The association of genetic factors with complications and outcome of allogeneic haematopoietic stem cell transplantation

PhD thesis

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1. INTRODUCTION

Allogeneic haematopoietic stem cell transplantation (allo-HSCT) provides the only curative treatment for many conditions including malignancies, hereditary and immune-mediated diseases. Worldwide there has been a linear increase in allo-HSCT activity. Estimations suggest that the one millionth HSCT (considering both autologous and allogeneic forms) in the world has been performed by the end of 2012. According to the most recent European Society for Blood and Marrow Transplantation (EBMT) survey the number of allogeneic transplants performed yearly in the more developed European countries is in the range of 201-300 per 10 million population. Although the outcome of patients undergoing the treatment has been gradually improving, allo-HSCT remains a high risk procedure with significant transplant-related mortality. The success of allo-HSCT in malignant disorders heavily relies on the beneficial post-transplant graft-versus-leukaemia/tumour effect that potently eradicates malignant cells. At the same time, the immunological attack delivered by donor cells on target recipient tissues can result in acute graft-versus-host disease (aGvHD). Acute GvHD is the leading cause of non-relapse mortality post-transplant and as such is the major impediment of allo-HSCT. Acute GvHD resembles an acute inflammatory reaction manifesting in the clinical picture of skin rash, nausea, vomiting, diarrhoea and liver dysfunction. Despite many novel modalities the outcome of aGvHD has not improved greatly. Extensive research efforts have been generated to predict and prevent the development of aGvHD. Numerous risk factors have been identified, and the ones that could potentially impact on donor selection preferences are of high

importance. With regards to genetic risk factors human leucocyte antigen (HLA) disparity between the recipient and the donor is the most relevant. On the other hand, high-resolution HLA matching cannot prevent the development of aGvHD, highlighting the involvement of other genetic systems. The discovery of genetic pathways involved in alloimmune reactions is of high significance that could help the prevention of complications via better donor selection and by shaping future drug development. Cytokines play an essential role in aGvHD reactions. Many of the cytokines with relevance for aGvHD relay their signals via the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway. Our hypothesis was that polymorphisms of the pathway, including Janus kinase 2 (JAK2) might affect the manifestation of aGvHD. Among JAK2 variants, the investigation of 46/1 haplotype seemed to be relevant as it has been reported to have functional consequences. Transplantation-associated thrombotic microangiopathy (TA-TMA) is another, feared complication of allo-HSCT due to its dismal prognosis (median mortality 75%). The key element of TA-TMA is endothelial dysfunction induced by various triggers such as infections, drugs or aGvHD. The clinical course presents with microangiopathic haemolytic anaemia, thrombocytopenia and organ dysfunction secondary to microthrombi with predilection in the kidneys. Giving the poor outcome of TA-TMA, the identification of risk factors and measures to prevent its development are of high clinical significance. HLA-DRB1*11 shows strong association with thrombotic thrombocytopenic purpura (TTP), another form of TMA. Giving the similarities of the clinical presentation of TTP and TA-TMA, we hypothesised that HLA-DRB1*11 could also be associated with TA-TMA.

2. OBJECTIVES

2.1. Establishment of database

The primary aim of our work was to set up a database including detailed patient-, donor-, and transplantation-related characteristics and outcome data of all first allogeneic haematopoietic stem cell transplantations performed in adult patients (n=425) for haematological malignancies in Hungary between 2007 and 2013.

Data were assembled for the following parameters;

a) Patient-, and disease-associated characteristics:

Patient age, gender, diagnosis, stage of disease, ABO and Rh blood groups, CMV serostatus, HLA type, karyotypic abnormalities in acute leukaemia;

b) Donor-specific features:

Related vs. unrelated donor type, donor sex, ABO and Rh blood groups, CMV serostatus, HLA type;

c) Transplantation-related characteristics: date of transplant, conditioning intensity and type, stem cell source, applied GvHD prophylactic regimen;

d) Outcome data: engraftment, relapse rate and timing, incidence, timing and severity of TA-TMA, aGvHD and CMV reactivation, overall survival, cause of death.

2.2. Transplantation-associated thrombotic microangiopathy (TA-TMA) study

This work aimed at addressing the following questions in association with TA-TMA development;

- What is the incidence and timing of TA-TMA?

- What proportion of patients develop organ damage?
- What are the risk factors for TA-TMA?
- Does HLA-DRB1*11 show an association with TA-TMA?
- Does the development of TA-TMA affect the survival?
- Does the prognosis differ in HLA-DRB1*11 carriers and non-carriers?

2.3. JAK2 46/1 haplotype study

Our second study assessed the association of recipient and donor Janus kinase 2 (JAK2) 46/1 haplotypes with transplantation outcome.

The following hypotheses were investigated;

- Are recipient and donor JAK2 46/1 haplotypes associated with aGvHD grades II-IV. development?
- Does the recipient/donor genotype combination affect the risk?
- Do the recipient and donor JAK2 46/1 haplotypes correlate with relapse?
- Are recipient and donor JAK2 46/1 haplotypes associated with survival?

3. METHODS

3.1. Patients

The entire cohort consisted of 425 consecutive adult patients who underwent first allo-HSCT between January 2007 and December 2013 for a haematological malignancy at the Department of Haematology and Stem Cell Transplantation, St Istvan and St Laszlo Hospital, Hungary. Giving that

this hospital was the only centre delivering this treatment modality at the time, all transplants performed on adults in Hungary were included. The indications of HSCT were the following; acute myeloid leukaemia (n=157), acute lymphoid leukaemia (n=75), non-Hodgkin lymphoma (n=42), myelodysplastic syndrome (n=35), chronic lymphocytic leukaemia (n=26), multiple myeloma (n=25), chronic myeloid leukaemia (n=24), myeloproliferative neoplasm (n=23) and Hodgkin lymphoma (n=18). Related and unrelated donor sources were close to equal (51.1% related and 48.9% unrelated). The majority of patients were treated with myeloablative conditioning (62.8%). GvHD prophylactic regimens were either cyclosporine (24%) or tacrolimus-based (76%). Our first study on TA-TMA was conducted in the whole cohort, whereas the second study on JAK2 46/1 haplotype included patients (n=124) transplanted for acute myeloid leukaemia (AML) in complete remission (CR).

3.2. Diagnosis of TA-TMA and acute GvHD

The definition for TA-TMA diagnosis was based on overall TMA criteria including probable TMA cases without organ damage proposed by Cho et al.: 1.) Normal coagulation assays; 2.) ≥ two schistocytes per high power field; 3.) Increased lactate dehydrogenase; 4.) Negative Coombs test; 5.) De novo, prolonged or progressive thrombocytopenia (< $50x10^9$/l or 50% or greater reduction from previous counts); 6.) Decrease in haemoglobin concentration; 7.) Decrease in serum haptoglobin. Patients with renal and/or neurological dysfunction (as defined by the Blood and Marrow Transplant Clinical Trials Network Toxicity Committee Consensus recommendations) were categorised as having TA-TMA with organ involvement.

Acute GvHD was defined and graded according to consensus criteria (modified Glucksberg).

3.3. Detection of JAK2 46/1 haplotype

For identification of JAK2 46/1 haplotype we tested SNP rs12343867, a fully-linked, tagging SNP to the haplotype. JAK2 rs12343867_C tags 46/1 haplotype: the nucleotide C represents the haplotype, while T determines non-46/1 haplotype. Melting curve analysis with hybridization probe detection format on LightCycler 480II (Roche Diagnostics) was performed to identify the alleles of rs12343867.

3.4. Statistical analysis

The statistical analyses were performed by SPSS Statistics software version 22 and STATA10 software. Categorical variables were compared using Chi-square or Fisher's exact tests, while continuous variables were tested by Mann-Whitney or Kruskal-Wallis tests. To assess associations of multiple factors with the outcome multivariate logistic regression was performed. Cumulative incidence was calculated by the Fine and Gray model. Survival between subgroups were compared by the log-rank test and estimated by the Kaplan-Meier method. Cox regression analysis was applied for the study of covariates in association with survival, hazard ratio (HR) and 95% confidence interval (95%CI) were calculated. All analyses were two-sided. P values of less than 0.05 were considered statistically significant, while p values between 0.05 and 0.1 were referred to as tendency.

4. RESULTS

4.1. TA-TMA STUDY

4.1.1. Incidence and timing of TA-TMA

TA-TMA was diagnosed in 18.8% of the patients (80/425), of whom half showed signs of organ damage (n=41, 51.3% of TA-TMA patients). The median time to TA-TMA manifestation from the day of transplant was 40 days (range 6-1670 days) and in the vast majority of patients (81.3%) TA-TMA presented within the first 100 days of transplantation.

4.1.2. Risk factors for TA-TMA manifestation

We validated the association of TA-TMA with several previously published risk factors. TA-TMA affected 27.4% of patients with an unrelated donor as opposed to only 10.6% in those with a sibling donor ($p<0.001$). TA-TMA was significantly more prevalent following the administration of myeloablative conditioning (MAC) (23.2% versus [vs.] 11.4% with reduced intensity conditioning [RIC], $p=0.003$). TA-TMA was diagnosed in 9.0% in the cyclosporine (CSA)-based aGvHD prophylactic subgroup, whereas the incidence increased with tacrolimus (TAC) administration (21.8%, $p=0.003$), irrespectively of the other agent given in combination with TAC. Among the 80 TA-TMA patients, 66 simultaneously presented with aGvHD during follow-up, and in 90.9% of these cases aGvHD preceded TA-TMA. While TA-TMA was rare in patients without aGvHD (7.8%), 34.9% and 55.0% were diagnosed among those who had grade II-III or IV aGvHD, respectively. CMV reactivation/disease were also proved to be risk factors for TA-TMA ($p=0.003$). Our study however failed to show association with other, implicated factors, such as recipient's age, gender, gender matching, diagnosis and ABO compatibility.

In addition to the above mentioned, documented risk factors, as a new finding the analyses confirmed that carriership for HLA-DRB1*11 was also significantly associated with the manifestation of TA-TMA. TA-TMA occurred in 24.8% of HLA-DRB1*11 positive vs. in 16.0% of HLA-DRB1*11 negative patients (p=0.034). The cumulative incidence of TA-TMA showed significant alteration according to HLA-DRB1*11 (p=0.026, **Figure 1.**).

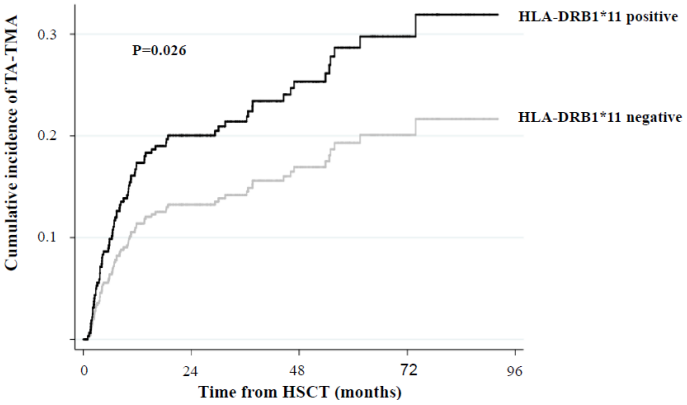

Figure 1. Cumulative incidence of TA-TMA in HLA-DRB1*11 carrier and non-carrier patients.

In multivariate analysis including the above parameters significantly associated in univariate testing the association of TA-TMA with donor type and grades II-IV aGvHD was independent, there was a tendency for association with CMV reactivation and HLA-DRB1*11, while conditioning intensity and type of GvHD prophylaxis failed to show independent association.

Separate analyses in sibling and unrelated transplants were performed due to uneven distribution of donor type among HLA-DRB1*11 carriers and non-carriers. The analyses showed that HLA-DRB1*11 carriership was significantly associated with the emergence of TA-TMA in the sibling subgroup (17.8% [10/56] in HLA-DRB1*11 positive versus 8.1% [13/161] in HLA-DRB1*11 negative cases; p=0.047), but not in the unrelated donor subset (29.6% [24/81] in HLA-DRB1*11 positive versus 26.0% [33/127] in HLA-DRB1*11 negative cases; p=0.633).

4.1.3. The influence of TA-TMA on post-transplant overall survival

The overall survival (OS) of patients who developed TA-TMA was significantly worse compared to patients without the complication (36-month OS: 27.4±5.3% vs. 55.3±2.9%, p<0.001). Within the TA-TMA group, the outcome of patients with organ damage was more unfavourable compared to individuals without organ involvement (p=0.025). The survival of patients without TA-TMA was significantly better compared to both subgroups, patients with TA-TMA without organ involvement (p=0.015) and with organ damage (p<0.001). The survival of patients not affected by TA-TMA was not influenced by HLA-DRB1*11 status (p=0.778), but carriers of HLA-DRB1*11 displayed superior OS within the cohort suffering from TA-TMA (p=0.003).

Cox regression analysis confirmed that TA-TMA was an independent adverse risk factor for overall survival (HR 1.9, 95% CI: 1.35-2.62). Among TA-TMA patients carriership of HLA-DRB1*11 was an independent predictor of better survival (HR: 0.46, 95% CI: 0.27-0.81, p=0.007).

4.2. JAK2 46/1 HAPLOTYPE STUDY

4.2.1. Outcome according to patients' JAK2 46/1 haplotype

Among the 124 transplant recipients (AML in CR) included in this study, 64 (51.6%) carried the TT, non-46/1 haplotype genotype, the number of heterozygous and homozygous carriers for the 46/1 haplotype was 49 (39.5%) and 11 (8.9%), respectively. In 46/1 haplotype carriers (R1) we observed significantly more grades II-IV aGvHD compared with non-carriers (R0). By 100 days post-transplant aGvHD grades II-IV was diagnosed in 41.7% of haplotype carriers as opposed to 18.8% of non-carriers (p=0.008, **Figure 2.**).

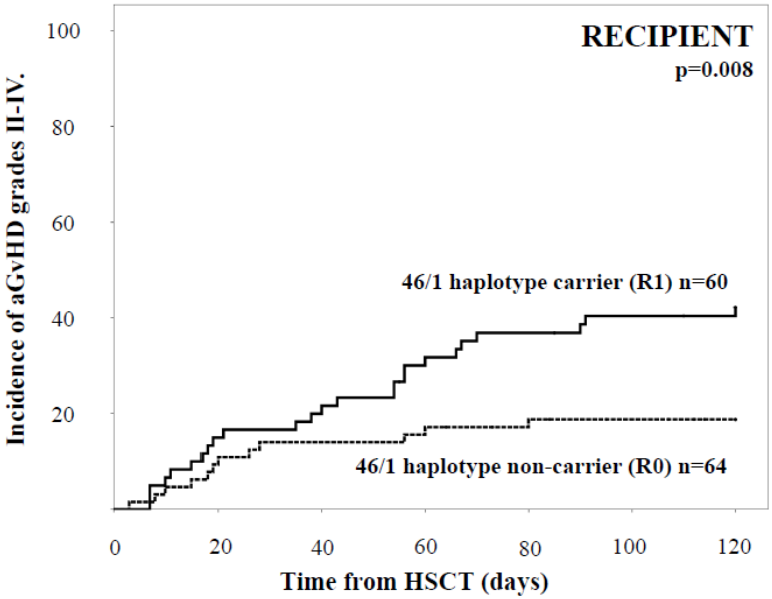

Figure 2. Incidence of aGvHD grades II-IV by 100 days was 41.7% in haplotype carriers (R1) in contrast to 18.8% in non-carriers (R0, p=0.008).

At 2 years the cumulative incidence of relapse was significantly lower in haplotype carriers (5.0%) in comparison with non-carriers (23.5%, p=0.008). Despite alterations in the causes of death and relapse rate according to the haplotype, OS was comparable in 46/1 haplotype carriers and non-carriers (2-year OS 69.2±6.1% in carriers vs. 60.7±6.7% in non-carriers, p=0.732).

4.2.2. Outcome according to donors' JAK2 46/1 haplotype

Similarly to recipient haplotype, moderate and severe aGvHD were more common in patients transplanted with haplotype carrier donors compared with patients transplanted with non-carrier donors by day 100 (39.7% vs. 21.2%, p=0.038). Relapse rates were comparable in patients transplanted from carrier and non-carrier donors (13.8% vs. 15.2%, p=1). The survival was not significantly different according to the donor haplotype, but there was a tendency for better OS among patients transplanted from non-46/1 haplotype carriers as opposed to haplotype carriers: 2-year OS 73.9±5.7 vs. 56.0±6.9% (p=0.056).

4.2.3. Outcome according to recipient/donor JAK2 46/1 haplotype combinations

As we observed that both recipient and donor haplotypes showed association with aGvHD, we elucidated how their combinations influenced the risk. The analysis confirmed significantly higher aGvHD risk if both, the recipient and the donor (R1D1) were 46/1 haplotype carriers in comparison with the non-carrier patient/donor (R0D0) constellation (OR=5.242, 95% CI=1.784-15.404, p=0.003). There was intermediate risk if either the recipient (R1D0) or donor (R0D1) was a carrier (**Figure 3.**).

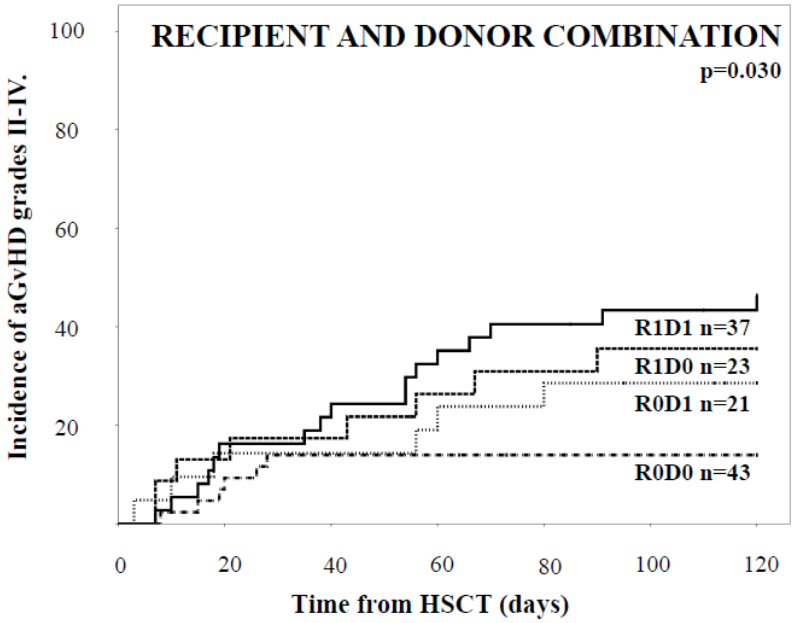

Figure 3. Incidence of aGvHD grades II-IV according to recipient/donor haplotype pairs. Incidences were 13.9% in the R0D0, 28.6% in the R0D1, 34.8% in the R1D0, and 45.9% in the R1D1 group (p=0.030).

The 2-year OS rates were not statistically different in the four above described recipient-donor groups (69.0±7.6% [R0D0], 82.1±8.1% [R1D0], 42.1±13.3% ([R0D1] and 61.1±8.2% ([R1D1], p=0.211).

In univariate analyses, aGvHD grades II-IV were significantly associated with unrelated donor type (p=0.031), myeloablative conditioning (p=0.036), recipient age at transplantation (p=0.046), JAK2 46/1 haplotype in the recipient (p=0.006) and in the donor (p=0.031). Multivariate analyses confirmed that recipient JAK2 46/1 haplotype was independently associated

with aGVHD (p=0.012), whereas the donor haplotype was not, however there was a tendency (p=0.08).

5. CONCLUSIONS

1. Our work identified two novel risk factors for the development of serious post-transplant complications, transplantation-associated thrombotic microangiopathy and acute graft-versus-host disease in this single centre cohort of adult Hungarian patients undergoing allogeneic HSCT.

2. TA-TMA was diagnosed in nearly the fifth (18.8%) of the patients with predominant presentation in the early post-transplant period (median 40 days, in 81% within first 100 days of transplantation). Organ damage was noted in 51.3% (41/80) of patients with TA-TMA. Unrelated donor type, acute GvHD grades II-IV, myeloablative conditioning, tacrolimus-based immunosuppression, CMV reactivation/disease and as a novel finding carriership for HLA-DRB1*11 were the identified risk factors for TA-TMA development.

3. TA-TMA was an independent adverse factor for overall survival. Within the TA-TMA group the presence of organ damage adversely affected the outcome, whereas the outcome was more favourable in HLA-DRB1*11 carriers.

4. In the AML cohort we confirmed as a new observation that both, recipient and donor JAK2 46/1 haplotypes were associated with the development of aGvHD grades II-IV. Moreover the impact of the recipient and donor genetic variant seemed to be cumulative giving that the highest incidence was disclosed among those, who themselves and their donors were both haplotype carriers. Multivariate analyses confirmed that the recipient JAK2 46/1 haplotype was independently associated with aGVHD, however the donor JAK2 46/1 haplotype was not.

5. Recipient JAK2 46/1 haplotype showed a favourable correlation with relapse rate, but the donor JAK2 46/1 haplotype had no effect on relapse incidence. The recipient and donor JAK2 46/1 haplotypes had no significant impact on post-transplant overall survival.

6. BIBLIOGRAPHY OF THE CANDIDATE'S PUBLICATIONS

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