Evaluation of Thrombotic Microangiopathy after Allogeneic Hematopoietic Stem Cell Transplantation

Ph.D. Thesis

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1. INTRODUCTION

Transplantation-associated thrombotic microangiopathy (TA-TMA) is a challenging complication following hematopoietic stem cell transplantation (HSCT). 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. However, the signs and symptoms of TA-TMA can be confused with other common transplantation associated or malignancy associated complications, such as acute graft-versus-host disease (GVHD), infections, graft failure, immune-mediated thrombocytopenia, venoocclusive disease, relapse, medication induced side-effects, such as hypertension or acute renal failure. 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.

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. 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. Jodele et al. 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.

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, eculizumab, a humanized monoclonal antibody against complement C5 was also evaluated in the setting of TA-TMA. 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. The diagnostic criteria used for TA-TMA recognition include hematologic and renal injury markers, reflecting the presence of already ongoing, clinically manifest organ damages. The recent modification of diagnostic criteria that included markers of complement activation, proteinuria, and hypertension, 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.

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.

2. AIMS

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

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). 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.

Informed consent was obtained in accordance with the Declaration of Helsinki and the study was approved by the institutional Ethics Committee on Human Research.

Determination of Complement and Other Laboratory and Clinical Parameters

Complement pathway activities, components, activation product levels, solubile terminal pathway activation marker (sC5b-9) levels and ADAMTS13 activity were systematically measured before transplantation and on day 28, 56 and 100 after HSCT. 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.

Definition of Transplant-Related Complications

Graft versus host disease (GVHD) was diagnosed using Glucksberg criteria, viral reactivation (Epstein-Bar virus, cytomegalovirus, adenovirus) was monitored. 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), 2) the clinical criteria of the International Working Group (IWG), 3) the diagnostic criteria proposed by Cho at al., 4) the diagnostic criteria of City of Hope, and 5) the diagnostic criteria proposed by

Jodele et al.. The date of diagnosis was defined as the date when all of the TA-TMA diagnostic criteria proposed by Jodele et al. were fulfilled.

Statistical Methods

Because most of the continuous variables showed skewed distribution, data are presented as median (range or interquartile range) and nonparametric tests were used for group comparisons. 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 and the GraphPad Prism 6.03 statistical software.

4. RESULTS

Study Cohort Demographics

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. Overall, 24 of 33 (73%) patient received treosulfan-based conditioning regimen

for malignant and non-malignant disorders. The main stem cell source was bone marrow (70%) from matched unrelated donors (76%). 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. Eleven (33%) patients experienced grade I or II acute GVHD.

Diagnosis of TA-TMA

Considering the five different diagnostic systems 2 of 33 subject met the BMT CTN , 7 of 33 the IWG criteria, 7 of 33 the diagnostic criteria of Cho et al., 3 of 33 the diagnostic criteria of City of Hope, and 10 of 33 patients met the diagnostic criteria for TA-TMA proposed by Jodele et al.. 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. Seven out of the 10 patients with TA-TMA fulfilled at least 3 TA-TMA criteria of the various diagnostic systems. 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. Surprisingly, grade I to II GVHD had almost the same incidence as TA-TMA after reduced toxicity conditioning regimen in our cohort.

Clinical Characteristics of Patients with 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. 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.

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

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. 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, $p<0.05$).

Importantly, 10 of 10 patients with later TA-TMA showed an early increase of sC5b-9. 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.

Next, we analyzed the relationship between early increase of sC5b-9 and engraftment and additional transplantation related complications. 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. 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 (p=0.001).

Hypertension until day 28 was the only TMA activity marker that was significantly associated with early increase of sC5b-9 levels (p=0.027), whereas presence of decreased haptoglobin concentrations showed a tendency for association (p=0.098).

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. Increase in sC5b-9 concentration had 100% sensitivity and 61% specificity for TA-TMA in our pediatric cohort.

Patient Survival

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. Seven of 10 patients (70%) with TA-TMA survived, compared with 17 of 23 (74%) patients without TA-TMA. Furthermore, early elevation of sC5b-9 showed no association with mortality.

5. CONCLUSIONS

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.

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.

6. 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: 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, doi: 10.1007/s12253-018-0403-y. Article in Press.
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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. J Immunother, 41: 158-163.
IF: 3.203 (2016)