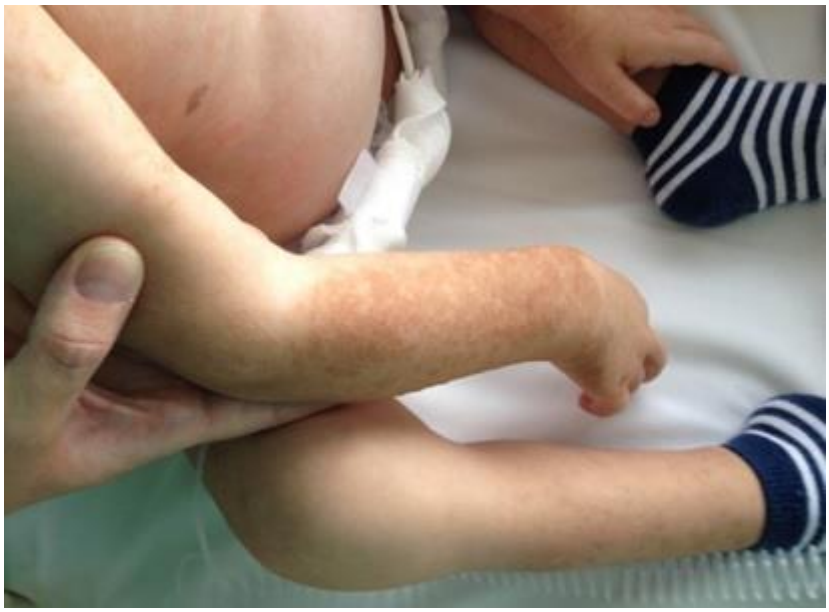


Figure 3

Acute Skin Graft-versus-host Disease in the Department

Despite of the use of prophylactic GVHD regimens, a significant proportion of transplant recipients will develop acute or chronic GVHD following HSCT. (69, 70) Corticosteroids are standard first-line therapy, but are only effective in roughly half of all cases with ~50% of patients going on to develop steroid-refractory disease, which increases the risk of nonrelapse mortality. (70, 71)

Patients with steroid-refractory disease require second-line therapies, with a combination of immunosuppressive agents, described previously in Immunosuppression chapter. (72) There is no standard therapy for steroid refractory GVHD. (73)

Therapeutic options include from increasing the dose of steroids to the addition of polyclonal or monoclonal antibodies, the use of immunotoxins, additional immunosuppressive/chemotherapeutic interventions, extracorporeal phototherapy, and other means. (74) The choice of initial therapy for patients with acute GVHD depends upon the organs involved, the severity of symptoms, the prophylactic regimen used, and, to some extent, the importance of a graft-versus-tumor effect. (75)

1.7.3. Veno-occlusive Disease

Hepatic veno-occlusive disease (VOD), also known as sinusoidal obstruction syndrome (SOS), is a potencially life-threatening complication, can develop primarily after myeloablative hematopoietic stem cell transplantation, but it can also occur after reduced-intensity conditioning. (76) The pathophysiological cascade of VOD involves endothelial-cell activation and damage, due to the conditioning regimen, and a prothrombotic-hypofibrinolytic state. (77, 78) Clinical diagnosis of hepatic VOD is based on the clinical triad of (1) painful hepatomegaly, (2) hyperbilirubinemia and (3) unexplained fluid retention. (79)

Defibrotide, a polydisperse mixture of single-stranded oligonucleotide with antithrombotic and fibrinolytic effects on microvascular endothelium, has emerged as an effective and safe therapy for patients with severe VOD. (80) Defibrotide can be also effective in preventing VOD, therefore this indication has now limitations due to the limited availability of the drug. (81, 82) Recent data suggest that the combination of high-dose steroids and defibrotide may be superior to defibrotide alone. (83)

1.7.4. Transplantation-associated Thrombotic Microangiopathy

Hematopoietic stem cell transplantation-associated thrombotic microangiopathy (TA-TMA) is a potencially severe complication of HSCT that can lead to high risk of death. (84) TA-TMA may have a benign clinical course, but in its most severe forms, manifesting with severe kidney injury, serositis, pulmonary hypertension, and multisystem organ failure, mortality rates are high. (84) In those who survive, TA-TMA may be associated with long-term morbidity including hypertension, chronic kidney disease, gastrointestinal or central nervous system disease, and pulmonary hypertension. (85)

However, the diagnosis of TA-TMA requires a very high index of suspicion. TA-TMA is identified as other thrombotic microangiopathies described in Thrombotic Microangiopathies Chapter. Patients present with microangiopathic hemolytic anemia, defined de novo acute anemia and thrombocytopenia not explained by another process, elevated lactate dehydrogenase (LDH), excessive transfusion requirements, and shistocytosis in the blood. (85) However, the signs and symptoms of TA-TMA can be confused with other common transplantation associated or malignancy associated complications, such as acute GVHD, infections, graft failure, immune-mediated thrombocytopenia, venoocclusive disease, relapse, medication induced side-effects, such as hypertension or acute renal failure. (85-87)

To to summarize the knowledge on TA-TMA with more detailes, a brief overview of thrombotic microangiopathies will be presented in Chapter Thrombotic Microangiopathies (1.8).

1.8. Thrombotic Microangiopathies

1.8.1. The Role of The Complement

The complement system is an ancient defense mechanism that stimulates the inflammatory response and destroys pathogens through opsonisation and lysis. (88, 89) In addition, it constitues a bridge between innate and adaptive immunity. (90) The responses to inflammatory triggers are mediated through a co-ordinated sequential enzyme cascade leading to clearance of foreign cells through pathogen recognition, opsonisation and lysis. (89) Complement also possesses anti-inflammatory functions: it binds to immune complexes and apoptotic cells, and assists in their removal from the circulation and damaged tissues. (91)

Complement system is increasingly being recognized as an important driver of human diseases. (92) Complement dysregulation plays an important role in the patomechanism of atypical hemolytic uremic syndrome (aHUS), Shiga-toxin–associated HUS (STEC-HUS), thrombotic thrombocytopenic purpura (TTP), antiphospholipid antibody syndrome, and the preeclampsia-related HELLP (hemolysis, elevated liver enzymes, and low platelet count) syndrome. (93, 94) Each one is characterized by an imbalance in the interrelated complement and coagulation cascades that fuels a vicious cycle of tissue damage. However, the clinical diversity of these disorders highlights important differences in the triggers and outcomes of complement dysregulation. (94)

The cell surface alternative pathway dysregulation plays an important role in TMAs with different etiologies (Figure 4). (95) Although C3 glomerulopathy and paroxysmal nocturnal hemoglobinuria (PNH) are also prototypical disorders of complement dysregulation with complement overactivation, cell surface alternative pathway dysregulation (aHUS), fluid phase alternative pathway dysregulation (C3 glomerulopathy), or terminal pathway dysregulation (PNH) predominates, resulting in very different phenotypes seen in these diseases. (95)

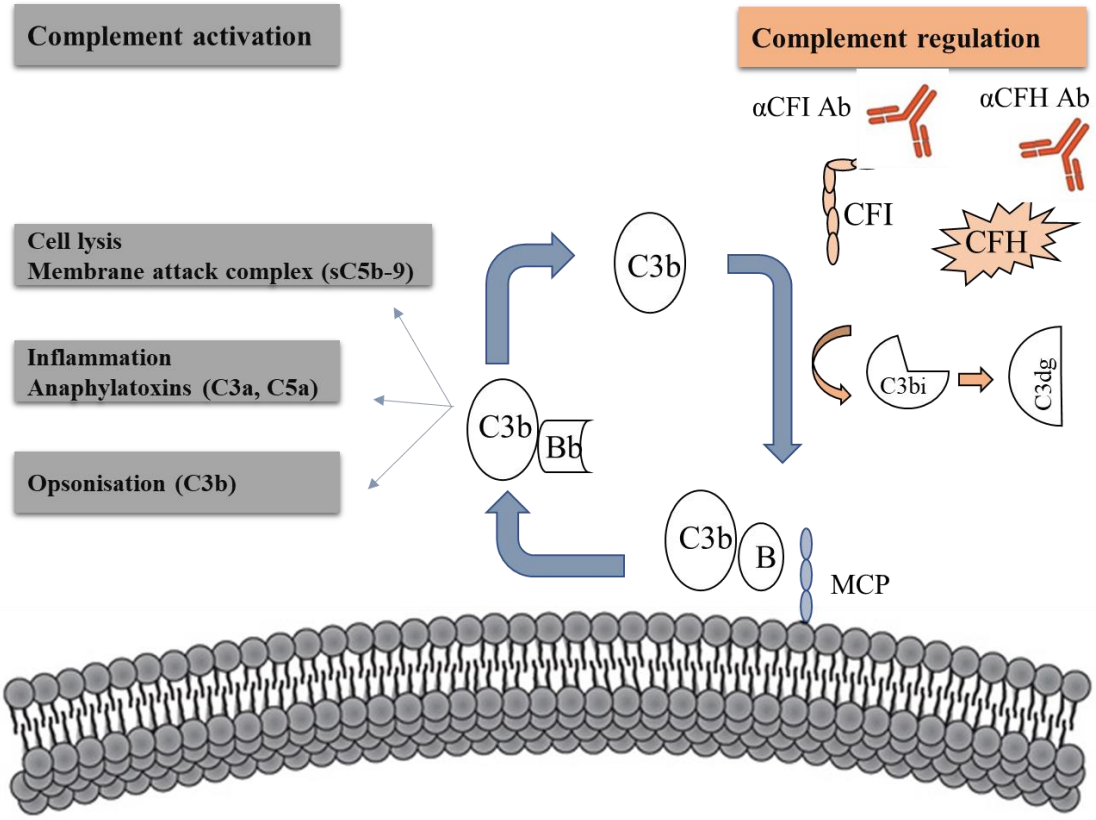


Figure 4

Complement Activation and Regulation (96)

The alternative pathway is a positive amplification loop. C3b interacts with factor B (B), which then is cleaved by factor D to form the alternative pathway C3 convertase (C3bBb). This enzyme complex is attached to the target covalently via C3b while Bb is the catalytic serine protease subunit. C3 is the substrate for this convertase, thus creating a powerful feedback loop. Unchecked, this leads to activation of the terminal complement pathway with generation of the effector molecules, the anaphylatoxin C5a, and the membrane attack complex (C5b-9). To protect host cells from damage, the alternative pathway is down-regulated by complement regulators, including CFH, CHI, and MCP. Activating mutations in *C3* and *CFB* and loss-of-function mutations in *CFH*, *CFI*, and *MCP*, in addition to autoantibodies to CFH and CFI, result in overactivation of the alternative pathway. (96)

CFB indicates complement factor B; CFH, complement factor H; CFI, complement factor I; MCP, membrane cofactor protein.

The diagnostic terms hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) are based on historical and overlapping clinical descriptions. (97, 98) HUS and TTP describe clinical presentations in the absence of knowledge about the cause. (97) Figure 5 gives a brief overview of the etiology of TMAs, marking hereditary, autoimmune, post-infectious and secondary conditions with different colours.

This chapter focuses on the clinical presentation, brief patomechanism, and complement pathway abnormalities of the TMAs, for the better understanding of TA-TMA.

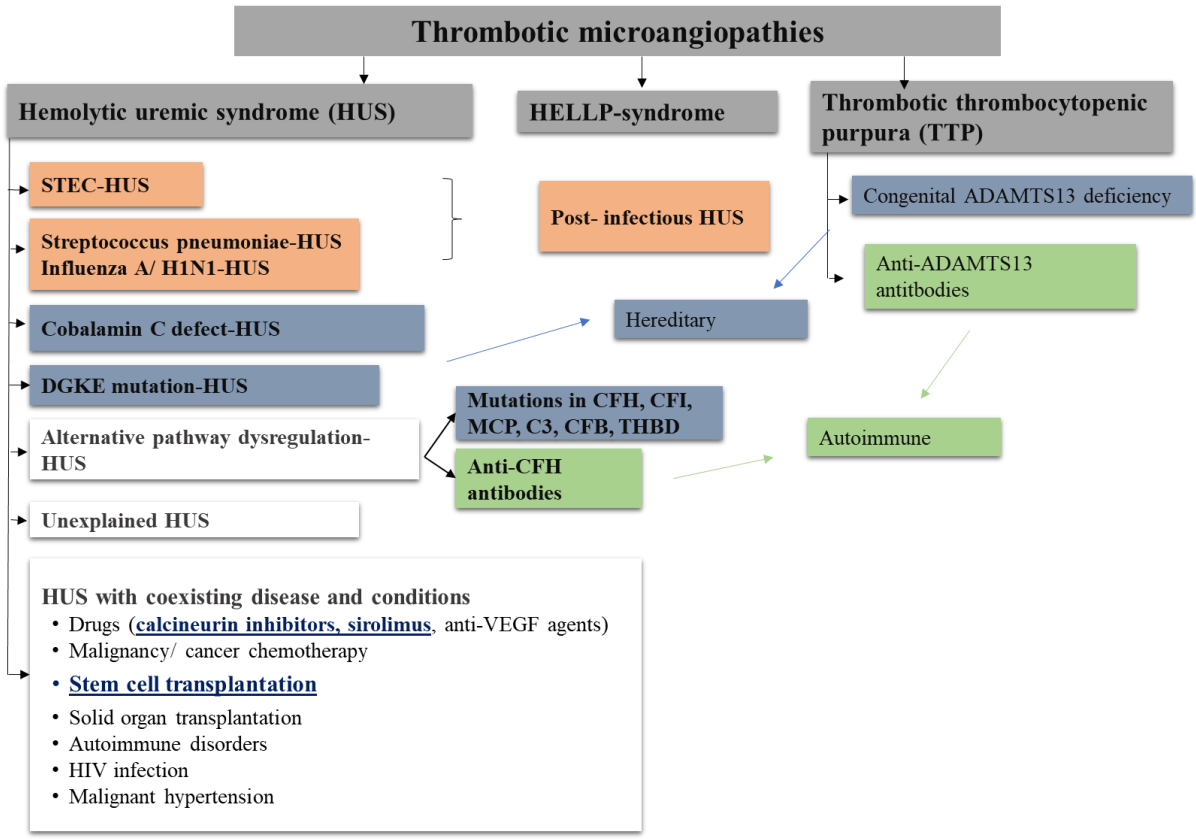


Figure 5

An Etiology-based Classification of the Various Forms of Thrombotic Microangiopathies. (99)

Blue, bold typeface marks the etiology of thrombotic microangiopathy of own research focus in the dissertation. Hereditary conditions marked with blue, post-infectious diseases with red, and autoimmune conditions with green.

ADAMTS 13 indicates a disintegrin and metalloprotease with thrombospondin repeats 13; CFB, complement factor B, CFH, complement factor H; CFI, complement factor I; DKGE, diacylglycerol kinase; HELLP, hemolysis, elevated liver enzymes, and low platelet count syndrome; HIV, human immunodeficiency virus; MCP, membrane cofactor protein (CD46); STEC, Shiga toxin- producing Escherichia coli; THBD, thrombomodulin; VEGF, vascular endothelium growth factor.

1.8.2. Thrombotic Thrombocytopenic Purpura

The first thrombotic thrombocytopenic purpura case was described by Eli Moschcowich in 1925 as a clinically and anatomically remarkable disease: „An Acute Febrile Pleiochromic Anemia with Hyaline Thrombosis of the Terminal Arterioles and Capillaries". (100) Since we know that ADAMTS13 (a distintegrin and metalloprotease with thrombospondin-1-like domains, member 13) activity is significantly decreased in TTP patients. (101, 102) Nonfamiliar TTP is due to an inhibitor of the von Willebrand factor cleaving protease, whereas the familiar form is caused by a constitutional deficiency of the protease. (101) TTP is clinically characterised by thrombocytopenia, microangiopathic hemolytic anemia, neurological symptoms, kidney failure and fever. (103) In laboratory testing, severely reduced ADAMTS13 activity (generally <10 percent) during an acute episode is a hallmark of acquired TTP. (104) Patients with hereditary TTP, ADAMTS13 testing shows severe deficiency without an inhibitor. (105)

1.8.3. HELLP-syndrome

HELLP is an acronym that refers to a syndrome characterized by Hemolysis with a microangiopathic blood smear, Elevated Liver enzymes, and a Low Platelet count. (106) HELLP develops in approximately 0.1 to 0.2 percent of pregnancies overall and in 10 to 20 percent of women with severe preeclampsia/eclampsia. (107) Upregulation of the alternative pathway of complement plays a role in HELLP syndrome. (108) HELLP syndrome follows a 2-hit disease model similar to aHUS, requiring both genetic susceptibility and an environmental risk factor. (109)

1.8.4. Hemolytic Uremic Syndrome

Hemolytic uremic syndrome is characterized by the triad of microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure. It has been classified as either diarrhoeal-associated or non-diarrhoeal/ atypical (aHUS). (110) In children, 90% of HUS cases are associated with a prodrome of diarrhea caused by infections with Shiga toxin- producing Escherichia coli that are able to attach to the intestinal wall; these are known as enterohemorrhagic E. coli (EHEC). (111)

The atypical form of HUS is characterized by complement overactivation. (96) As shown on Figure 5 previously, inherited defects in complement genes, and aquired autoantibodies against complement regulatory proteins have been described. (112, 113) Incomplete penetrance of mutations in all predisposing genes are reported, suggesting that a precipitating event or trigger is required to unmask the complement regulatory deficiency. (96) Overactivity of the alternative pathway is central to the pathogenesis of aHUS. (95)

Patients with Streptococcal-induced HUS (pHUS) typically present with either meningitis or pneumonia before progressing to microangiopathic hemolytic anemia, thrombocytopenia and acute kidney injury. (114) The incidence of pHUS has been increasing in the recent years. (115) The pathogenesis of of pHUS is attributed to the enzyme-, neuraminidase, which is produced by the Streptococcus pneumoniae bacteria. (116) It cleaves the N-acetilneuraminic acid residues on the surface of the red blood cells, glomerular endothelial cells, and platelets, thereby exposing the hidden T-antigen. (117)

Cobalamin C-defect is a genetic disorder of cobalamin (vitamin B12) metabolism and is a rare cause of HUS. (99, 118) This metabolic disease is characterized by marked heterogeneity of neurocognitive disease (microcephaly, seizures, developmental delay, ataxia, hypotonia) and variable other organ system involvement (failure to thrive, cardiovascular, renal, ocular) manifesting predominantly early in life, sometimes during gestation. (119)

Certain conditions such as infection (streptococcus pneumonia, HIV), connective tissue disease, pregnancy, malignancy, and drugs (bleomycin, cisplatin, gemcitabine, mitomycin C, tacrolimus, cyclosporine A, anti-vascular endothelial growth factor agents, interferon, etc.) may also predispose to TMA. (120) Patients during hematopoietic stem cell transplantation also have various risk factors to develop TMA.

1.8.5. Transplantation- associated Thrombotic Microangiopathy

The poor understanding of the pathophysiology and the lack of an accepted definition of TMA have led to a large variety in the published incidence (0,5%-63,6%) and inconsistent risk factors. (121) Patients after HSCT often develop anemia, thrombocytopenia, fever, renal dysfunction, elevated LDH and fragmented red blood

cells from many competing causes. (85-87) In addition, schistocytosis can be absent in severe forms of TA-TMA, because of the high vascular permeability and extravasation of erythrocytes observed in TA-TMA. (122)

TA-TMA was first recognised in 1980, as a side effect of cyclosporin A administration for GVHD prophylaxis in allogeneic HSCT. (123) Cyclosporin A is a calcineurin inhibitor that suppresses immune response by downregulating the transcription of various cytokine genes. Since then, calcineurin inhibitors have been linked to TA-TMA as a potential trigger. (124) Additionaly, clinical studies have identified a lot of potencial risk factors for TA-TMA: age, the use of unrelated donors, HLA mismatch, AB0 incompatibility, the carriage of HLA-DRB1*11 allele, composition of the conditioning regimen, mTOR inhibitors (mechanistic target of rapamycin), development of acute GVHD, and viral and fungal infections. (125-128) Similarly, liver dysfunction and gastric bleeding are prognostic factors for TA-TMA. (129) However, the occurence of these risk factors in all patients after HSCT is high, making the role of the triggers in the pathomechanism hard to determine. (125) Furthermore, TA-TMA and GVHD have similar triggers and some manifestations are common, and often occur together. (122)

It is not clear whether one factor alone could trigger the manifestation of TA-TMA. As an example, calcineurin inhibitors are widely used in patients after organ and stem cell transplantation. (130) Calcineurin inhibitors have been identified as a risk factor also after organ transplantation. (130, 131) TA-TMA after kidney transplantation is rare but severe condition with poor graft outcomes. (131) Interestingly, calcineurin inhibitors have several hematological and immunological indications, such as in asplastic anemia or nephrosis syndrome, where secondary TMA have not been observed. (132, 133)

Therefore, the pathomechanism of TA-TMA remain poorly understood until the 2010's, and complement measurements are recommended only in the recently published criteria in 2015 by Jodele et al. (134) Incidence of TA-TMA depends on the diagnostic criteria applied. The next chapter gives a brief overview of the changes and the conclusions of the different diagnostic criteria of TA-TMA.

1.8.5.1. Changes in Diagnostic Criteria

Several societies have attempted to create criteria for diagnosing TA-TMA (Table 2). The International Working Group (IWG) proposed that the diagnosis of TMA requires the fulfillment of all of the following criteria: 1) >4% schistocytes in blood; 2) de novo, prolonged or progressive thrombocytopenia (platelet count <50x10_9/L or 50% or greater reduction of previous counts); 3) sudden and persistent increase of LDH concentration; 4) decrease in the hemoglobin concentration or increased transfusion requirement; and 5) decrease in serum haptoglobin. (135)

Therefore, the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) Toxicity Committee Consensus Definition for TMA proposed the 4 following diagnostic criteria for TA-TMA in 2005: 1) red blood cell fragmentation and ≥2 schistocytes per high-power field on peripheral smear; 2) concurrent increased serum LDH above institutional baseline; 3) concurrent renal and/ or neurologic dysfunction without other explanations; 4) negative direct and indirect Coombs test results. The panel decided against using thrombocytopenia as a diagnostic criteria, because transplant recipients often have low platelet counts from various causes during the early post-transplantation period. (136)

According to the Overall Thrombotic Microangiopathy Grouping (O-TMA) by Cho et al., all of the seven criteria must be met; 1) ≥2 schistocytes per high-power field on peripheral smear; 2) increased serum LDH above institutional baseline; 3) de novo, prolonged or progressive thrombocytopenia (platelet count <50x10_9/L or 50% or greater reduction of previous counts); 4) decrease in the hemoglobin concentration; 5) decrease in serum haptoglobin; 6) negative direct and indirect Coombs test results; 7) normal coagulation assays. Overall Thrombotic Microangiopathy Grouping is similar to the International Working Group criteria and the BMT CTN criteria, including all components of the proposed criteria, except it does not include transfusion status and instead requires a negative Coombs test and normal coagulation assays to exclude disseminated intravascular coagulation. (121)

A forth set of diagnostic criteria created by the transplantation center City of Hope included 1) the presence of schistocytes and persistent nucleated RBCs, 2) prolonged or progressive thrombocytopenia (<50x10_9 G/L or >50% decrease); 3) LDH greater than twice the upper limit of normal; 4) a decrease in the serum

haptoglobin; and 5) increase of serum creatinin above ≥50% baseline. Patients were defined definite TA-TMA if they fulfilled all the four criteria. Patients were considered to have probable TA-TMA if they met three of the four criteria. Patients who met the characteristics of TMA secondary of disease relapse or progression were not classified as TMA cases. (124)

Jodele et al. used first the O-TMA diagnostic criteria to identify TA-TMA in pediatric HSCT population. (137) Based on their work on TA-TMA pathophysiology in children and young adults, seven diagnostic criteria of TA-TMA were decribed in Cincinnati: 1) elevated LDH (above the upper limit of normal for age); 2) proteinuria (a random urinalysis protein concentration (≥30mg/dl)); 3) hypertension (a blood pressure at the 95th percentile value for age, sex and height); 4) de novo thrombocytopenia (platelet count <50x10_9/L or 50% or greater reduction of previous counts);. 5) de novo anemia (a hemoglobin level below the lower limit of normal for age or anemia requiring transfusion support); 6) the presence of schistocytes in the peripherial blood or histologic evidence of microangiopathy on a tissue specimen; 7) terminal complement activation (elevated plasma concentration of sC5b-9 above upper normal laboratory limit). (134) Proteinuria (≥30mg/dl) and elevated marker of complement activation (sC5b-9) at TA-TMA diagnosis were found to be associated with poor outcome, and are additional criteria to diagnose high risk TA-TMA. (138) Important to note, that Jodele et al. reported first complement measurements for the diagnosis of TA-TMA. (138) Serum haptoglobin was not included as a criteria in Cincinnatti, but low haptoglobin was described as a poor prognostic marker in patients with TA-TMA. (134) The modification of diagnostic criteria that included markers of complement activation, proteinuria, and hypertension (85), led to an improved and earlier detection of the developing clinical TA-TMA.

In conclusion, both diagnostic criteria have limitations due to the wide spectrum from mild to severe forms of TA-TMA cases. The diagnostic criteria used for TA-TMA recognition include hematologic and renal injury markers, reflecting the presence of already ongoing, clinically manifest organ damages. Therefore, there is a demanding clinical need to recognize, or to predict TA-TMA as early as possible, but current possibilities to achieve these goals are largely limited.

Table 2

Five Different Diagnostic Criteria for TA-TMA (121, 124, 135, 136, 138)

Parameter	BMT CTN	IWG	O-TMA criteria	City of Hope	Jodele et al.
LDH	above upper limit of normal	above upper limit of normal	above upper limit of normal	>2x above upper limit of normal	above upper limit of normal of age
Thrombo-cytopenia	No	<50x10_9/L or 50% or greater reduction of previous counts	<50x10_9/L or 50% or greater reduction of previous counts	<50x10_9 G/L or >50% decrease	<50x10_9/L or 50% or greater reduction of previous counts
Anemia	No	decreased hemoglobin or increased transfusion requirement	decrease in the hemoglobin concentration	No	hemoglobin level below the lower limit of normal for age or anemia requiring transfusion support
Schistocytes	>2 per high power field	>4%	>2 per high power field	presence of shisctocytes and persistent nucleated RBCs	>2 per high power field or presence of shistocytes on tessiu specimen

Parameter	BMT CTN	IWG	O-TMA criteria	City of Hope	Jodele et al.
Hapto-globin	No	Decreased	Decreased	No	No
Kidney function	Serum creatinin 2x above baseline (or neurological dysfunction)	No	No	Serum creatinin 1,5x above baseline	proteinuria (random >30mg/dl; high risk criteria)
Negative Coombs test	Yes	No	Yes	No	No
Hyper-tension	No	No	No	No	Yes (pediatric)
Terminal comple-ment complex	No	No	No	No	Elevated (high risk criteria)

BMT CTN indicates Blood and Marrow Transplant Clinical Trials Network; IWG, International Working Group; LDH, lactate dehydrogenase; O-TMA, Overall Thrombotic Microangiopathy Grouping.

1.8.5.2. Tissue Biopsy

Inappropriate complement activation or insufficient inhibition can result in vascular endothelial injury and thrombotic microangiopathy. (139) The adverse effects of TA-TMA come from platelet aggregation with microthrombi formation leading to tissue ischemia and hypoxaemia. The multi-organ injury can involve the kidney, the gastrointestinal tract, and can lead to pulmonary, cardiac and neurology dysfunction. (134, 140, 141) TA-TMA may involve the intestinal vasculature and can present with bleeding and ischemic colitis. (142) In spite of the organ involvement, tissue biopsy is rarely performed after HSCT in the cases with decreased kidney function or lung involvement. On the other hand, bowel biopsy in routinly performed to diagnose and grade graft-versus-host disease. (143, 144) Mucosal petechiae or hemorrhage may reflect significant bowel vascular injury in patients with systemic high risk TA-TMA. (142, 145) Defined colonoscopic findings would improve early clinical diagnosis of intestinal TA-TMA.

1.8.5.3. Complement Dysregulation in TA-TMA

Although significant advances have been made in understanding the pathogenesis of other thrombotic microangiopathies, TA-TMA remains poorly understood until the 2010's.

As our understanding in aHUS has evolved, TA-TMA seems to resemble more aHUS than other TMAs. (125) The working group in Cincinnati published several studies, highlighting the role of complement dysregulation in the patomechanism of TA-TMA. Excessive complement activation (as reflected by increased levels of anaphylatoxins C3a and C5a, and terminal pathway complement complex) are known to activate-, among others-, platelets, granulocytes, endothelial cellsand coagulation, leading to cellular injury and development of TA-TMA. (146-148) Recent observations about the close association of increased terminal complement pathway activation complex sC5b-9 levels and worse outcome of TA-TMA are supporting the importance of complement activation in the pathogenesis of TA-TMA. (134) Jodele et al. (138) reported that evidence of terminal complement activation (elevated sC5b-9 levels) in the blood at the time of TMA diagnosis were associated with very poor survival.

Many different variants in complement genes have been reported to predispose to aHUS, leading to the overactivation of the alternative pathway. (112, 113) In their early works, Jodele et al. identified a high prevalence of deletions in CFH-related genes 3 and 1 (delCFHR3-CFHR1) in patients with TA-TMA. (137) Later, they also performed a hypothesis-driven analysis of 17 different genes that participate in complement activation in pediatric patients after HSCT. They used gene expression profiling to examine the functional significance of variance in these genes. 65% of the patients with TA-TMA had at least one gene variant, as compared with 9% of patients without TA-TMA. (149) In addition, variants in three or more genes were associated with increased mortality. (149) These results has not been validated in other studies. Therefore, studies to understand better the importance of identifying genetic mutations before HSCT remain to be done. (86)

1.8.5.4. Treatment

Currently available therapeutic modalities for TA-TMA include modification of drug therapies (ie. withdrawal of calcineurin inhibitors), plasma exchange, and application of intravenous immune globulin, rituximab, defibrotide, pravastatin and cidofovir. (150)

However, based on the recent recognition of complement alternative pathway dysregulation after HSCT, and on the promising results of complement inhibition in various forms of TMA besides atypical hemolytic uremic syndrome (151, 152), eculizumab, a humanized monoclonal antibody against complement C5 was also evaluated in the setting of TA-TMA.

Eculizumab was first shown to be effective and well tolerated in patients with paroxysmal nocturnal hemoglobinuria (PNH). (153, 154) In PNH patients, eculizumab improves anemia, and decreases transfusion requirements. (154) Eculizumab has been also approved to use in aHUS from the 2010's (155). Eculizumab treatment should begin immediately when the diagnosis of aHUS is confirmed. (156) For children, a pediatric weight-based dosing shedule has been established. (157)

In the recent years, several case reports (158, 159) and case-series studies (160-162) that eculizumab, having an acceptable toxicity profile, may be also a promising (163) therapeutic option for patients with TA-TMA. Therefore, eculizumab

pharmacokinetics in HSCT patients differ significantly from reports in other diseases like aHUS and paroxysmal nocturnal hemoglobinuria. (164)

Patients undergoing eculizumab therapy has an increased risk for meningococcal infections without adequately functioning complement system. (165) Meningococcal vaccine is indicated for patients with other indications, but patients with TA-TMA are severely immunocompromised, and are not able to mount a response to vaccines. (166) On the other hand, Jodele et. al showed that terminal complement blockade in the early post-transplant period can be performed without meningococcal vaccination while using appropriate antimicrobial prophylaxis until complement function is restored after therapy completion. (163) On the other hand, a high rate of fungal and bacterial infections were observed among HSCT patients during eculizumab treatment, leading to therapy failure. (167)

In summary, complement blockade has shown some promising rates of hematologic responses, however further studies are needed to confirm the role of complement activation in TA-TMA, and to enhance diagnostic strategy. A number of complement inhibitors are in the development and may change treatment paradigm. (168)

2. AIMS

Transplantation-associated thrombotic microangiopathy (TA-TMA) is a challenging complication following HSCT. Incidence rates of TA-TMA after HSCT are reported between 0.5 and 63.6%, this wide range is related to factors including the inability to obtain a tissue biopsy, the various and changing diagnostic criteria used to define TA-TMA, and the changes of conditioning regimens in the past years. (86)

The pathogenesis of TA-TMA is related to endothelial injury, only partially explaining the systemic nature of this complication. Recent observations suggested that complement alternative pathway dysregulation may also be involved in the pathogenesis of TA-TMA. (137) Jodele et al. (138) reported that evidence of terminal complement activation (elevated sC5b-9 levels) in the blood at the time of TMA diagnosis were associated with very poor survival. However, consecutive changes in complement profile and complement activation product levels, and their relationship to complications following HSCT, are scarcely known today.

The recent modification of diagnostic criteria that included markers of complement activation, proteinuria, and hypertension (134), led to an improved and earlier detection of the developing clinical TA-TMA. However, to the best of our knowledge, there are only a few studies that investigated the potential role of the biomarkers for TA-TMA in the post-HSCT setting, such as soluble adhesion molecule-, serum neutrophil extracellular trap- and complement activation product levels. (138, 169-171)

Based on the recent diagnostic criteria for TA-TMA, and the description of alternative pathway dysregulation in TA-TMA patients, on the high risk of developing TMA in patients with elevated terminal pathway activation marker levels, and on the first promising results with anti-C5 complement inhibition in TA-TMA, we decided to focus on potential predictive complement biomarkers in the post-HSCT setting.

The aims of the work presented in this thesis are:

1) To identify patients with TA-TMA using five different diagnostic TA-TMA criteria published in literature and to compare the various groups for TA-TMA parameters.

2) To analyze longitudinal changes of complement profile after HSCT in a prospective, consecutive pediatric cohort.

3) To identify potential complement biomarkers of TA-TMA development.

3. METHODS

3.1. Patient Population, Sample, and Data Collection

Forty pediatric patients underwent allogeneic stem cell transplantation for malignant (n=20) and non-malignant (n=20) indications at our center in Budapest from November 2013 to June 2015. Thirty-three of the 40 patients fulfilled inclusion criteria and were included in further data evaluation and complement measurements. Exclusion criterias in this prospective cohort were weight below 10 kg (5 patients were excluded) and early death (<30 days after transplantation, 2 patients were excluded). The study flow chart is presented on Figure 6. Informed consent was obtained in accordance with the Declaration of Helsinki and the study was approved by the institutional Ethics Committee on Human Research.

Samples were systematically collected before transplantation, representing baseline level, and on day 28, 56 and 100 after HSCT. Blood sampling followed the predefined protocol, and no extra samples were taken, if a transplantation related complication occurred. Samples (serum, EDTA-anticoagulated plasma and sodium-citrate anticoagulated plasma) were taken from antecubital venipuncture or from central venous catheter. Cells and supernatants were separated by centrifugation and shipped on ice for processing. The aliquoted samples were stored below -70°C until use.

3.2. Clinical Monitoring

Demographic, clinical, and laboratory information was collected from the medical records. The following TMA activity markers were registered: serum LDH, creatinine and haptoglobin levels, new onset anemia, thrombocytopenia, fragmentocytes (determined by automated hematology analyzer), Coombs test, proteinuria and hypertension. Elevation of serum creatinine was regarded as doubling of serum creatinine from baseline pretransplantation level. Proteinuria was defined as a random urinalysis protein concentration of 30 mg/dL. Hypertension was defined as the blood pressure above the 95th percentile value for age, sex, and height.

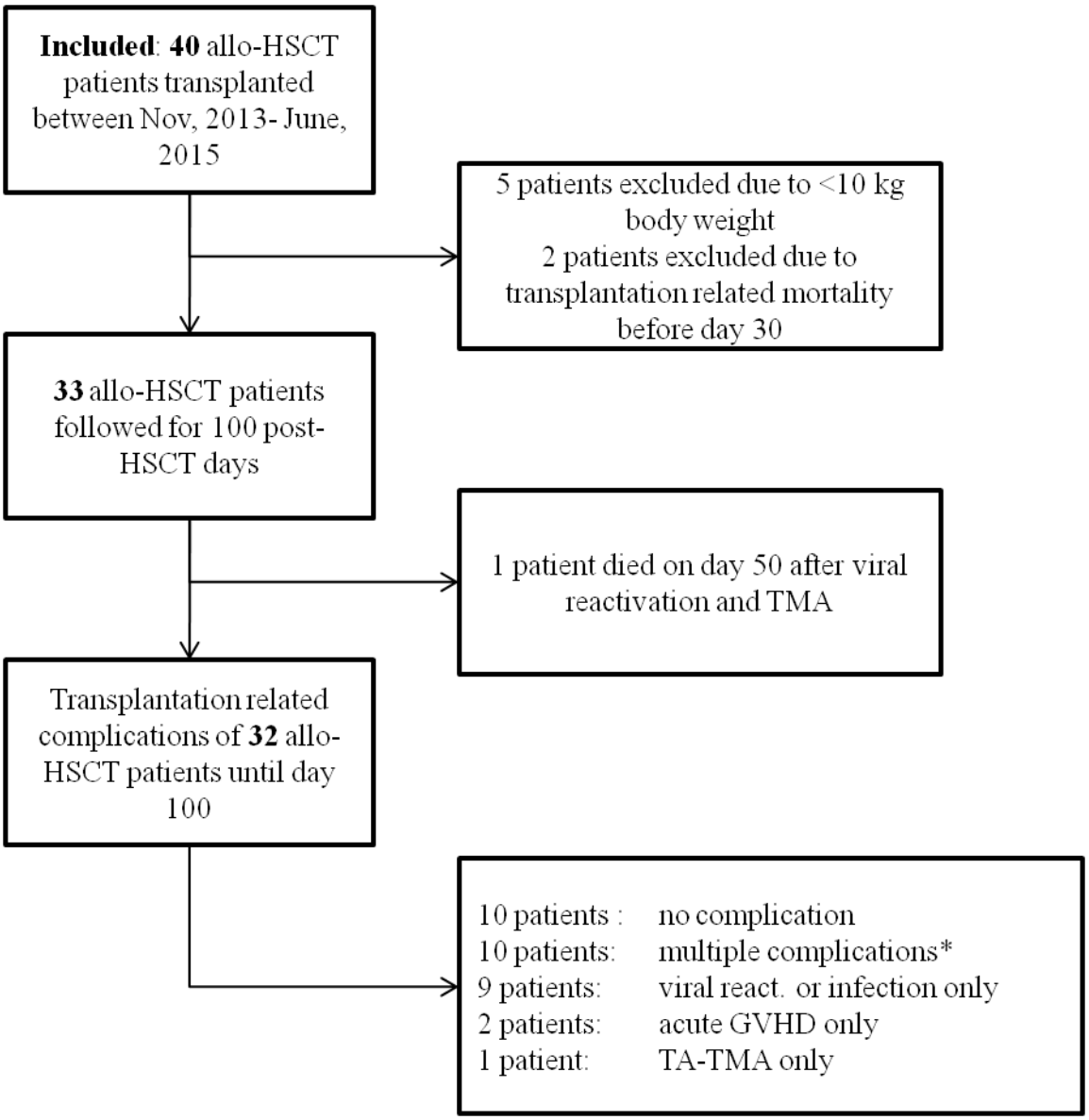


Figure 6

Study Flow Chart.

*The sequence of multiple complications, including acute GVHD (G), viral reactivation or new viral infection (V), and TA-TMA (T) were as follows: 3 patients G-T; 2 patients V-G-T; 2 patients G-V-T; 1-1 patient each: V-T, G-V, V-G.

GVHD indicates graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; PTLD, post-transplantation lymphoproliferative disorder; TA-TMA, transplantation-associated thrombotic microangiopathy.

3.3. Determination of Complement and Other Laboratory Parameters

Complement measurements were performed after the collection of the last follow-up sample. Complement C3 (reference range 0.9 to 1.8 g/L) and C4 (reference range, 0.15 to 0.55 g/L, Beckman Coulter), factor I and factor B (radial immunodiffusion, reference range, 70 to 130% for both), factor H (sandwich enzyme-linked immunosorbent assay (ELISA), 250 to 880 mg/L) levels, total classical (sheep red blood cell hemolytic titration, reference range, 48-103 CH50/mL) and alternative pathway activities (Wieslab Comp AP330 kit, 70-125%, Euro Diagnostica Malmo Sweden) were measured in serum samples. As complement activation products, levels of fragments C3a (MicroVue C3a desarg EIA A031, range, 70 to 270 ng/mL), C4d (MicroVue C4d EIA, A008, range, 0.7 to 6.3 μg/mL), and sC5b-9 (MicroVue sC5b-9 Plus EIA, A020, range, 110 to 252 ng/mL) were determined with commercial kits (Quidel, San Diego, CA, USA) according to the manufacturer's instructions from EDTA plasma samples.

The fluorigenic substrate, FRETS-VWF73, was applied for the determination of ADAMTS13 enzyme activity (reference range, 67 to 151%) from sodium-citrate plasma samples. The methods were described previously in full detail. (172)

3.4. Definition of Transplant-Related Complications

Acute GVHD was graded according to the Glucksberg criteria. (68) Quantitative PCR assays (cytomegalovirus (CMV), Epstein-Barr virus (EBV), and adenovirus were performed weekly to detect viral reactivation or primary infection. We defined viral infection (n=17) after transplantation as CMV, EBV or adenovirus reactivation (n=15) or primary infection (n=2) during the first 100 days after HSCT. Acyclovir was started on the first day of conditioning therapy as herpes virus prophylaxis, and levofloxacin as antibacterial prophylaxis. All patients received posaconazol or micafungin fungal prophylaxis. Rituximab, foscarnet, ganciclovir and cidofovir were used as preemptive therapy. Transplantation failure (TF) was defined if relapse, rejection or transplantation-related mortality occurred.

Five sets of diagnostic criteria were used to define TA-TMA: 1) the diagnostic criteria of Blood and Marrow Transplant Clinical Trials Network Toxicity Committee Consensus Summary (BMT CTN), (136) 2) the clinical criteria of the International

Working Group (IWG), (135) 3) the diagnostic criteria proposed by Cho at al., (121) 4) the diagnostic criteria of City of Hope, (124) and 5) the diagnostic criteria proposed by Jodele et al. (134). The date of diagnosis was defined as the date when all of the TA-TMA diagnostic criteria proposed by Jodele et al. were fulfilled. In case of early signs of a developing TA-TMA, withdrawal or change of calcineurin inhibitors was the initial step to control the process in our clinical practice. Eculizumab as a therapeutic option for TA-TMA was not available during the whole study period in Hungary.

3.5. Statistical Methods

Because most of the continuous variables showed skewed distribution, and failed Shapiro- Wilk test, data are presented as median (range or interquartile range) and nonparametric tests were used for group comparisons (we used Mann-Whitney U test for two independent groups, and for repeated measures we used the Wilcoxon matched pairs test for two groups, or Friedman test for multiple groups). Fisher's exact test was used to compare categorical variables. The Kaplan-Meier method was used for estimating TA-TMA event- free survival and the log-rank test was used to compare survival curves. Two-sided p- values were calculated and $p< 0.05$ was considered statistically significant. Analyses were performed using the Statistica 8.0 (StatSoft Inc., Tulsa, OK, USA) and the GraphPad Prism 6.03 (GraphPad Software Inc., San Diego, CA, USA) statistical software.

4. RESULTS

4.1. Study Cohort Demographics

Enrollment of the 33 pediatric study patients undergoing allogeneic stem cell transplantation is shown on Figure 7. Forty-eight percent of patients underwent HSCT for nonmalignant disorders, including bone marrow failure syndromes (82%), immunodeficiencies (12%) and metabolic disease (6%). Malignant indications (52%) included acute lymphoblastic leukemia (ALL, 29%), acute myeloid leukemia (AML, 41%), Hodgkin lymphoma (HL, 6 %), non-Hodgkin lymphoma (NHL, 12%) and juvenile myelomonocytic leukemia (JMML, 12%). The majority of myeloablative regimens (96%) were treosulfan based reduced toxicity conditioning therapy. Overall, 24 of 33 (73%) patient received treosulfan-based conditioning regimen for malignant and non-malignant disorders. Busulphan was used in one JMML case. Reduced intensity conditioning regimens were used in in 8 of 33 (24%) cases. The main stem cell source was bone marrow (70%) from matched unrelated donors (76%). Haploidentical donor was used in one malignant case. Conditiong regimens, indications and stem cell sources are shown in Table 5 in full detail. Patients were followed for 100 days after transplantation and transplantation related complications were monitored.

Viral reactivation was detected in 15 of 33 patients and was the most common transplant-related complication in our study population. In addition, 1 patient had primary CMV infection and 1 patient had primary EBV infection during the study period.

Eleven (33%) patients experienced grade I or II acute GVHD. All of the 11 patients had Grade I-II GVHD and no patients developed severe GVHD (grade III to IV) in the study period. Thirty-one of 33 patients received cyclosporine A, 1 of 33 patients received tacrolimus due to previous hypertension during the first HSCT, and 1 patient received mycofenolate mofetil after haploidentical HSCT as GVHD prophylaxis with or without methotrexate or anti-thymocyte globulin. Sirolimus was used in 3 cases as GVHD prophylaxis or treatment.

4.2. Diagnosis of TA-TMA

All components of the five diagnostic criteria for TA-TMA and all TA-TMA activity parameters were consecutively monitored.

Table 3

TA-TMA Activity Parameters in the 33 Allogeneic HSCT Patients.

Days after HSCT are shown, if the activity parameters fulfilled the criteria for TA-TMA.

Patient code	Elevated LDH 2x	Schistocytes	Anemia	Thrombocytopenia	Decreased haptoglobin	Hypertension	Creatinine 2x	Proteinuria	Other complication
1	47				28				relapse
2	20		47						GVHD
3		12							
4		16			28				
5	12	5	14	16	14	5	7		GVHD
6							21		EBV, adenovirus
7	68	33	83	77	100	29			GVHD
8	21			66	56		33		CMV, relapse
9					56				relapse
10	34	37							
11	25	4	27	31	28	26			CMV, EBV, PTLD
12	23	20							
13	25	28	98	98		67			GVHD, adenovirus
14					56				
15	43	58	65	65		60		59	GVHD, EBV
16	33	47					40		GVHD

Patient code	Elevated LDH 2x	Schistocytes	Anemia	Thrombocytopenia	Decreased haptoglobin	Hypertension	Creatinine 2x	Proteinuria	Other complication
17	18	32	56	53		-4		32	GVHD, CMV, adeno, rejection
18	7	31	27	31	28	14			GVHD
19									CMV, relapse
20	39								CMV
21	26	26	44	43	40	40	34	26	EBV, adeno, PTLD, MOF
22	102	63	86			27		32	CMV, relapse
23		24						24	GVHD, CMV
24	40	36							EBV, PTLD, relapse
25	34						27		CMV
26		46		78	56				CMV
27	48	27	90	90	28	48			GVHD, CMV, relapse
28									
29						65			GVHD, CMV
30	30								CMV
31	54				28				
32	29	15			28				
33	48	72	72	83	28	60		73	relapse

Patients are highlighted with blue, if any of the five different diagnostic criteria for TA-TMA was fulfilled.

CMV indicates cytomegalovirus; creatinin 2x, doubling of baseline pretransplantation creatinie level; EBV, Epstein-Barr virus; GVHD, graft-versus-host disease; LDH, lactate dehydrogenase (>2x upper limit of normal); MOF, multiple organ failure; PTLD, post-transplantation lymphoproliferative disorder.

Considering these five different diagnostic systems 2 of 33 subject met the BMT CTN (136), 7 of 33 the IWG criteria (135), 7 of 33 the diagnostic criteria of Cho et al. (121), 3 of 33 the diagnostic criteria of City of Hope (124) and 10 of 33 patients met the diagnostic criteria for TA-TMA proposed by Jodele et al.. (134) Altogether, incidence of TA-TMA was observed in the range of 6% (2 of 33) to 30% (10 of 33) depending on the diagnostic criteria applied. Figure 7 shows the cumulative incidence of TA-TMA with the five different diagnostic criteria. Seven out of the 10 patients with TA-TMA fulfilled at least 3 TA-TMA criteria of the various diagnostic systems. As marked in Table 4, all patients met the diagnostic criteria for TA-TMA proposed by Jodele et al., who met any of the other four diagnostic criteria. Three patients had normal haptoglobin level and normal kidney function (no doubling of serum creatinine) during the whole transplantation period and would be defined as TA-TMA only according to Jodele et al. and/ or City of Hope. (124, 134) All patients developed Coombs-negative hemolytic anemia, therefore patients fulfilled the IWG (135) and O-TMA (121) criteria on the same days after transplantation. TA-TMA was typically diagnosed on day 61 after transplantation (median, range, 16-98) according to Jodele et al.. (134) As presented on Figure 7, all TA-TMA cases occurred within the first 100 days after HSCT. Different times of diagnosis are detailed in Table 4 with the five different diagnostic systems. Surprisingly, grade I to II GVHD had almost the same incidence as TA-TMA after reduced toxicity conditioning regimen in our cohort.

Table 4

Diagnosis of TA-TMA with the Five Different Diagnostic Criteria. Numbers show the day after transplantation, when the diagnostic criteria of TA-TMA was fulfilled.

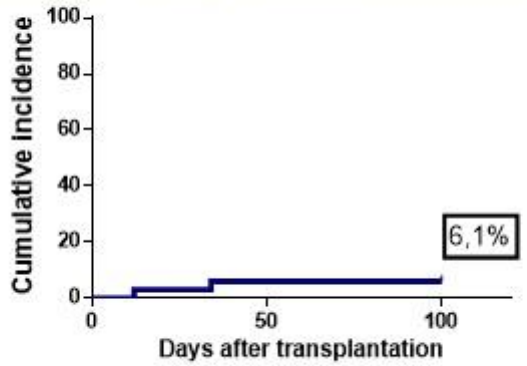
Patient code	Sex	HSCT indication	Age	Diagnosis of TMA				
				BMT CTN	IWG	O-TMA	City of Hope	Jodele et al.
5	**M**	Malignant	9y	**12**	**16**	**16**	**16**	**16**
7	**M**	Nonmalignant	4y		**83**	**83**		**83**
11	**M**	Malignant	7y		**31**	**31**		**31**
13	**M**	Nonmalignant	1y					**96**
15	**M**	Nonmalignant	10y					**65**
17	**F**	Nonmalignant	9y				**56**	**56**
18	**F**	Nonmalignant	4y		**31**	**31**		**31**
21	**M**	Nonmalignant	12y	**34**	**44**	**44**	**43**	**44**
27	**M**	Malignant	6y		**90**	**90**		**90**
33	**M**	Malignant	14y		**83**	**83**		**83**

BMT CTN indicates Blood and Marrow Transplant Clinical Trials Network; IWG, International Working Group; O-TMA, Overall Thrombotic Microangiopathy Grouping.

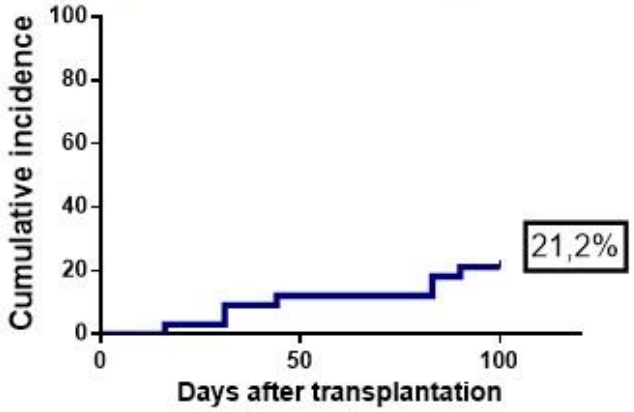
Evaluation of the different TMA activity markers revealed (Table 3), that typically elevated LDH, hypertension and proteinuria occurred earlier. Most often, patients with TA-TMA developed new onset anemia and trhombocytopenia after hypertension and elevated LDH. Acute kidney injury was observed only in 2 out of 10 patients, who also fulfilled the diagnostic criteria for TA-TMA according to Blood and Marrow Transplant Clinical Trials Network. (136) Interestingly, the other 8 patients had normal kidney function, but hypertension was detected on median 35 (range 4-67) day. In addition, as a high risk criteria proposed by Jodele et al., 4 of 10 patients had proteinuria during the study period, typically before the development of anemia and thrombocytopenia. All 10 patiens required again red blood cell transfusions, and 6 of 10 patients required thrombocyte transfusions. Severe neurological sympmtoms were not observed, 3 of 10 patients were apathetic at the diagnosis of TA-TMA. There was no severe gastrointestinal bleeding as a sign of intestinal TA-TMA during the study period.

In case of early signs of a developing TA-TMA, withdrawal or change of calcineurin inhibitors were the initial steps to control the process in our clinical practice. Calcineurin inhibitor was discontinued (n=5) or changed (n=5) by the treating physicians if hypertension, relapse, minimal residual disease or rapidly elevating LDH occurred. Defibrotide was used in one case for venooclusive disease therapy, UPN (unique patient number) 21 had ongoing TA-TMA with acute kidney injury, and died of multiple organ failure after post-transplantation lymphoproliferative disorder.

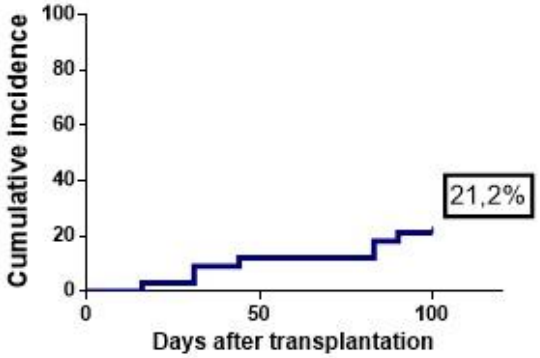
1) BMT CTN Toxicity Committee Consensus Definition

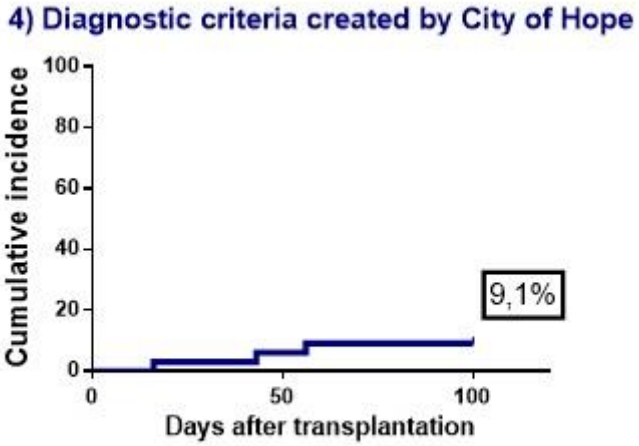


2) International Working Group



3) Overall Thrombotic Microangiopathy Grouping





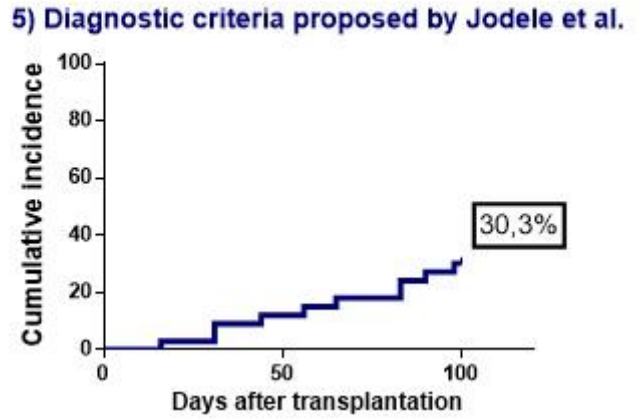


Figure 7

Cumulative Incidence of TA-TMA with the Five Different Diagnostic Criteria

BMT CTN indicates Blood and Marrow Transplant Clinical Trials Network.

4.3. Clinical Characteristics of Patients with TA-TMA

Tables 5 and 6 show the clinical and biomarker characteristics of patient groups with or without TA-TMA. To include every patient with TA-TMA, we used all available diagnostic criteria, and altogether 10 of 33 patients were defined as TA-TMA from mild to severe cases for further data evaluation. There was no association between TA-TMA and age, gender, transplant history, donor type, stem cell source, conditioning therapy, time of engraftment and viral infections (Table 5). However, TA-TMA was clearly associated with acute GVHD. TA-TMA occurred in 7 of 10 (70%) cases with GVHD, whereas only 4 of the 23 patients without TA-TMA developed GVHD (17%, $p<0.006$, Table 5). Viral infection was slightly more frequent in patients with TA-TMA compared with those without (6/10 vs. 11/23), but this difference was statistically not significant ($p=0.710$). However, double virus (CMV, EBV, or adenovirus) reactivation was registered for 5 of the 17 cases with viral infection, out of which 4 patients also developed TA-TMA. TA-TMA was preceded by acute GVHD in 3 of 10, by viral reactivation in 2 of 10, or by both in 4 of 10 cases.

Table 5.

Basic Demographic, Therapy and Transplantation-Related Complication Characteristics for the 33 Pediatric Allogeneic HSCT Patients

	Patients with TA-TMA (n=10)	**Patients without TA-TMA (n=23)**	**p value**
Age (y)	8.2 (1.8-14.2)	10.9 (2.5-17.4)	0.15
Male/ Female	8/2	15/8	0.36
Indication of transplantation			
Malignancy	4 (40%)	13 (57%)	0.628
Bone marrow failure	5 (50%)	9 (39%)	
Other (Immune deficiency or metabolic disease)	1 (10%)	1 (4%)	
Recent transplantations			
0	8 (80%)	20 (87%)	0.328 (for trend)
1	1 (10%)	3 (13%)	
2	1 (10%)	0 (0%)	
Donor type			
Related identical or haploidentical	4 (40%)	4 (17%)	0.205
Unrelated	6 (60%)	19 (83%)	
Stem cell source			
Bone marrow	7 (70%)	16 (70%)	0.961
PBSC	2 (20%)	4 (17%)	
Cord blood	1 (10%)	3 (13%)	
Conditioning therapy			
Myeloablative	7 (70%)	19 (83%)	0.646
Reduced intensity	3 (30%)	4 (17%)	
Engraftment			
Engraftment (day)	17 (17-21)	21 (19-25)	0.131
HSCT related complications			
Acute GVHD before day 100	7 (70%)	4 (17%)	**0.006**
GVHD before day 28	4 (40%)	1 (4%)	**0.019**
Viral infection before day 100	6 (60%)	11 (48%)	0.710
Viral infection before day 28	3 (30%)	3 (13%)	
None of the two	1 (10%)	10 (43%)	**0.015** (trend)
GVHD or viral reactivation	5 (50%)	11 (48%)	
GVHD and viral reactivation	4 (40%)	2 (9%)	
Relapse-related mortality	2 (20%)	5 (22%)	0.648
Transplantation-related mortality	1 (10%)	1 (4%)	0.520

Data presented are n (%) unless otherwise indicated. Bold typeface indicates statistical significance. PBSC indicates peripheral stem cell.

4.4. Longitudinal Analysis of Complement Activation During the First 100 Days after HSCT

Our first objective was to describe changes in complement activities, component-, regulator-, and activation product levels during the first 100 days after transplantation, and to identify associations between complement activation and TA-TMA (Table 6). Complement terminal pathway activation complex sC5b-9 level was the only parameter that showed significant changes of the measured complement parameters. A clear and significant association with TA-TMA was observed, with peak sC5b-9 levels on day 28 in the TA-TMA group (Table 6 and Figure 8). Baseline sC5b-9 levels did not differ in patients without (200 (144-266) ng/mL, median (interquartile range)), or with (208 (166- 271) ng/mL) subsequent TA-TMA. However on day 28, patients who later developed TA-TMA had sC5b-9 concentrations of 411 ng/mL (337-472, median, interquartile range), whereas patients without TA-TMA had 201 (185-290, $p<0.05$).

Table 6.

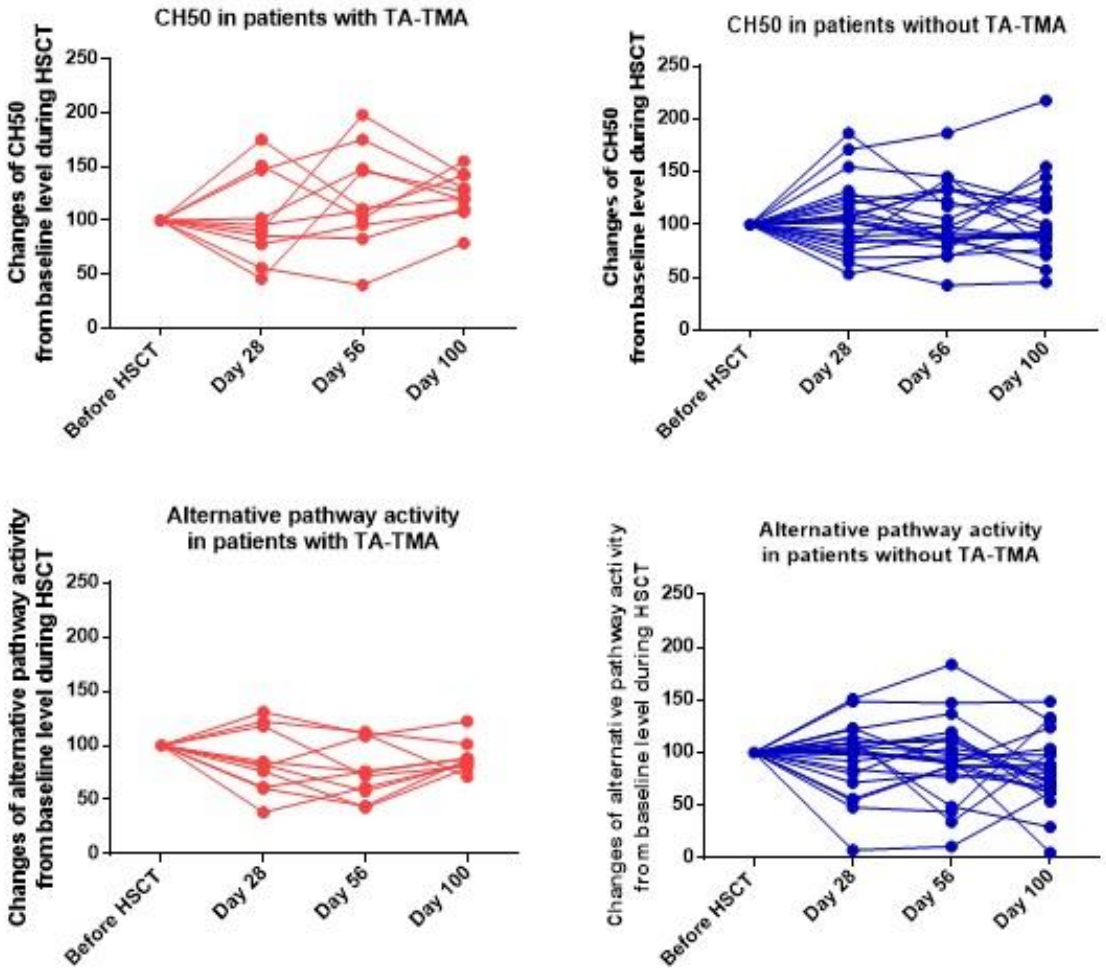
Complement Pathway Activities and Activation Product Levels at Different Time Points Before and After Transplantation

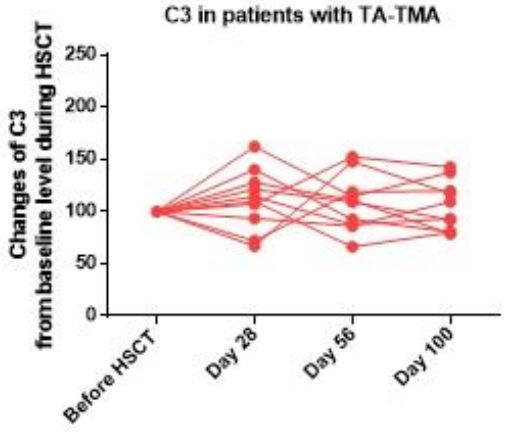
Parameter	Before HSCT	Day 28	Day 56	Day 100	p values
Classical pathway activity (CH50/mL)					
TA-TMA	59 (54-65)	58 (49-70)	71 (56-86)	75 (70-76)	ns
No TA-TMA	61 (56-74)	67 (57-79)	62 (52-77)	66 (60-68)	
Alternative pathway activity (%)					
TA-TMA	73 (62-82)	58 (48-70)	54 (44-77)	62 (60-70)	ns
No TA-TMA	81 (74-93)	82 (67-96)	78 (70-92)	64 (64-76)	
Complement C3 (mg/L)					
TA-TMA	1.39 (1.27-1.61)	1.73 (1.3-1.8)	1.5 (1.41-1.54)	1.5 (1.31-1.53)	ns
No TA-TMA	1.49 (1.3-1.66)	1.44 (1.23-1.79)	1.33 (1.12-1.5)	1.3 (1.2-1.3)	
Anaphylatoxin C3a (ng/mL)					
TA-TMA	86.3 (78.9-114.8)	161.4 (141.9-312.3)	102.9 (75.6-147.1)	93.1 (87.1-94.3)	ns
No TA-TMA	113.4 (92.7-164)	119.1 (88.3-238.3)	110.2 (77.5-176.7)	140 (75.4-190.5)	
C4d (ug/mL)					
TA-TMA	5.4 (3.2-7.2)	5.9 (3.7-6)	4.7 (4-6.2)	6.8 (4.9-8.1)	ns
No TA-TMA	5.3 (4.1-6.3)	6.0 (3.8-7.15)	5.1 (3-6.6)	3.7 (3.3-3.8)	
sC5b-9 (ng/mL)					
TA-TMA	208.4 (166-270.6)	410.7 (336.5-471.2)	324.1 (298.3-371.1)	323.5 (197.3-348.1)	**p=0.005 for TA-TMA; p=0.013 for time**
No TA-TMA	199.8 (143.6-265.5)	201.2 (184.8-290.3)	239.2 (191.8-381.2)	232 (204.9-232)	
ADAMTS13 activity (%)					
TA-TMA	110 (89-136)	87 (68-108)	92 (79-99)	102 (93-116)	ns
No TA-TMA	95 (79-120)	78 (63-92)	87 (78-119)	100 (89-103)	

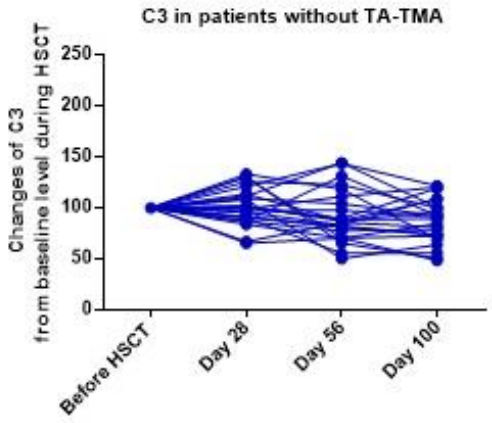
P values were obtained with two-way analysis of variance.
Data are presented as median (interquartile range). Bold typeface indicates statistical significance.
Complement normal values are shown in Methods.
ns indicates not significant.

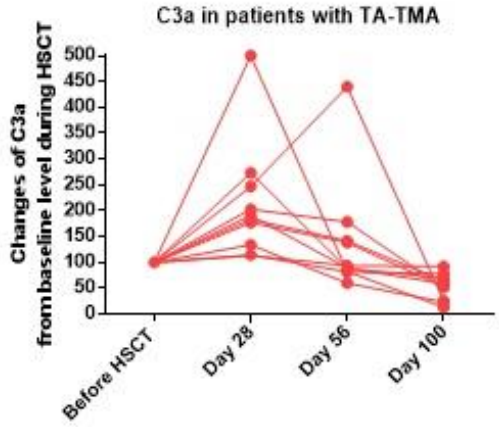
Importantly, 10 of 10 patients with later TA-TMA showed an early increase of sC5b-9 (Table 7 and Figure 8). On day 28, sC5b9 levels of 9 of 10 future TA-TMA patients exceeded 252 ng/mL, the upper level of reference range, and of 6/10 even 400 ng/mL, whereas among the remaining 23 patients 8 of 23 showed an increase of sC5b-9 by day 28 but the upper limit of the reference range was exceeded by only 5 of 23 patients (p=0.0004).

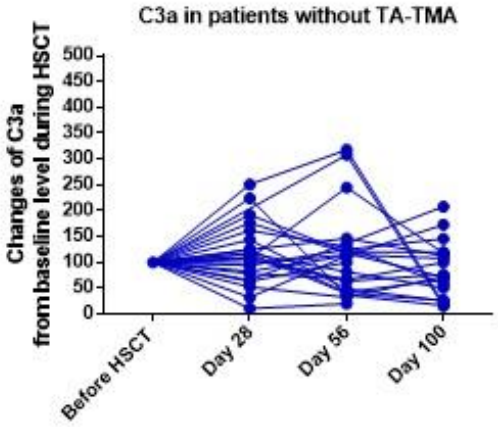
There was no significant association of TA-TMA with changes in classical and alternative pathway activities, C3, C4, C4d, and C3a concentrations, and ADAMTS13 activity. Figure 8 illustrates the changes of the complement parameters and ADAMTS13 activity from the pre-transplantation baseline level (100%) to day 28, day 56 and day 100.

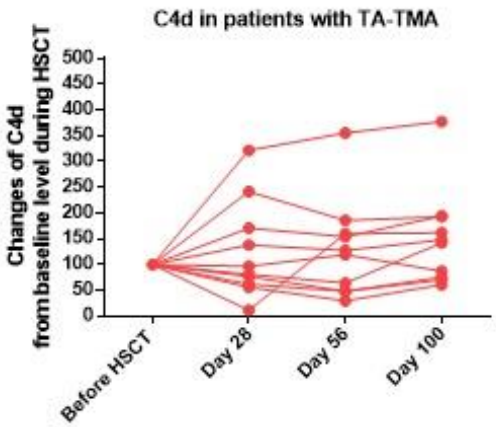


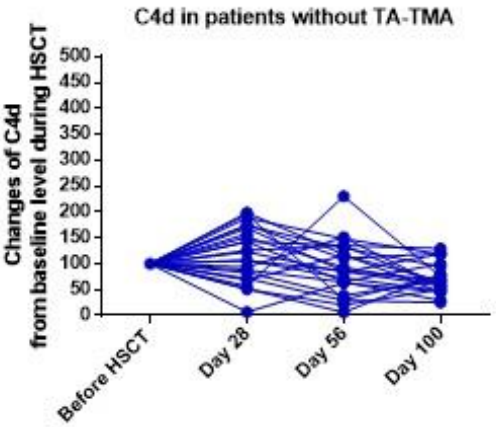

C3 in patients with TA-TMA
Changes of C3 from baseline level during HSCT
250
200
150
100
50
0
Before HSCT
Day 28
Day 56
Day 100


C3 in patients without TA-TMA
Changes of C3 from baseline level during HSCT
250
200
150
100
50
0
Before HSCT
Day 28
Day 56
Day 100


C3a in patients with TA-TMA
Changes of C3a from baseline level during HSCT
500
450
400
350
300
250
200
150
100
50
0
Before HSCT
Day 28
Day 56
Day 100


C3a in patients without TA-TMA
Changes of C3a from baseline level during HSCT
500
450
400
350
300
250
200
150
100
50
0
Before HSCT
Day 28
Day 56
Day 100


C4d in patients with TA-TMA
Changes of C4d from baseline level during HSCT
500
450
400
350
300
250
200
150
100
50
0
Before HSCT
Day 28
Day 56
Day 100


C4d in patients without TA-TMA
Changes of C4d from baseline level during HSCT
500
450
400
350
300
250
200
150
100
50
0
Before HSCT
Day 28
Day 56
Day 100

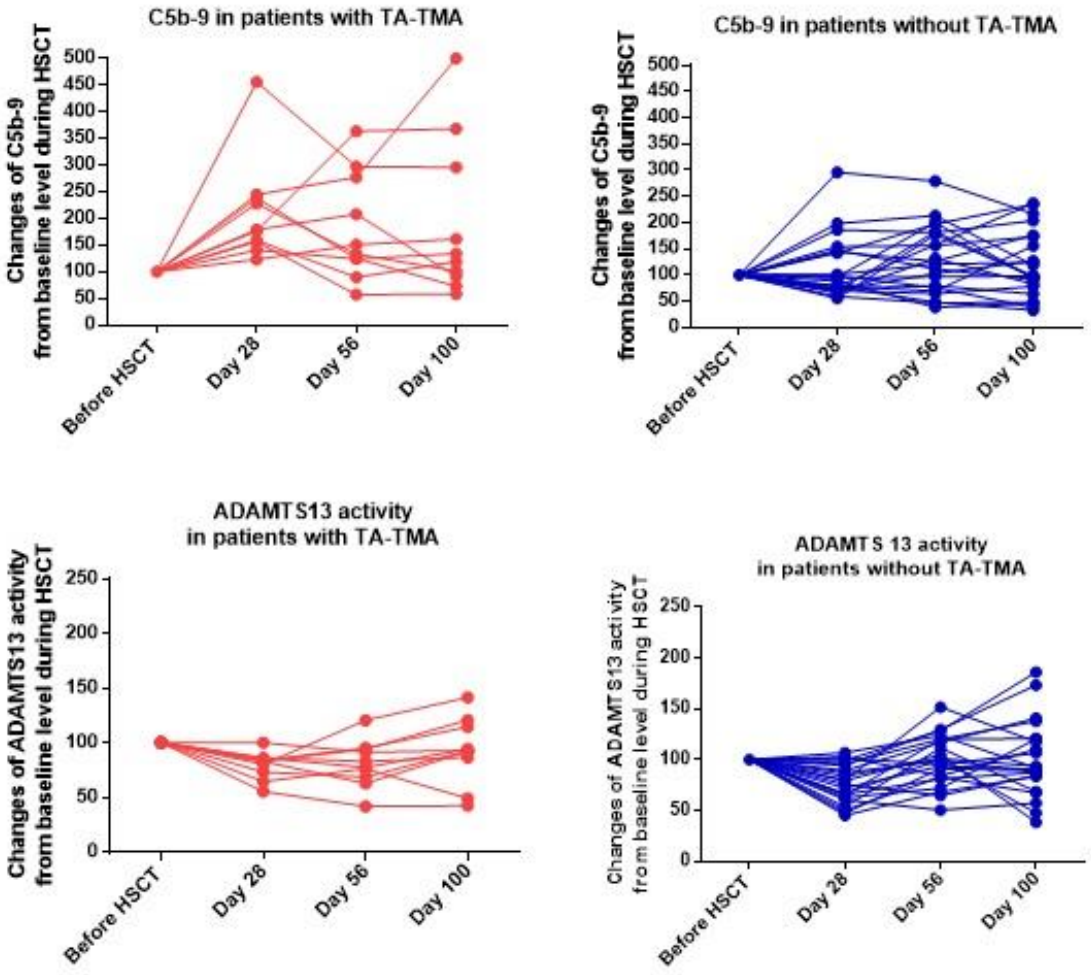


Figure 8

Changes of the Complement Parameters, Activation Product Levels and ADAMTS13 Activity from Baseline Pre-transplantation Level (100%) during the First 100 days after Transplantation in Patiens with or TMA and without TA-TMA.

CH50 indicates total complement activity; HSCT, hematopoietic stem cell transplantation; sC5b-9, solubile terminal pathway activation marker; TA-TMA, transplantation-associated thrombotic microangiopathy.

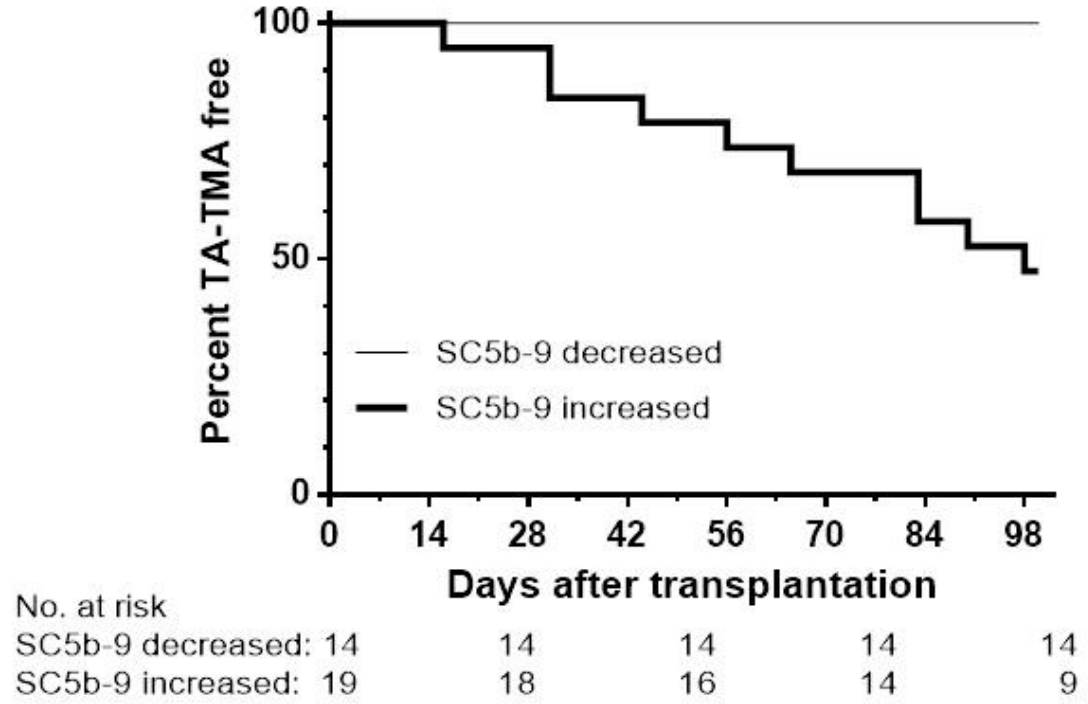


Figure 9.

Development of Thrombotic Microangiopathy after HSCT in Relation to Changes of Terminal Pathway Activation Marker sC5b-9 from Baseline to Day 28. Kaplan Meier plot showing percentage of TA-TMA event free patients, p=0.0016, log-rank test.

Next, we analyzed the relationship between early increase of sC5b-9 and engraftment and additional transplantation related complications (Table 7). Patients were stratified according to changes between baseline and day 28 sC5b-9 levels, and TA-TMA showed a clear and remarkable association with early increase of terminal pathway activation (Figure 9). Ten patients of 19, who had an early increase in sC5b-9, developed later TA-TMA, whereas none of the 14 patients without an increase of sC5b-9 developed TA-TMA. Acute GVHD, viral reactivation and/or infection, severe mucositis, fever, need for major analgetics or intensive care unit treatment, blood stream infection or transplant failure showed no significant association with early increase of sC5b-9 levels (Table 7).

Table 7.

Time of Engraftment and Transplantation- Related Complications of Patients with or without Early Elevation of sC5b-9

	sC5b-9 elevation from baseline to day 28 n=19	**No change or decrease of sC5b-9 from baseline to day 28 n=14**	**p value**
Engraftment day	21 (17-26)	21 (18-22)	ns
TA-TMA before day 100	10 (53%)	0 (0%)	**0.001**
TA-TMA before day 28	1 (5%)	0 (0%)	
Acute GVHD before day 100	9 (47%)	2 (14%)	ns
Acute GVHD before day 28	6 (32%)	1 (7%)	ns
Viral reactivation and/or new infection before day 100	11 (58%)	6 (43%)	ns
Viral reactivation and/or new infection before day 28	4 (21%)	2 (14%)	ns
Fever on day 28	6 (32%)	5 (36%)	ns
Severe mucositis	5 (26%)	3 (21%)	ns
Need of major analgetics during aplasia	14 (74%)	6 (43%)	ns
Blood stream infection	4 (21%)	3 (21%)	ns
Intensive care treatment	3 (16%)	3 (21%)	ns
Transplant failure before 100 days	5 (26%)	1 (7%)	ns

Data presented are median (interquartile range) or n (%). P values were obtained by Fisher's exact test. Bold typeface indicates statistical significance.

Intrigued by the clear association between TA-TMA and early increase of sC5b-9 levels we looked if early terminal pathway activation was associated with early laboratory or clinical markers of TA-TMA. As presented in Table 8, hypertension until day 28 was the only TMA activity marker that was significantly associated with early increase of sC5b-9 levels, whereas presence of decreased haptoglobin concentrations showed a tendency for association.

Table 8.

Activity Markers of TA-TMA with or without Early Elevation of sC5b-9 Until Day 28

	sC5b-9 elevation from baseline to day 28 n=19	**No change or decrease of sC5b-9 from baseline to day 28 n=14**	**p value**
Elevated LDH until day 28	7 (37%)	2 (14%)	ns
New onset anemia until day 28	1 (5%)	0 (0%)	ns
New onset thrombocytopenia until day 28	1 (5%)	0 (0%)	ns
Low haptoglobin on day 28	7 (37%)	1 (7%)	0.098
Fragmentocytes until day 28	8 (42%)	2 (14%)	ns
Proteinuria until day 28	0 (0%)	1 (7%)	ns
Hypertension until day 28	6 (32%)	0 (0%)	**0.027**

Data presented are n (%). P values were obtained by Fisher's exact test.
Bold typeface indicates statistical significance.
LDH indicates lactate dehydrogenase.

Finally, we analyzed if early increase of sC5b-9 levels may help clinical management of HSCT patients and calculated sensitivity and specificity values to predict development of TA-TMA. Early sC5b9 elevation was a 100% sensitive predictive marker for the later development of TA-TMA (10/10 patients with TA-TMA had early elevation of sC5b9, Figure 6), but it was less specific (10/19 patients with early sC5b9 increase had TA-TMA events translating to 61% specificity).

4.5. Patient Survival

Due to the high proportion of treosulfan-based conditioning regimens (24 of 33) in our cohort, the rate of significant early transplant- related complications was low, and non-relapse mortality did not associate with TA-TMA (Table 5). Overall survival after a median 2.6 (interquartile range, 1.5-2.9) year follow-up time was 24 of 33 (73%). Overall survival was 15 of 16 (94%) in nonmalignant patients. Accordingly, in patients with malignancy and with treosulfan- based myeloablative regimen, relapse rate was high, 7 of 17 patients died of relapse, and 1 of 17 patient in transplant-related mortality (TRM). TRM rate was low (2 of 33; 6%) after day 30. Two patients died due to TRM before day 30 without any signs-of TA-TMA, and were excluded from the complement measurements. One patient died on day 50 during the follow-up period of our study due to multiple organ failure during ongoing TA-TMA and post-transplantation lymphoproliferative disorder. This patient fulfilled all five diagnostic criteria of TA-TMA, and had acute kidney failure. Other cause of TRM was also due to PTLD, multiple organ failure, without any signs of TA-TMA. Seven of 10 patients (70%) with TA-TMA survived, compared with 17 of 23 (74%) patients without TA-TMA. Two patients, who were excluded for early (< day 30) non-relapse mortality, had only available pre-transplantation sC5b-9 levels, and showed no clinical signs of developing TA-TMA.

Overall survival in our cohort for those with or without TA-TMA is the same with the diagnostic criteria of the IWG (4/7 vs. 20/26; p=0.35), with the widely used diagnostic criteria proposed by Cho et. al. (4/7 vs. 20/26; p=0.35) and according to Jodele et al. (7 of 10 vs. 17 of 23; ns). As peresented on Figure 10, early elevation of sC5b-9 showed no association with mortality.

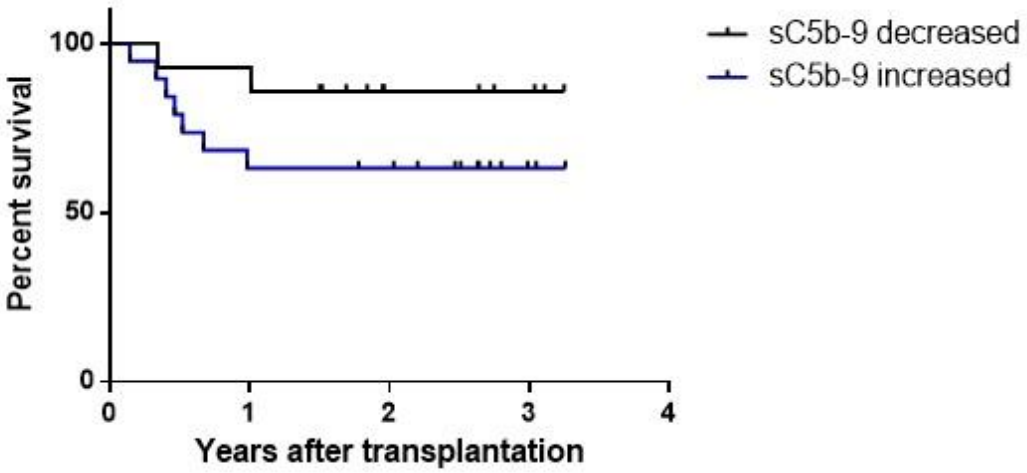


Figure 10

Survival in Patient with or without the Eary Elevation of sC5b-9. Kaplan Meier plot showing percentage of survival. p= 0.1478, log-rank test

5. DISCUSSION

The aim of my dissertation was to provide observational data about the incidence of TA-TMA in our pediatric cohort with different diagnostic criteria, to observe the complement biomarker changes after HSCT, and to investigate the possible link between the development of TA-TMA and complement activation. Our results demonstrate a remarkably strong association between the presence of an early increase of terminal complement pathway activation and later development of TA-TMA, translating to 100% sensitivity to predict the occurrence of this complication.

Clinical manifestation of TA-TMA may include thrombocytopenia, Coombs negative microangiopathic hemolysis, schistocytes on preiferial blood smear, acute renal failure, mental status changes, pulmonary complications such as severe pulmonary arterial hypertension, and dysfunction of other organs causing multiple organ failure. (134, 168, 173) Interestingly, these severe clinical manifestations are not evident in all patients with TA-TMA, as a number of them resolve after withdrawal of calcineurin inhibitors. (125) In addition, such manifestations may be seen in patients with other HSCT-related complications, including GVHD, CMV infection and sepsis. (85-87) These facts highlight the need of reliable diagnostic criteria and predictive markers of TA-TMA.

Therefore, we found five different diagnostic criteria of TA-TMA in literature (121, 124, 134-136), and aimed to compare the incidence of TA-TMA in our pediatric cohort. Overall, 10 of 33 patients fulfilled any of the diagnostic criteria of TA-TMA. All patients fulfilled the recent published modification of TA-TMA criteria, that included markers of complement activation, proteinuria, and hypertension, and excluded decreased haptoglobin (134). We observed similar incidence of TA-TMA (30%), as described by Jodele et. al. (134). Similarly to Jodele's observations (138), elevated LDH, and as clinical symptoms systolic hypertension and proteinuria occurred earlier in our patients with TA-TMA. Most often, patients with TA-TMA developed new onset anemia and trhombocytopenia only after hypertension and elevated LDH. These observation would help to guide physicians to recognise the developing TA-TMA in time, before any signs of end-organ damage. On the other hand, patients developed TA-TMA later, typically on day 61.

In contrast, acute kidney injury, as a sign of organ injury was observed only in 2 of 10 patients, who also fulfilled the diagnostic criteria for TA-TMA according to Blood and Marrow Transplant Clinical Trials Network. (136) The lower incidence of TA-TMA in our cohort with BMT CTN criteria (136), requiring concurrent organ dysfunction for the diagnosis of TA-TMA, is comparable to other results in adult cohorts. (121, 174, 175) In addition, incidence of TA-TMA (21%) in our pediatric cohort is similar to the previously described incidence (19%) in the adult unit in our hospital. (128), using the O-TMA diagnostic criteria proposed by Cho et al. (121). Three patients had normal haptoglobin levels during the whole study period, despite of the changes of other TMA activity markers. The diversity among diagnostic criteria based on clinical symptoms may lead to difficulty to understanding clinically important cases that would require immediate intensification of treatment. Altogether, we identified 10 patients with TA-TMA from mild to severe cases. After reduced toxicity conditioning regimen, in majority of patients TA-TMA was a mild, self-limiting form, without any sign of organ damage, and resolved after the withdrawal or change of calcineurin inhibitor.

Our pediatric data suggest, that incidence and time of the diagnosis may depend on different diagnostic criteria used. To identify potential complement biomarkers of TA-TMA development, we aimed to define every patients with TA-TMA form mild to severe cases. To include every patient with TA-TMA for complement measurements, we used all available diagnostic criteria, and altogether 10 patients were defined as TA-TMA from mild to severe cases.

After the accumulation of evidences about the involvement of complement activation in the pathogenesis of TA-TMA, and with the advent of novel therapeutic possibilities including complement inhibitory therapy, the clinical interest and awareness of early diagnosis and possible prediction of TA-TMA largely increased. Quickly and easily measurable predictive biomarkers or surrogate markers of TA-TMA could be optimized for prediction or rapid diagnosis, the current list of such markers include soluble adhesion molecules, serum neutrophil extracellular traps and complement activation marker levels. (138, 169-171) Moreover, the modified Ham test has been used to prove complement activation in vitro. (176) Our data further support the potential utility of terminal pathway activation marker sC5b-9 in this setting, as this

is an easily measureable and well-controlled marker that has recently been included in the international external quality control program of diagnostic complement laboratories. (177)

The observation of the association between the early increase of sC5b-9 and later development of TA-TMA is intriguing. Jodele et al. observed elevated median 333 ng/mL concentration of sC5b-9 at the time of TMA diagnosis, and median 201 ng/mL in those without this complication. (138) Our results (Table 6) are very similar to these values and provide independent confirmation of these original observations. Important to note, that complement terminal pathway activation complex sC5b-9 level was the only parameter that showed significant changes of the measured complement parameters. There was no significant association of TA-TMA with changes in classical and alternative pathway activities, other complement activation products, and ADAMTS13 activity.

Furthermore, Jodele et al. described that the earliest sign of TA-TMA was hypertension. (138) In accord with this observation, it is interesting to note that hypertension was the only TMA activity marker that showed significant association with early increase of sC5b-9. Taken together, we provide independent confirmation that increased terminal pathway activation is characteristic of the development of TA-TMA and is associated with its earliest activity marker, hypertension.

We found that early (i.e. from baseline to day 28) increase of sC5b-9, before the development of most of the complications, can predict later TA-TMA (typically diagnosed on day 61). This observation is especially interesting in light of the recently described possible model for the mechanistic link between endothelial injury and TA-TMA. (171) Gloude et al. proposed that chemotherapy, radiation, and infections lead to endothelial injury during the early stages of HSCT resulting in IL-8 release, neutrophil activation and release of neutrophil extracellular traps (NET). These processes may jointly lead to complement activation, and because NETs are known to activate complement (178), their amount and presence may determine the extent of complement activation. Our results about the remarkable strong association between early increase of complement activation (as reflected by sC5b-9 levels) and later development of TA-TMA are in line with this model. Therefore, due to the high proportion of treosulfan-based conditioning regimens (24 of 33) in our cohort the rate of significant early

transplantation- related complications was reasonably low, (179, 180, 181) and nonrelapse mortality did not associate with TA-TMA (Table 5). After more aggressive or toxic conditioning regimens more severe TA-TMA cases could be observed, and further studies would be needed to validate these observations on adult and on other conditioning regimen cohorts. TA-TMA defining signs or activity markers, such as anemia, thrombocytopenia, kidney function, or proteinuria, can be influenced by other transplant-related complications and/or side effects of drugs. Therefore, our observations (if validated in an independent cohort) on a predictive marker, analyzed early before and during engraftment period, when most of the previously mentioned complications are not present, may bear clinically valuable information.

The dissertation has strengths and limitations. An important strength of our study is that it was a consecutive and prospective study measuring complement profile and activation in the whole cohort, independently from any HSCT complications. This design allowed us to register longitudinal alterations of complement markers, and link changes with later complications. Furthermore, we used multiple, well-controlled and reliable assays to directly measure and follow complement activation. The pediatric cohort with reduced toxicity treosulfan-based conditioning therapy and the sample size of 33 are the main limitations of our results, making it important to note that results should be considered as preliminary, until independent confirmation is published, and it is possible that different results will be observed in adult cohorts and with more aggressive or toxic conditioning regimens.

6. CONCLUSION

My Ph.D. dissertation focuses on the better understanding of transplantation-associated thrombotic microangiopathy.

The conclusions of the dissertation are summerized below:

(1) Based on our observations, thrombotic microangiopathy is a frequent complication of hematopoietic stem cell transplantation. Incidence of TA-TMA was observed in the range of 6% (2 of 33) to 30% (10 of 33) depending on the diagnostic criteria applied. Altogether, we identified 10 of 33 patients, from mild to severe TA-TMA cases in our pediatric cohort. TA-TMA was diagnosed typically on day 61 (range, 16-98) after HSCT. Incidence of TA-TMA and time of diagnosis is influenced by the diagnostic criteria used.

(2) TA-TMA occurred typically after GVHD and/or viral infection or reactivation. TA-TMA was preceded by acute GVHD in 3 of 10, by viral reactivation in 2/ of 10, or by both in 4 of 10 cases. After reduced toxicity conditioning regimen, in majority of patients TA-TMA was a mild, self-limiting form, without any sign of organ damage, and resolved after the withdrawal or change of calcineurin inhibitor.

(3) Complement terminal pathway activation complex sC5b-9 level was the only parameter that showed significant changes of the measured complement parameters. There was no significant association of TA-TMA with changes in classical and alternative pathway activities, C3, C4, C4d, and C3a concentrations, and ADAMTS13 activity.

(4) We report results of the first consecutive, longitudinal study on complement activation during the first 100 days after HSCT, and describe the remarkable close association between early increase of terminal pathway activation marker levels and later development of TA-TMA. We found that early (i.e. from

baseline to day 28) increase of sC5b-9, before the development of most of the complications, can predict later TA-TMA. Increase in sC5b-9 concentration had 100% sensitivity and 61% specificity for TA-TMA in our pediatric cohort. Baseline sC5b-9 levels did not differ in patients without (200 (144-266) ng/mL, median (interquartile range)), or with (208 (166- 271) ng/mL) subsequent TA-TMA, however, on day 28 significant differences were observed (201 (185-290) ng/mL versus 411 (337-471) ng/mL, p=0.004). Importantly, all 10 patients with TMA showed increase in sC5b-9 level from baseline level to day 28, whereas in patients without TMA the same tendency was observed for only 9/23 (p=0.031).

(5) Due to the high proportion of treosulfan-based conditioning regimens (24 of 33) in our cohort the rate of significant early transplantation- related complications was reasonably low, and nonrelapse mortality did not associate with TA-TMA. If our observations can be validated in an independent cohort with more aggressive or toxic conditioning regimens, the measurement of complement activation marker sC5b-9 may be useful, in order to identify patients with early increase of sC5b-9, as a sign of increased risk of developing later TA-TMA. Early monitoring of complement activation marker sC5b-9 and activity markers of TA-TMA would guide physicians to facilitate subsequent therapy decisions, on time. Further studies enrolling higher number of patients are necessary to determine if terminal pathway activation is an independent predictor of TMA development after HSCT.

The main conclusions of the dissertation can be summerized in the following three points:

(1) Based on our observations, thrombotic microangiopathy is a frequent complication of hematopoietic stem cell transplantation. Development of TA-TMA occurred in 30% of our patients, typically after GVHD and/or viral reactivation. In majority of patients TA-TMA was a mild, self-limiting form, without any sign of organ damage. Incidence of TA-TMA and time of diagnosis is influenced by the diagnostic criteria used.

(2) Complement terminal pathway activation complex sC5b-9 level was the only parameter that showed significant changes of the measured complement parameters. No additional complement parameters were closely associated with the development of TA-TMA.

(3) Early (i.e. from baseline to day 28) increase of sC5b-9, before the development of most of the complications, was predictive for later development of TA-TMA, and should therefore be considered as an alarming sign necessitating a careful monitoring of all TA-TMA activity markers. If our observations can be validated in an independent cohort, early monitoring of complement activation marker sC5b-9 and activity markers of TA-TMA would guide physicians to facilitate subsequent therapy decisions, on time.

7. SUMMERY

Transplantation-associated thrombotic microangiopathy (TA-TMA) is a challenging complication following hematopoietic stem cell transplantation (HSCT). Over the past several decades, the cause of TA-TMA has remained unknown, and its prediction is largely unresolved. Therefore, during my Ph.D. studies we aimed to identify patients with TA-TMA using five different diagnostic TA-TMA criteria, and analyze changes of complement profile after HSCT in order to identify potential markers of TA-TMA development. Thirty- three consecutive pediatric patients (9.6 ± 4.4 years old) were included in this study who underwent allogeneic HSCT due to malignant (n=17) or non malignant (n=16) indications. Five different TA-TMA diagnostic criteria were applied and all important clinical and laboratory parameters of TA-TMA activity were registered. Complement pathway activities, components and terminal pathway activation marker (sC5b-9) levels were systematically measured before transplantation and on day 28, 56 and 100 after HSCT. During the first 100 days after HSCT, 1 out of 33 patients patient died (day 50, multiple organ failure), whereas altogether 10 subjects met the criteria for TA-TMA, typically on day 61 (median, range 16-98). TA-TMA was preceded by acute graft-versus-host disease in 3 of 10, by viral reactivation in 2 of 10, or by both in 4 of 10 cases. After reduced toxicity conditioning regimen, in majority of patients TA-TMA was a mild, self-limiting form, without any sign of organ damage, and resolved after the withdrawal or change of calcineurin inhibitor. We found that early (i.e. from baseline to day 28) increase of sC5b-9, before the development of most of the complications, can predict later TA-TMA. Increase in sC5b-9 concentration had 100% sensitivity and 61% specificity for TA-TMA in our pediatric cohort. Importantly, 10 of 10 patients with later TA-TMA showed an early increase of sC5b-9. No additional complement parameters were closely associated with the development of TA-TMA.

Incidence of TA-TMA is influenced by the diagnostic criteria used. Altogether, development of TA-TMA occurred in 30% of our patients. Early raise of sC5b-9 activation marker was predictive for later development of TA-TMA, and should therefore be considered as an alarming sign necessitating a careful monitoring of all TA-TMA activity markers. Further studies enrolling higher number of patients are necessary to determine if terminal pathway activation is an independent predictor of TA-TMA.

8. ÖSSZEFOGLALÁS

Az őssejt-transzplantációhoz társult thromboticus microangiopathia (TA-TMA) felismerése és kezelése nagy kihívást jelent őssejt-transzplantáció (tx) után. Pontos patomechanizmusa és prediktív tényezői azonban még nagyrészt ismeretlenek. Ph.D. munkám során célul tűztük ki a TA-TMA-s betegek definiálását öt különböző diagnosztikus kritériumrendszer segítségével. Emellett vizsgáltuk a komplementprofil változásainak szerepét a TA-TMA kialakulásában, és célul tűztük ki a változások prediktív tényezőként való értékelését. Prospektíven harminc-három (9.6 ± 4.4 év) gyermeket vizsgáltunk, akik malignus (n=17) és nem malignus indikáció (n=16) miatt estek át allogén őssejt-tx-en. A TA-TMA-t öt diagnosztikus kritériumrendszer segítségével definiáltuk, valamint monitoriztuk a TA-TMA aktivitását jelző klinikai és laboratóriumi paramétereket. A tx előre meghatározott időpontjaiban (kondícionáló kezelés előtt, +28., +56. és +100. napon) meghatároztuk a komplement paramétereket (komplement út aktivitások, komponenesek, szolúbilis terminális komplement út aktivációs komplex (sC5b-9)). A tx utáni első 100 napban 1/33 beteg meghalt (50. nap, sokszervi elégtelenség), míg összesen tipikusan a +61. napon (medián, 16-98) 10/33 beteg teljesítette a TA-TMA diagnosztikus kritériumait. Jellemzően a TA-TMA graft-versus host betegség és/vagy vírus reaktiváció után jelentkezett. A csökkentett toxicitású kondícionáló kezelés után a betegek nagy részénél a TA-TMA egy enhye, önlimitáló formában zajlott, szervkárosodásra utaló jelek nélkül, és calcineurin inhibitorok elhagyása vagy váltása után a betegek nagy részénél oldódott. Az sC5b-9 korai emelkedése (kiindulási szintről a +28. napra), a legtöbb szövődmény kialakulása előtt, előre jelezte a későbbi TA-TMA-t. A sC5b-9 szint korai emelkedése 100%-os szenzitiviással és 61%-os specifitással prediktálta a TA-TMA kialakulását. Továbbá mind a 10/10 később TA-TMA-s betegnél korai sC5b-9 szint emelkedést detektáltunk. Más komplement paraméter nem mutatott szorosabb összefüggést a TA-TMA kialakulásával.

A TA-TMA incidenciáját befolyásolja, hogy mely diagnosztikus kritériumrendszer alapján definiáljuk. Összesen a betegek 30%-ánál alakult ki TA-TMA. A sC5b-9 aktivációs marker korai emelkedése prediktív tényezőként értékelhető a TA-TMA kialakulása szempontjából, és felhívhatja a figyelmet a többi TA-TMA-t jelző aktivációs termék szoros monitorizálására. További nagy esetszámú vizsgálatok szükségesek annak igazolására, hogy a sC5b-9 korai emelkedése a TA-TMA független prediktora.

9. REFERENCES

1. Okamoto S. (2017) Current indication for hematopoietic cell transplantation in adults. Hematol Oncol Stem Cell Ther, 10: 178-183.
2. Thomas E, Storb R, Clift RA, Fefer A, Johnson FL, Neiman PE, Lerner KG, Glucksberg H and Buckner CD. (1975) Bone-marrow transplantation (first of two parts). N Engl J Med, 292: 832-43.
3. Thomas ED, Storb R, Clift RA, Fefer A, Johnson L, Neiman PE, Lerner KG, Glucksberg H and Buckner CD. (1975) Bone-marrow transplantation (second of two parts). N Engl J Med, 292: 895-902.
4. Laberko A and Gennery AR. (2018) Clinical considerations in the hematopoietic stem cell transplant management of primary immunodeficiencies. Expert Rev Clin Immunol, 14: 297-306.
5. Locatelli F and Strahm B. (2018) How I treat myelodysplastic syndromes of childhood. Blood, 131: 1406-1414.
6. Eisengart JB, Rudser KD, Tolar J, Orchard PJ, Kivisto T, Ziegler RS, Whitley CB and Shapiro EG. (2013) Enzyme replacement is associated with better cognitive outcomes after transplant in Hurler syndrome. J Pediatr, 162: 375-380.
7. Shenoy S. (2007) Has stem cell transplantation come of age in the treatment of sickle cell disease? Bone Marrow Transplant, 40: 813-821.
8. La Nasa G, Giardini C, Argiolu F, Locatelli F, Arras M, De Stefano P, Ledda A, Pizzati A, Sanna MA, Vacca A, Lucarelli G and Contu L. (2002) Unrelated donor bone marrow transplantation for thalassemia: the effect of extended haplotypes. Blood, 99: 4350-4356.
9. Ljungman P, Bregni M, Brune M, Cornelissen J, de Witte T, Dini G, Einsele H, Gaspar HB, Gratwohl A, Passweg J, Peters C, Rocha V, Saccardi R, Schouten H, Sureda A, Tichelli A, Velardi A and Niederwieser D. (2010) Allogeneic and autologous transplantation for haematological diseases, solid tumours and immune disorders: current practice in Europe 2009. Bone Marrow Transplant, 45: 219-234.
10. Tyndall A and Saccardi R. (2005) Haematopoietic stem cell transplantation in the treatment of severe autoimmune disease: results from phase I/II studies, prospective randomized trials and future directions. Clin Exp Immunol, 141: 1-9.

11. Singh AK and McGuirk JP. (2016) Allogeneic Stem Cell Transplantation: A Historical and Scientific Overview. Cancer Res, 76: 6445-6451.

12. LaBelle JL and Cunningham JM. (2013) Stem cell transplant as an immunomodulatory tool for children with hematologic malignancies. American Society of Clinical Oncology educational book American Society of Clinical Oncology Meeting, 33: 347.

13. Stary J and Hrusak O. (2016) Recent advances in the management of pediatric acute lymphoblastic leukemia. F1000Res, 5: 2635.

14. Mehta PA and Davies SM. (2008) Allogeneic transplantation for childhood ALL. Bone Marrow Transplant, 41: 133-139.

15. Umeda K, Hiramatsu H, Kawaguchi K, Iwai A, Mikami M, Nodomi S, Saida S, Heike T, Ohomori K and Adachi S. (2016) Impact of pretransplant minimal residual disease on the post-transplant outcome of pediatric acute lymphoblastic leukemia. Pediatr Transplant, 20: 692-696.

16. Schweitzer J, Zimmermann M, Rasche M, von Neuhoff C, Creutzig U, Dworzak M, Reinhardt D and Klusmann JH. (2015) Improved outcome of pediatric patients with acute megakaryoblastic leukemia in the AML-BFM 04 trial. Ann Hematol, 94: 1327-1336.

17. Rasche M, Zimmermann M, Borschel L, Bourquin JP, Dworzak M, Klingebiel T, Lehrnbecher T, Creutzig U, Klusmann JH and Reinhardt D. (2018) Successes and challenges in the treatment of pediatric acute myeloid leukemia: a retrospective analysis of the AML-BFM trials from 1987 to 2012. Leukemia, Article in Press.

18. Gross TG, Hale GA, He W, Camitta BM, Sanders JE, Cairo MS, Hayashi RJ, Termuhlen AM, Zhang MJ, Davies SM and Eapen M. (2010) Hematopoietic stem cell transplantation for refractory or recurrent non-Hodgkin lymphoma in children and adolescents. Biol Blood Marrow Transplant, 16: 223-230.

19. Yaniv I, Krauss AC, Beohou E, Dalissier A, Corbacioglu S, Zecca M, Afanasyev BV, Berger M, Diaz MA, Kalwak K, Sedlacek P, Varotto S, Peters C and Bader P. (2018) Second Hematopoietic Stem Cell Transplantation for Post-Transplantation Relapsed Acute Leukemia in Children: A Retrospective EBMT-PDWP Study. Biol Blood Marrow Transplant, Article in Press.

20. Dalle JH and Peffault de Latour R. (2016) Allogeneic hematopoietic stem cell transplantation for inherited bone marrow failure syndromes. Int J Hematol, 103: 373-379.

21. Bacigalupo A. (2017) How I treat acquired aplastic anemia. Blood, 129: 1428-1436.

22. Bacigalupo A, Giammarco S and Sica S. (2016) Bone marrow transplantation versus immunosuppressive therapy in patients with acquired severe aplastic anemia. Int J Hematol, 104: 168-174.

23. Niemeyer CM and Baumann I. (2008) Myelodysplastic syndrome in children and adolescents. Semin Hematol, 45: 60-70.

24. Schmidt RE, Grimbacher B and Witte T. (2017) Autoimmunity and primary immunodeficiency: two sides of the same coin? Nat Rev Rheumatol, 14: 7-18.

25. Gaspar HB, Qasim W, Davies EG, Rao K, Amrolia PJ and Veys P. (2013) How I treat severe combined immunodeficiency. Blood, 122:3749-3758.

26. Boelens JJ, Orchard PJ and Wynn RF. (2014) Transplantation in inborn errors of metabolism: current considerations and future perspectives. Br J Haematol, 167: 293-303.

27. Tanaka A, Okuyama T, Suzuki Y, Sakai N, Takakura H, Sawada T, Tanaka T, Otomo T, Ohashi T, Ishige-Wada M, Yabe H, Ohura T, Suzuki N, Kato K, Adachi S, Kobayashi R, Mugishima H and Kato S. (2012) Long-term efficacy of hematopoietic stem cell transplantation on brain involvement in patients with mucopolysaccharidosis type II: a nationwide survey in Japan. Mol Genet Metab, 107: 513-20.

28. Eisengart JB, Rudser KD, Tolar J, Orchard PJ, Kivisto T, Ziegler RS, Whitley CB and Shapiro EG. (2013) Enzyme replacement is associated with better cognitive outcomes after transplant in Hurler syndrome. J Pediatr, 162: 375-380

29. Riezzo I, Pascale N, La Russa R, Liso A, Salerno M and Turillazzi E. (2017) Donor Selection for Allogenic Hemopoietic Stem Cell Transplantation: Clinical and Ethical Considerations. Stem Cells Int, 2017: 5250790.

30. Kindwall-Keller TL and Ballen KK. (2017) Alternative Donor Graft Sources for Adults with Hematologic Malignancies: A Donor for All Patients in 2017! Oncologist, 22: 1125-1134.

31. Thomas ED. (1994) Stem cell transplantation: past, present and future. Stem cells, 12: 539-544.

32. Altaf SY, Apperley JF and Olavarria E. (2016) Matched unrelated donor transplants-State of the art in the 21st century. Semin Hematol, 53: 221-229.

33. Rocha V and Locatelli F. (2008) Searching for alternative hematopoietic stem cell donors for pediatric patients. Bone Marrow Transplant, 41: 207-214.

34. Vriesendorp HM. (2003) Aims of conditioning. Exp Hematol, 31: 844-854.

35. Jethava YS, Sica S, Savani B, Socola F, Jagasia M, Mohty M, Nagler A and Bacigalupo A. (2017) Conditioning regimens for allogeneic hematopoietic stem cell transplants in acute myeloid leukemia. Bone Marrow Transplant, 52: 1504-1511.

36. Bacigalupo A, Ballen K, Rizzo D, Giralt S, Lazarus H, Ho V, Apperley J, Slavin S, Pasquini M, Sandmaier BM, Barrett J, Blaise D, Lowski R and Horowitz M. (2009) Defining the intensity of conditioning regimens: working definitions. Biol Blood Marrow Transplant, 15: 1628-1633.

37. Cutler C and Ballen K. (2009) Reduced-intensity conditioning and umbilical cord blood transplantation in adults. Bone Marrow Transplant, 44: 667-671.

38. Mohty M, Labopin M, Volin L, Gratwohl A, Socie G, Esteve J, Tabrizi R, Nagler A and Rocha V. (2010) Reduced-intensity versus conventional myeloablative conditioning allogeneic stem cell transplantation for patients with acute lymphoblastic leukemia: a retrospective study from the European Group for Blood and Marrow Transplantation. Blood, 116: 4439-4443.

39. Boztug H, Sykora KW, Slatter M, Zecca M, Veys P, Lankester A, Cant A, Skinner R, Wachowiak J, Glogova E, Potschger U and Peters C. (2016) European Society for Blood and Marrow Transplantation Analysis of Treosulfan Conditioning Before Hematopoietic Stem Cell Transplantation in Children and Adolescents With Hematological Malignancies. Pediatr Blood Cancer, 63: 139-148.

40. Vertes AA. (2015) The potential of cytotherapeutics in hematologic reconstitution and in the treatment and prophylaxis of graft-versus-host disease. Chapter I: current practice and remaining unmet medical needs. Regen Med, 10: 331-343.

41. Servais S, Beguin Y and Baron F. (2013) Emerging drugs for prevention of graft failure after allogeneic hematopoietic stem cell transplantation. Expert Opin Emerg Drugs, 18 :173-192.

42. Martino R, Romero P, Subira M, Bellido M, Altes A, Sureda A, Brunet S, Badell I, Cubells J and Sierra J. (1999) Comparison of the classic Glucksberg criteria and the IBMTR Severity Index for grading acute graft-versus-host disease following HLA-identical sibling stem cell transplantation. International Bone Marrow Transplant Registry. Bone Marrow Transplant, 24: 283-287.

43. Kharfan-Dabaja M, Mhaskar R, Reljic T, Pidala J, Perkins JB, Djulbegovic B and Kumar A. (2014) Mycophenolate mofetil versus methotrexate for prevention of graft-versus-host disease in people receiving allogeneic hematopoietic stem cell transplantation. Cochrane Database Syst Rev, 2014:Cd010280.

44. Gandhi S, Kulasekararaj AG, Mufti GJ and Marsh JC. (2013) Allogeneic stem cell transplantation using alemtuzumab-containing regimens in severe aplastic anemia. Int J Hematol, 97: 573-580.

45. Mussetti A, Greco R, Peccatori J and Corradini P. (2017) Post-transplant cyclophosphamide, a promising anti-graft versus host disease prophylaxis: where do we stand? Expert Rev Hematol, 10: 479-492.

46. Lutz M and Mielke S. (2016) New perspectives on the use of mTOR inhibitors in allogeneic haematopoietic stem cell transplantation and graft-versus-host disease. Br J Clin Pharmacol, 82: 1171-1179.

47. Atiq F, Hameli E, Broers AEC, Doorduijn JK, Van Gelder T, Andrews LM, Koch BCP, Versmissen J and de Winter BCM. (2018) Converting cyclosporine A from intravenous to oral administration in hematopoietic stem cell transplant recipients and the role of azole antifungals. Eur J Clin Pharmacol, Article in Press.

48. Hamilton BK, Rybicki L, Dean R, Majhail NS, Haddad H, Abounader D, Hanna R, Sobecks R, Duong H, Hill BT, Copelan E, Bolwell B and Kalaycio M. (2015) Cyclosporine in combination with mycophenolate mofetil versus methotrexate for graft versus host disease prevention in myeloablative HLA-identical sibling donor allogeneic hematopoietic cell transplantation. Am J Hematol, 90: 144-148.

49. Holtick U, Frenzel LP, Shimabukuro-Vornhagen A, Theurich S, Claasen J, Scheid C, von Bergwelt-Baildon M, Frohlich H, Wendtner CM and Chemnitz JM. (2015) CD4+ T cell counts reflect the immunosuppressive state of CD4 helper cells in patients after allogeneic stem cell transplantation. Ann Hematol, 94: 129-137.

50. Ogonek J, Kralj Juric M, Ghimire S, Varanasi PR, Holler E, Greinix H and Weissinger E. (2016) Immune Reconstitution after Allogeneic Hematopoietic Stem Cell Transplantation. Front Immunol, 7: 507.

51. Seggewiss R and Einsele H. (2010) Immune reconstitution after allogeneic transplantation and expanding options for immunomodulation: an update. Blood, 115: 3861-3868.

52. Servais S, Hannon M, Peffault de Latour R, Socie G and Beguin Y. (2017) Reconstitution of adaptive immunity after umbilical cord blood transplantation: impact on infectious complications. Stem Cell Investig, 4: 40.

53. Admiraal R, de Koning CCH, Lindemans CA, Bierings MB, Wensing AMJ, Versluys AB, Wolfs TFW, Nierkens S and Boelens JJ. (2017) Viral reactivations and associated outcomes in the context of immune reconstitution after pediatric hematopoietic cell transplantation. The J Allergy Clin Immunol, 140: 1643-1650.

54. Sahin U, Toprak SK, Atilla PA, Atilla E and Demirer T. (2016) An overview of infectious complications after allogeneic hematopoietic stem cell transplantation. J Infect Chemother, 22: 505-514.

55. Omrani AS and Almaghrabi RS. (2017) Complications of hematopoietic stem cell transplantation: Bacterial infections. Hematol Oncol Stem Cell Ther, 10: 228-232.

56. Omrani AS and Almaghrabi RS. (2017) Complications of hematopoietic stem transplantation: Fungal infections. Hematol Oncol Stem Cell Ther, 10: 239-244.

57. Ferreira AM, Moreira F, Guimaraes T, Spadao F, Ramos JF, Batista MV, Filho JS, Costa SF and Rocha V. (2018) Epidemiology, risk factors and outcomes of multi-drug resistant bloodstream infections in hematopoietic stem cell transplant recipients: importance of previous gut colonisation. J Hosp Infect, Article in Press.

58. Locatelli F, Bertaina A, Bertaina V and Merli P. (2016) Cytomegalovirus in hematopoietic stem cell transplant recipients - management of infection. Expert Rev Hematol, 9: 1093-1105.

59. Chan ST and Logan AC. (2017) The clinical impact of cytomegalovirus infection following allogeneic hematopoietic cell transplantation: Why the quest for meaningful prophylaxis still matters. Blood reviews, 31: 173-183.

60. Liu L, Zhang X and Feng S. (2018) Epstein-Barr Virus-Related Post-Transplantation Lymphoproliferative Disorders After Allogeneic Hematopoietic Stem Cell Transplantation. Biol Blood Marrow Transplant, Article in Press.

61. Morscio J and Tousseyn T. (2016) Recent insights in the pathogenesis of post-transplantation lymphoproliferative disorders. World J Transplant, 6: 505-516.

62. Kosulin K, Berkowitsch B, Matthes S, Pichler H, Lawitschka A, Potschger U, Fritsch G and Lion T. (2018) Intestinal Adenovirus Shedding Before Allogeneic Stem Cell Transplantation Is a Risk Factor for Invasive Infection Post-transplant. EbioMedicine, 28: 114-119.

63. Feuchtinger T, Lang P and Handgretinger R. (2007) Adenovirus infection after allogeneic stem cell transplantation. Leuk Lymphoma, 48: 244-255.

64. Levine JE, Logan B, Wu J, Alousi AM, Ho V, Bolanos-Meade J and Weisdorf D. (2010) Graft-versus-host disease treatment: predictors of survival. Biol Blood Marrow Transplant, 16: 1693-1699.

65. Blazar BR, Murphy WJ and Abedi M. (2012) Advances in graft-versus-host disease biology and therapy. Nat Rev Immunol, 12: 443-458.

66. van Bekkum DW, de Vries MJ and van der Waay D. (1967) Lesions characteristic of secondary disease in germfree heterologous radiation chimeras. J Natl Cancer Inst, 38: 223-231.

67. Billingham RE. (1966) The biology of graft-versus-host reactions. Harvey Lect, 62: 21-78.

68. Cahn JY, Klein JP, Lee SJ, Milpied N, Blaise D, Antin JH, Leblond V, Ifrah N, Jouet JP, Loberiza F, Ringden O, Barrett AJ, Horowitz MM and Socie G. (2005) Prospective evaluation of 2 acute graft-versus-host (GVHD) grading systems: a joint Societe Francaise de Greffe de Moelle et Therapie Cellulaire (SFGM-TC), Dana Farber Cancer Institute (DFCI), and International Bone Marrow Transplant Registry (IBMTR) prospective study. Blood, 106: 1495-500.

69. Flinn AM and Gennery AR. (2017) Treatment of Pediatric Acute Graft-versus-Host Disease-Lessons from Primary Immunodeficiency? Front Immunol, 8: 328.

70. Hill L, Alousi A, Kebriaei P, Mehta R, Rezvani K and Shpall E. (2018) New and emerging therapies for acute and chronic graft versus host disease. Ther Adv Hematol, 9: 21-46.

71. Holtan SG and MacMillan ML. (2016) A risk-adapted approach to acute GVHD treatment: are we there yet? Bone Marrow Transplant, 51: 172-175.

72. Dignan FL, Clark A, Amrolia P, Cornish J, Jackson G, Mahendra P, Scarisbrick JJ, Taylor PC, Hadzic N, Shaw BE and Potter MN. (2012) Diagnosis and management of acute graft-versus-host disease. Br J Haematol, 158: 30-45.

73. Wolff D, Ayuk F, Elmaagacli A, Bertz H, Lawitschka A, Schleuning M, Meyer RG, Gerbitz A, Hilgendorf I, Hildebrandt GC, Edinger M, Klein S, Halter J, Mousset S, Holler E and Greinix HT. (2013) Current practice in diagnosis and treatment of acute graft-versus-host disease: results from a survey among German-Austrian-Swiss hematopoietic stem cell transplant centers. Biol Blood Marrow Transplant, 19: 767-776.

74. Deeg HJ. (2007) How I treat refractory acute GVHD. Blood, 109: 4119-4126.

75. Martin PJ, Rizzo JD, Wingard JR, Ballen K, Curtin PT, Cutler C, Litzow MR, Nieto Y, Savani BN, Schriber JR, Shaughnessy PJ, Wall DA and Carpenter PA. (2012) First- and second-line systemic treatment of acute graft-versus-host disease: recommendations of the American Society of Blood and Marrow Transplantation. Biol Blood Marrow Transplant, 18: 1150-1163.

76. Dalle JH and Giralt SA. (2016) Hepatic Veno-Occlusive Disease after Hematopoietic Stem Cell Transplantation: Risk Factors and Stratification, Prophylaxis, and Treatment. Biol Blood Marrow Transplant, 22: 400-409.

77. Salat C, Holler E, Reinhardt B, Kolb HJ, Seeber B, Ledderose G, Mittermueller J, Duell T, Wilmanns W and Hiller E. (1994) Parameters of the fibrinolytic system in patients undergoing BMT: elevation of PAI-1 in veno-occlusive disease. Bone Marrow Transplant, 14: 747-750.

78. Jevtic D, Zecevic Z, Veljkovic D, Dopsaj V, Radojicic Z and Elezovic I. (2011) Veno-occlusive disease in pediatric patients after hematopoietic stem cell transplantation: relevance of activated coagulation and fibrinolysis markers and natural anticoagulants. J Pediatr Hematol Oncol, 33: 227-234.

79. Ho VT, Revta C and Richardson PG. (2008) Hepatic veno-occlusive disease after hematopoietic stem cell transplantation: update on defibrotide and other current investigational therapies. Bone Marrow Transplant, 41: 229-237.

80. Richardson PG, Smith AR, Triplett BM, Kernan NA, Grupp SA, Antin JH, Lehmann L, Shore T, Iacobelli M, Miloslavsky M, Hume R, Hannah AL, Nejadnik B

and Soiffer RJ. (2017) Defibrotide for Patients with Hepatic Veno-Occlusive Disease/Sinusoidal Obstruction Syndrome: Interim Results from a Treatment IND Study. Biol Blood Marrow Transplant, 23: 997-1004.

81. Qureshi A, Marshall L and Lancaster D. (2008) Defibrotide in the prevention and treatment of veno-occlusive disease in autologous and allogeneic stem cell transplantation in children. Pediatr Blood Cancer, 50: 831-832.

82. Chalandon Y, Roosnek E, Mermillod B, Newton A, Ozsahin H, Wacker P, Helg C and Chapuis B. (2004) Prevention of veno-occlusive disease with defibrotide after allogeneic stem cell transplantation. Biol Blood Marrow Transplant, 10: 347-354.

83. Gloude NJ, Jodele S, Teusink-Cross A, Grimley M, Davies SM, Lane A and Myers KC. (2018) Combination of High-Dose Methylprednisolone and Defibrotide for Veno-Occlusive Disease in Pediatric Hematopoietic Stem Cell Transplant Recipients. Biol Blood Marrow Transplant, 24: 91-95.

84. George JN, Li X, McMinn JR, Terrell DR, Vesely SK and Selby GB. (2004) Thrombotic thrombocytopenic purpura-hemolytic uremic syndrome following allogeneic HPC transplantation: a diagnostic dilemma. Transfusion, 44: 294-304.

85. Jodele S, Dandoy CE, Myers KC, El-Bietar J, Nelson A, Wallace G and Laskin BL. (2016) New approaches in the diagnosis, pathophysiology, and treatment of pediatric hematopoietic stem cell transplantation-associated thrombotic microangiopathy. Transfus Apher Sci, 54: 181-190.

86. Obut F, Kasinath V and Abdi R. (2016) Post-bone marrow transplant thrombotic microangiopathy. Bone Marrow Transplant, 51: 891-897.

87. Holbro A and Passweg JR. (2015) Management of hemolytic anemia following allogeneic stem cell transplantation. Hematology Am Soc Hematol Educ Program., 2015: 378-384.

88. Ferreira VP, Pangburn MK and Cortes C. (2010) Complement control protein factor H: the good, the bad, and the inadequate. Mol Immunol, 47: 2187-2197.

89. Walport MJ. (2001) Complement. First of two parts. N Engl J Med, 344: 1058-1066.

90. Nonaka M. (2014) Evolution of the complement system. Subcell Biochem, 80: 31-43.

91. Nesargikar PN, Spiller B and Chavez R. (2012) The complement system: history, pathways, cascade and inhibitors. Eur J Microbiol Immunol, 2: 103-111.
92. Brodsky RA. (2015) Complement in hemolytic anemia. Blood, 126: 2459-2465.
93. Sakari Jokiranta T, Viklicky O, Al Shorafa S, Coppo R, Gasteyger C, Macia M, Pankratenko T, Shenoy M, Soylemezoglu O, Tsimaratos M, Wetzels J and Haller H. (2017) Differential diagnosis of thrombotic microangiopathy in nephrology. BMC nephrology, 18: 324.
94. Ricklin D and Cines DB. (2013) TMA: beware of complements. Blood, 122: 1997-1999.
95. Wong EKS and Kavanagh D. (2018) Diseases of complement dysregulation-an overview. Semin Immunopathol, 40: 49-64.
96. Kavanagh D, Goodship TH and Richards A. (2013) Atypical hemolytic uremic syndrome. Semin Nephrol, 33: 508-530.
97. Besbas N, Karpman D, Landau D, Loirat C, Proesmans W, Remuzzi G, Rizzoni G, Taylor CM, Van de Kar N and Zimmerhackl LB. (2006) A classification of hemolytic uremic syndrome and thrombotic thrombocytopenic purpura and related disorders. Kidney Int, 70: 423-431.
98. Prohaszka Z. (2008) [Haemolytic uremic syndrome and thrombotic thrombocytopenic purpura: classification based on molecular etiology and review of recent developments in diagnostics]. Orv Hetil, 149: 1251-1261.
99. Loirat C, Fakhouri F, Ariceta G, Besbas N, Bitzan M, Bjerre A, Coppo R, Emma F, Johnson S, Karpman D, Landau D, Langman CB, Lapeyraque AL, Licht C, Nester C, Pecoraro C, Riedl M, van de Kar NC, Van de Walle J, Vivarelli M and Fremeaux-Bacchi V. (2016) An international consensus approach to the management of atypical hemolytic uremic syndrome in children. Pediatr Nephrol, 31: 15-39.
100. Moschcowitz E. (1952) An acute febrile pleiochromic anemia with hyaline thrombosis of the terminal arterioles and capillaries; an undescribed disease. Am J Med, 13: 567-569.
101. Furlan M, Robles R, Galbusera M, Remuzzi G, Kyrle PA, Brenner B, Krause M, Scharrer I, Aumann V, Mittler U, Solenthaler M and Lammle B. (1998) von Willebrand factor-cleaving protease in thrombotic thrombocytopenic purpura and the hemolytic-uremic syndrome. N Engl J Med, 339: 1578-1584.

102. Tsai HM and Lian EC. (1998) Antibodies to von Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura. N Engl J Med, 339: 1585-1594.

103. Kremer Hovinga JA, Heeb SR, Skowronska M and Schaller M. (2018) Pathophysiology of thrombotic thrombocytopenic purpura and hemolytic uremic syndrome.: J Thromb Haemost, 16: 618-629.

104. Bendapudi PK, Hurwitz S, Fry A, Marques MB, Waldo SW, Li A, Sun L, Upadhyay V, Hamdan A, Brunner AM, Gansner JM, Viswanathan S, Kaufman RM, Uhl L, Stowell CP, Dzik WH and Makar RS. (2017) Derivation and external validation of the PLASMIC score for rapid assessment of adults with thrombotic microangiopathies: a cohort study. Lancet Haematol, 4: 157-164.

105. Lotta LA, Wu HM, Mackie IJ, Noris M, Veyradier A, Scully MA, Remuzzi G, Coppo P, Liesner R, Donadelli R, Loirat C, Gibbs RA, Horne A, Yang S, Garagiola I, Musallam KM and Peyvandi F. (2012) Residual plasmatic activity of ADAMTS13 is correlated with phenotype severity in congenital thrombotic thrombocytopenic purpura. Blood, 120: 440-448.

106. Stone JH. (1998) HELLP syndrome: hemolysis, elevated liver enzymes, and low platelets. Jama, 280: 559-562.

107. Abildgaard U and Heimdal K. (2013) Pathogenesis of the syndrome of hemolysis, elevated liver enzymes, and low platelet count (HELLP): a review. Eur J Obstet Gynecol Reprod Biol, 166: 117-123.

108. Vaught AJ, Gavriilaki E, Hueppchen N, Blakemore K, Yuan X, Seifert SM, York S and Brodsky RA. (2016) Direct evidence of complement activation in HELLP syndrome: A link to atypical hemolytic uremic syndrome. Exp Hematol, 44: 390-398.

109. Vaught AJ, Braunstein EM, Jasem J, Yuan X, Makhlin I, Eloundou S, Baines AC, Merrill SA, Chaturvedi S, Blakemore K, Sperati CJ and Brodsky RA. (2018) Germline mutations in the alternative pathway of complement predispose to HELLP syndrome. JCI Insight, Article in Press.

110. Kavanagh D and Goodship T. Genetics and complement in atypical HUS. (2010) Pediatr Nephrol, 25: 2431-2442.

111. Siegler RL. (1995) The hemolytic uremic syndrome. Pediatr Clin North Am, 42: 1505-1529.

112. Noris M, Bresin E, Mele C and Remuzzi G. Genetic Atypical Hemolytic-Uremic Syndrome. In: M. P. Adam, H. H. Ardinger, R. A. Pagon, S. E. Wallace, L. J. H. Bean, K. Stephens and A. Amemiya, eds. GeneReviews Seattle: University of Washington, Seattle, ınitial Posting: November 16, 2007; Last Update: June 9, 2016. Ebook.

113. Zhang T, Lu J, Liang S, Chen D, Zhang H, Zeng C, Liu Z and Chen H. (2016) Comprehensive Analysis of Complement Genes in Patients with Atypical Hemolytic Uremic Syndrome. Am J Nephrol, 43:160-169.

114. Weintraub L, Ahluwalia M, Dogra S, Uehlinger J, Skversky A and Vasovic L. (2014) Management of streptococcal pneumoniae-induced hemolytic uremic syndrome: a case report. Clin Nephrol Case Stud, 2:9-17.

115. Copelovitch L and Kaplan BS. (2010) Streptococcus pneumoniae--associated hemolytic uremic syndrome: classification and the emergence of serotype 19A. Pediatrics, 125: 174-182.

116. Cochran JB, Panzarino VM, Maes LY and Tecklenburg FW. (2004) Pneumococcus-induced T-antigen activation in hemolytic uremic syndrome and anemia. Pediatr Nephrol, 19: 317-321.

117. Huang DT, Chi H, Lee HC, Chiu NC and Huang FY. (2006) T-antigen activation for prediction of pneumococcus-induced hemolytic uremic syndrome and hemolytic anemia. Pediatr Infect Dis J, 25:608-610.

118. Adrovic A, Canpolat N, Caliskan S, Sever L, Kiykim E, Agbas A and Baumgartner MR. (2016) Cobalamin C defect-hemolytic uremic syndrome caused by new mutation in MMACHC. Pediatr Int, 58: 763-765.

119. Beck BB, van Spronsen F, Diepstra A, Berger RM and Komhoff M. (2017) Renal thrombotic microangiopathy in patients with cblC defect: review of an under-recognized entity. Pediatr Nephrol, 32: 733-741.

120. Kavanagh D, Goodship TH and Richards A. (2006) Atypical haemolytic uraemic syndrome. Br Med Bull, 77-78: 5-22.

121. Cho BS, Yahng SA, Lee SE, Eom KS, Kim YJ, Kim HJ, Lee S, Min CK, Cho SG, Kim DW, Lee JW, Min WS and Park CW. (2010) Validation of recently proposed consensus criteria for thrombotic microangiopathy after allogeneic hematopoietic stem-cell transplantation. Transplantation, 90: 918-926.

122. Laskin BL, Goebel J, Davies SM and Jodele S. (2011) Small vessels, big trouble in the kidneys and beyond: hematopoietic stem cell transplantation-associated thrombotic microangiopathy. Blood, 118: 1452-1462.

123. Powles RL, Clink HM, Spence D, Morgenstern G, Watson JG, Selby PJ, Woods M, Barrett A, Jameson B, Sloane J, Lawler SD, Kay HE, Lawson D, McElwain TJ and Alexander P. (1980) Cyclosporin A to prevent graft-versus-host disease in man after allogeneic bone-marrow transplantation. Lancet, 1:327-329.

124. Garcia-Martin P, Alarcon-Payer C, Lopez-Fernandez E, Moratalla L, Romero A, Sainz J, Rios R and Jurado M. (2015) Transplantation-Associated Thrombotic Microangiopathy in Patients Treated With Sirolimus and Cyclosporine as Salvage Therapy for Graft-Versus-Host Disease. Ann Pharmacother, 49: 986-994.

125. Gavriilaki E, Sakellari I, Anagnostopoulos A and Brodsky RA. (2017) Transplant-associated thrombotic microangiopathy: opening Pandora's box. Bone Marrow Transplant, 52: 1355-1360.

126. Shayani S, Palmer J, Stiller T, Liu X, Thomas SH, Khuu T, Parker PM, Khaled SK, Forman SJ and Nakamura R. (2013) Thrombotic microangiopathy associated with sirolimus level after allogeneic hematopoietic cell transplantation with tacrolimus/sirolimus-based graft-versus-host disease prophylaxis. Biol Blood Marrow Transplant, 19: 298-304.

127. Labrador J, Lopez-Corral L, Lopez-Godino O, Vazquez L, Cabrero-Calvo M, Perez-Lopez R, Diez-Campelo M, Sanchez-Guijo F, Perez-Lopez E, Guerrero C, Alberca I, Del Canizo MC, Perez-Simon JA, Gonzalez-Porras JR and Caballero D. (2014) Risk factors for thrombotic microangiopathy in allogeneic hematopoietic stem cell recipients receiving GVHD prophylaxis with tacrolimus plus MTX or sirolimus. Bone Marrow Transplant, 49: 684-690.

128. Balassa K, Andrikovics H, Remenyi P, Batai A, Bors A, Kiss KP, Szilvasi A, Rajczy K, Inotai D, Gopcsa L, Lengyel L, Barta A, Reti M, Tordai A and Masszi T. (2015) The potential role of HLA-DRB1*11 in the development and outcome of haematopoietic stem cell transplantation-associated thrombotic microangiopathy. Bone Marrow Transplant, 50: 1321-1325.

129. Ye Y, Zheng W, Wang J, Hu Y, Luo Y, Tan Y, Shi J, Zhang M and Huang H. (2017) Risk and prognostic factors of transplantation-associated thrombotic

microangiopathy in allogeneic haematopoietic stem cell transplantation: a nested case control study. Hematol Oncol, 35: 821-827.

130. Verbiest A, Pirenne J and Dierickx D. (2014) De novo thrombotic microangiopathy after non-renal solid organ transplantation. Blood Rev, 28: 269-279.

131. Caires RA, Marques ID, Repizo LP, Sato VA, Carmo LP, Machado DJ, de Paula FJ, Nahas WC and David-Neto E. (2012) De novo thrombotic microangiopathy after kidney transplantation: clinical features, treatment, and long-term patient and graft survival. Transplant Proc, 44: 2388-2390.

132. Scheinberg P and Young NS. (2012) How I treat acquired aplastic anemia. Blood, 120: 1185-1196.

133. Shah SR, Altaf A, Arshad MH, Mari A, Noorani S, Saeed E, Mevawalla AA, Haq ZU and Faquih ME. (2015) Use of Cyclosporine Therapy in Steroid Resistant Nephrotic Syndrome (SRNS): A Review. Glob J Health Sci, 8: 136-141.

134. Jodele S, Laskin BL, Dandoy CE, Myers KC, El-Bietar J, Davies SM, Goebel J and Dixon BP. (2015) A new paradigm: Diagnosis and management of HSCT-associated thrombotic microangiopathy as multi-system endothelial injury. Blood Rev, 29: 191-204.

135. Ruutu T, Barosi G, Benjamin RJ, Clark RE, George JN, Gratwohl A, Holler E, Iacobelli M, Kentouche K, Lammle B, Moake JL, Richardson P, Socie G, Zeigler Z, Niederwieser D and Barbui T. (2007) Diagnostic criteria for hematopoietic stem cell transplant-associated microangiopathy: results of a consensus process by an International Working Group. Haematologica, 92: 95-100.

136. Ho VT, Cutler C, Carter S, Martin P, Adams R, Horowitz M, Ferrara J, Soiffer R and Giralt S. (2005) Blood and marrow transplant clinical trials network toxicity committee consensus summary: thrombotic microangiopathy after hematopoietic stem cell transplantation. Biol Blood Marrow Transplant, 11: 571-575.

137. Jodele S, Licht C, Goebel J, Dixon BP, Zhang K, Sivakumaran TA, Davies SM, Pluthero FG, Lu L and Laskin BL. (2013) Abnormalities in the alternative pathway of complement in children with hematopoietic stem cell transplant-associated thrombotic microangiopathy. Blood, 122: 2003-2007.

138. Jodele S, Davies SM, Lane A, Khoury J, Dandoy C, Goebel J, Myers K, Grimley M, Bleesing J, El-Bietar J, Wallace G, Chima RS, Paff Z and Laskin BL. (2014)

Diagnostic and risk criteria for HSCT-associated thrombotic microangiopathy: a study in children and young adults. Blood, 124: 645-653.

139. Tsai HM. (2013) Untying the knot of thrombotic thrombocytopenic purpura and atypical hemolytic uremic syndrome. Am J Med 126: 200-209.

140. Jodele S, Hirsch R, Laskin B, Davies S, Witte D and Chima R. (2013) Pulmonary arterial hypertension in pediatric patients with hematopoietic stem cell transplant-associated thrombotic microangiopathy. Biol Blood Marrow Transplant, 19: 202-207.

141. Lerner D, Dandoy C, Hirsch R, Laskin B, Davies SM and Jodele S. (2014) Pericardial effusion in pediatric SCT recipients with thrombotic microangiopathy. Bone Marrow Transplant, 49 :862-863.

142. El-Bietar J, Warren M, Dandoy C, Myers KC, Lane A, Wallace G, Davies SM and Jodele S. (2015) Histologic Features of Intestinal Thrombotic Microangiopathy in Pediatric and Young Adult Patients after Hematopoietic Stem Cell Transplantation. Biol Blood Marrow Transplant, 21: 1994-2001.

143. Crowell KR, Patel RA, Fluchel M, Lowichik A, Bryson S and Pohl JF. (2013) Endoscopy in the diagnosis of intestinal graft-versus-host disease: is lower endoscopy with biopsy as effective in diagnosis as upper endoscopy combined with lower endoscopy? Pediatr Blood Cancer, 60:1798-800.

144. Johansson JE, Nilsson O and Stotzer PO. (2015) Colonoscopy and Sigmoidoscopy are Equally Effective for the Diagnosis of Colonic Acute Graft-versus-Host Disease in Patients with Diarrhea after Allogeneic Stem Cell Transplantation: A Prospective Controlled Trial. Biol Blood Marrow Transplant, 21: 2086-2090.

145. Yamada-Fujiwara M, Miyamura K, Fujiwara T, Tohmiya Y, Endo K, Onishi Y, Ishizawa K, Kameoka J, Ito M and Harigae H. (2012) Diagnosis of intestinal graft-versus-host disease and thrombotic microangiopathy after allogeneic stem cell transplantation. Tohoku J Exp Med, 227: 31-37.

146. Boyce S, Eren E, Lwaleed BA and Kazmi RS. (2015) The Activation of Complement and Its Role in the Pathogenesis of Thromboembolism. Semin Thromb Hemost, 41: 665-672.

147. Vercellotti GM, Moldow CF and Jacob HS. (2012) Complement, oxidants, and endothelial injury: how a bedside observation opened a door to vascular biology. J Clin Invest, 122: 3044-3045.

148. Sacks T, Moldow CF, Craddock PR, Bowers TK and Jacob HS. (1978) Oxygen radicals mediate endothelial cell damage by complement-stimulated granulocytes. An in vitro model of immune vascular damage. J Clin Invest, 61: 1161-1167.

149. Jodele S, Zhang K, Zou F, Laskin B, Dandoy CE, Myers KC, Lane A, Meller J, Medvedovic M, Chen J and Davies SM. (2016) The genetic fingerprint of susceptibility for transplant-associated thrombotic microangiopathy. Blood, 127: 989-996.

150. Kim SS, Patel M, Yum K and Keyzner A. (2015) Hematopoietic stem cell transplant-associated thrombotic microangiopathy: review of pharmacologic treatment options. Transfusion, 55: 452-458.

151. Lapeyraque AL, Malina M, Fremeaux-Bacchi V, Boppel T, Kirschfink M, Oualha M, Proulx F, Clermont MJ, Le Deist F, Niaudet P and Schaefer F. (2011) Eculizumab in severe Shiga-toxin-associated HUS. N Engl J Med, 364: 2561-2563.

152. Chapin J, Weksler B, Magro C and Laurence J. (2012) Eculizumab in the treatment of refractory idiopathic thrombotic thrombocytopenic purpura. Br J Haematol, 157: 772-774.

153. Hillmen P, Young NS, Schubert J, Brodsky RA, Socie G, Muus P, Roth A, Szer J, Elebute MO, Nakamura R, Browne P, Risitano AM, Hill A, Schrezenmeier H, Fu CL, Maciejewski J, Rollins SA, Mojcik CF, Rother RP and Luzzatto L. (2006) The complement inhibitor eculizumab in paroxysmal nocturnal hemoglobinuria. N Engl J Med, 355: 1233-1243.

154. Brodsky RA, Young NS, Antonioli E, Risitano AM, Schrezenmeier H, Schubert J, Gaya A, Coyle L, de Castro C, Fu CL, Maciejewski JP, Bessler M, Kroon HA, Rother RP and Hillmen P. (2008) Multicenter phase 3 study of the complement inhibitor eculizumab for the treatment of patients with paroxysmal nocturnal hemoglobinuria. Blood, 111: 1840-1847.

155. Kose O, Zimmerhackl LB, Jungraithmayr T, Mache C and Nurnberger J. (2010) New treatment options for atypical hemolytic uremic syndrome with the complement inhibitor eculizumab. Semin Thromb Hemost, 36: 669-372.

156. Ebrahem R, Kadhem S and Truong Q. (2017) Treatment of Atypical Hemolytic-Uremic Syndrome in the Era of Eculizumab. Cureus, 9: e1111.

157. Schmidtko J, Peine S, El-Housseini Y, Pascual M and Meier P. (2013) Treatment of atypical hemolytic uremic syndrome and thrombotic microangiopathies: a focus on eculizumab. Am J Kidney Dis, 61: 289-299.

158. Fernandez C, Lario A, Fores R and Cabrera R. (2015) Eculizumab Treatment in a Patient with Hematopoietic Stem Cell Transplantation-Associated Thrombotic Microangiopathy and Steroid-Refractory Acute Graft Versus Host Disease. Hematol Rep, 7: 6107.

159. Peffault de Latour R, Xhaard A, Fremeaux-Bacchi V, Coppo P, Fischer AM, Helley D and Socie G. (2013) Successful use of eculizumab in a patient with post-transplant thrombotic microangiopathy. Br J Haematol, 161: 279-280.

160. Jodele S, Fukuda T, Vinks A, Mizuno K, Laskin BL, Goebel J, Dixon BP, Teusink A, Pluthero FG, Lu L, Licht C and Davies SM. (2014) Eculizumab therapy in children with severe hematopoietic stem cell transplantation-associated thrombotic microangiopathy. Biol Blood Marrow Transplant, 20: 518-525.

161. de Fontbrune FS, Galambrun C, Sirvent A, Huynh A, Faguer S, Nguyen S, Bay JO, Neven B, Moussi J, Simon L, Xhaard A, Resche-Riggon M, O'Meara A, Fremeaux-Bacchi V, Veyradier A, Socie G, Coppo P and de Latour RP. (2015) Use of Eculizumab in Patients With Allogeneic Stem Cell Transplant-Associated Thrombotic Microangiopathy: A Study From the SFGM-TC. Transplantation, 99: 1953-1959.

162. Dhakal P, Giri S, Pathak R and Bhatt VR. (2017) Eculizumab in Transplant-Associated Thrombotic Microangiopathy. Clin Appl Thromb Hemost, 23: 175-180.

163. Jodele S, Dandoy CE, Danziger-Isakov L, Myers KC, El-Bietar J, Nelson A, Wallace G, Teusink-Cross A and Davies SM. (2016) Terminal Complement Blockade after Hematopoietic Stem Cell Transplantation Is Safe without Meningococcal Vaccination. Biol Blood Marrow Transplant, 22: 1337-1340.

164. Jodele S, Fukuda T, Mizuno K, Vinks AA, Laskin BL, Goebel J, Dixon BP, Chima RS, Hirsch R, Teusink A, Lazear D, Lane A, Myers KC, Dandoy CE and Davies SM. (2016) Variable Eculizumab Clearance Requires Pharmacodynamic Monitoring to Optimize Therapy for Thrombotic Microangiopathy after Hematopoietic Stem Cell Transplantation. Biol Blood Marrow Transplant, 22: 307-315.

165. Benamu E and Montoya JG. (2016) Infections associated with the use of eculizumab: recommendations for prevention and prophylaxis. Curr Opin Infect Dis, 29: 319-329.

166. Alashkar F, Vance C, Herich-Terhurne D, Preising N, Duhrsen U and Roth A. (2017) Serologic response to meningococcal vaccination in patients with paroxysmal nocturnal hemoglobinuria (PNH) chronically treated with the terminal complement inhibitor eculizumab. Ann Hematol, 96: 589-596.

167. Bohl SR, Kuchenbauer F, von Harsdorf S, Kloevekorn N, Schonsteiner SS, Rouhi A, Schwarzwalder P, Dohner H, Bunjes D and Bommer M. (2017) Thrombotic Microangiopathy after Allogeneic Stem Cell Transplantation: A Comparison of Eculizumab Therapy and Conventional Therapy. Biol Blood Marrow Transplant, 23: 2172-2177.

168. Dhakal P and Bhatt VR. (2017) Is complement blockade an acceptable therapeutic strategy for hematopoietic cell transplant-associated thrombotic microangiopathy? Bone Marrow Transplant, 52: 352-356.

169. Matsuda Y, Hara J, Osugi Y, Tokimasa S, Fujisaki H, Takai K, Ohta H, Kawa-Ha K and Okada S. (2001) Serum levels of soluble adhesion molecules in stem cell transplantation-related complications. Bone Marrow Transplant, 27: 977-892.

170. Arai Y, Yamashita K, Mizugishi K, Watanabe T, Sakamoto S, Kitano T, Kondo T, Kawabata H, Kadowaki N and Takaori-Kondo A. (2013) Serum neutrophil extracellular trap levels predict thrombotic microangiopathy after allogeneic stem cell transplantation. Biol Blood Marrow Transplant, 19: 1683-1689.

171. Gloude NJ, Khandelwal P, Luebbering N, Lounder DT, Jodele S, Alder MN, Lane A, Wilkey A, Lake KE, Litts B and Davies SM. (2017) Circulating dsDNA, endothelial injury, and complement activation in thrombotic microangiopathy and GVHD. Blood, 130: 1259-1266.

172. Reti M, Farkas P, Csuka D, Razso K, Schlammadinger A, Udvardy ML, Madach K, Domjan G, Bereczki C, Reusz GS, Szabo AJ and Prohaszka Z. (2012) Complement activation in thrombotic thrombocytopenic purpura. J Thromb Haemost, 10: 791-798.

173. Dandoy CE, Davies SM, Hirsch R, Chima RS, Paff Z, Cash M, Ryan TD, Lane A, El-Bietar J, Myers KC and Jodele S. (2015) Abnormal echocardiography 7 days after

stem cell transplantation may be an early indicator of thrombotic microangiopathy. Biol Blood Marrow Transplant, 21: 113-118.

174. Kennedy GA, Bleakley S, Butler J, Mudie K, Kearey N and Durrant S. (2009) Posttransplant thrombotic microangiopathy: sensitivity of proposed new diagnostic criteria. Transfusion, 49: 1884-1889.

175. Willems E, Baron F, Seidel L, Frere P, Fillet G and Beguin Y. (2010) Comparison of thrombotic microangiopathy after allogeneic hematopoietic cell transplantation with high-dose or nonmyeloablative conditioning. Bone Marrow Transplant, 45: 689-693.

176. Rotz SJ, Luebbering N, Dixon BP, Gavriilaki E, Brodsky RA, Dandoy CE, Jodele S and Davies SM. (2017) In vitro evidence of complement activation in transplantation-associated thrombotic microangiopathy. Blood Adv, 1: 1632-1634.

177. Prohaszka Z, Nilsson B, Frazer-Abel A and Kirschfink M. (2016) Complement analysis 2016: Clinical indications, laboratory diagnostics and quality control. Immunobiology, 221: 1247-1258.

178. Yuen J, Pluthero FG, Douda DN, Riedl M, Cherry A, Ulanova M, Kahr WH, Palaniyar N and Licht C. (2016) NETosing Neutrophils Activate Complement Both on Their Own NETs and Bacteria via Alternative and Non-alternative Pathways. Front Immunol, 7: 137.

179. Burroughs LM, Shimamura A, Talano JA, Domm JA, Baker KK, Delaney C, Frangoul H, Margolis DA, Baker KS, Nemecek ER, Geddis AE, Sandmaier BM, Deeg HJ, Storb R and Woolfrey AE. (2017) Allogeneic Hematopoietic Cell Transplantation Using Treosulfan-Based Conditioning for Treatment of Marrow Failure Disorders. Biol Blood Marrow Transplant, 23: 1669-1677.

180. Strocchio L, Zecca M, Comoli P, Mina T, Giorgiani G, Giraldi E, Vinti L, Merli P, Regazzi M and Locatelli F. (2015) Treosulfan-based conditioning regimen for allogeneic haematopoietic stem cell transplantation in children with sickle cell disease. Br J Haematol, 169: 726-736.

181. Burroughs LM, Nemecek ER, Torgerson TR, Storer BE, Talano JA, Domm J, Giller RH, Shimamura A, Delaney C, Skoda-Smith S, Thakar MS, Baker KS, Rawlings DJ, Englund JA, Flowers ME, Deeg HJ, Storb R and Woolfrey AE. (2014) Treosulfan-

based conditioning and hematopoietic cell transplantation for nonmalignant diseases: a prospective multicenter trial. Biol Blood Marrow Transplant, 20: 1996-2003.

10. LIST OF PUBLICATIONS

Publications related to this thesis:

Horváth O, Kállay K, Csuka D, Mező B, Sinkovits G, Kassa C, Stréhn A, Csordás K, Sinkó J, Prohászka Z, Kriván G. (2018) Early Increase in Complement Terminal Pathway Activation Marker sC5b-9 Is Predictive for the Development of Thrombotic Microangiopathy after Stem Cell Transplantation. Biol Blood Marrow Transplant, 24: 989-996.
IF: 4.704 (2016)

Horváth O, Prohászka Z, Kállay K, Kassa C, Stréhn A, Csordás K, Sinkó J, Kriván G. (2017) [Changes in diagnostic criteria of thrombotic microangiopathy after stem cell transplantation]. Orv Hetil, 158: 1043-1050. Hungarian.
IF: 0.349 (2016)

Other publications:
Horváth O, Kállay K, Kriván G. (2015) [A vashiányos anaemia korszerű kezelése gyermekkorban] Gyermekgyógyásztai Továbbképző Szemle, 20(5): 211-213. Hungarian.

Kállay K, Zakariás D, Csordás K, Benyó G, Kassa C, Sinkó J, Stréhn A, Horváth O, Vásárhelyi B, Kriván G. (2018) Antithymocyte Globuline Therapy and Bradycardia in Children. Pathol Oncol Res. Mar 9. doi: 10.1007/s12253-018-0403-y. Article in Press.
IF: 1.736 (2016)

Kállay K, Kassa C, Réti M, Karászi É, Sinkó J, Goda V, Stréhn A, Csordás K, Horváth O, Szederjesi A, Tasnády S, Hardi A, Kriván G. (2018) Early Experience With CliniMACS Prodigy CCS (IFN-gamma) System in Selection of Virus-specific T Cells From Third-party Donors for Pediatric Patients With Severe Viral Infections After Hematopoietic Stem Cell Transplantation, 41(3): 158-163.
IF: 3.203 (2016)

11. ACKNOWLEDGEMENTS

There are a lot of people to thank who in different ways have contributed to finish my dissertation. I would like to say thank for all the physicians and nurses who participated in the patient care and follow-up. I would specially like to thank:

Gergely Kriván, my supervisor. Thank you for being supportive, that you encouraged my ideas, and introduced me to the field of stem cell transplantation. Thank you, that you have been always ready for a brainstorming, and let me have own ideas and decisions as a young physician.

Zoltán Prohászka, my external mentor. Thank you that I could always ask for your advice during the years, and you always encouraged me to concentrate on scientific research, and facilitated my scientific work.

Krisztián Kállay, for being a great boss. Thank you for your sence of humor, and that you created a pleasant, supportive and inspirating working atmosphere in the department.

I would like to thank all my co-authors and collagues from the department and from the lab who contributed to the present projects: Csaba Kassa, János Sinkó, Katalin Csordás, Anita Stréhn, Vera Goda, Gabriella Kertész, Gábor Benyó, Éva Karászi, Dorottya Csuka, Blanka Mező and György Sinkovits.

I would like to thank my supervisor during the university years, György Reusz, by whom I started to learn about TMAs, and who initiated to complete a validated scientific work, and who always provided me valuable pieces of advice.

Special thanks to Sonata Jodele, my supervisor during my short term visit in Cincinnati, who showed me how to perform high quality scientific work and patient care at the same time, and who encouraged me to publish my papers.

Finally, I would like to express my special thank to my family and to my friends for their support and understanding for many years. I am especially grateful for my parents, who have been continuously providing my a supporting enviroment, and always encouraging me to learn and to do whatever I am interested in.