Premature cyclosporine cessation and TBI-containing conditioning regimen increase the risk of acute GvHD in children undergoing unrelated donor hematopoietic stem cell transplantation

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Abstract

Background. Acute graft-versus-host disease (aGvHD) is a potentially fatal complication after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Identifying its risk factors would enable the proper prophylaxis and management, which may significantly improve the general outcome of children treated with HSCT.

Objectives. The aim of this single-center, retrospective cohort study was to assess the potential risk factors for grades II–IV of aGvHD in children after the 1st allo-HSCT from an unrelated donor (UD), performed as a result of an underlying malignant disease.

Material and methods. From among patients who received HSCT in our center in the years 2004–2015, 237 were included in the study cohort. All the patients received standard aGvHD prophylaxis consisting of cyclosporine (CsA) and a short course of methotrexate (MTX). Various clinical and epidemiological features, the transplant proceedings, graft composition, conditioning regimens, as well as the duration and coherence of aGvHD prophylaxis were analyzed as potential risk factors for aGvHD.

Results. The incidence of II–IV aGvHD in the study cohort was 58.6%. The median time of the diagnosis of aGvHD was 18 days post-HSCT. In the multivariate analysis, risk factors significantly associated with grades II–IV of aGvHD were: myeloablative conditioning regimen containing total body irradiation (TBI-MAC) (RR (relative risk): 1.69; $p = 0.03$), premature termination of CsA administration due to its toxicity (RR: 1.99; $p = 0.0003$) and HSCT performed before the year 2009 (RR: 1.97; $p = 0.0001$). Donor and recipient age, donor–recipient sex mismatch, stem cell source, risk of disease, and amount of infused CD34+ cells seem to be insignificant as risk factors for aGvHD. The overall survival (OS) of patients with aGvHD was noticeably worse than in those who were aGvHD-free: 60.8% vs 74.1% ($p = 0.08$).

Conclusions. The conditioning regimen and the proper aGvHD prophylaxis, including continuous CsA administration, have a major impact on aGvHD occurrence. According to our results, the termination of CsA therapy should be carefully considered, and avoided if possible.

Key words: risk factors, acute graft-versus-host disease, hematopoietic stem cell transplantation, prophylaxis

Introduction

Acute graft-versus-host disease (aGvHD) remains one of the most common life-threatening complications after allogeneic hematopoietic stem cells transplantation (allo-HSCT), contributing significantly to morbidity and mortality.¹ Several studies have reported numerous risk factors associated with increased incidence of aGvHD in the adult population. Among them, human leukocyte antigen (HLA) mismatch, type of the conditioning regimen, female donor to male recipient, and higher recipient age have proven to be the most valid.^{2–6} However, it needs to be highlighted that pediatric HSCTs differ remarkably from those in the adult population, particularly regarding the indications for transplant, existing comorbidities, previous treatment, and transplantation regimens.

In the pediatric population, the reported incidence of aGvHD varies from 30% to 80%, despite given prophylaxis.^{2,7–10} First-line treatment for aGvHD is based mostly on high-dose steroids and turns out to be abortive in about 50% of cases. There are many possible second-line treatment agents and protocols, but, even when providing promising results, none of them have been proven unequivocally effective.^{11,12} On that account, not only is an intensive quest for successful second-line treatment for aGvHD needed, but first and foremost, a precise evaluation of its risk factors, especially for a pediatric cohort of patients.

The aim of this single-center, retrospective cohort study was to assess which potential risk factors have a significant influence on the frequency of grades II–IV of aGvHD in children with a malignant disease after the 1st HSCT from an unrelated donor (UD). An analysis of the general outcome, overall survival (OS) and transplant-related mortality (TRM) in the studied cohort was the secondary aim of this research.

Patients and methods

A retrospective analysis of the medical records of the patients who underwent HSCT in the Department of Pediatric Hematology, Oncology and Bone Marrow Transplantation of Wrocław Medical University (Poland) in the years 2004–2015 was performed. According to the inclusion criteria, all patients under 21 years who were suffering from a malignant disease and underwent the 1st allo-HSCT procedure from an UD were considered in this study. Due to their small number, patients who received cord blood were excluded from the study. The final cohort included a total amount of 237 children and young adults (Table 1).

The grafted cells were obtained from human leukocyte antigen (HLA)-allele matched or mismatched UD (age: 19–56 years; median age: 31 years). The HLA typing was performed at the high-resolution level (4 digits) in A*, B*,

Table 1. Study cohort characteristics and analyzed risk factors for aGvHD

Patients' characteristics	n	%
Diagnosis		
acute leukemia	162	68.4
chronic myeloblastic leukemia	28	11.8
myelodysplastic syndrome	32	13.5
lymphoma	15	6.3
Patient sex		
male	148	6.3
female	89	37.6
Donor–recipient sex mismatch		
matched	142	59.9
male to female	44	18.6
female to male	51	21.5
Patient age [years]		
≤5	53	22.4
>5 and ≤15	129	54.4
>15	55	23.2
Donor age [years] (median: 31)		
≤median	123	51.9
>median	114	48.1
Amount of CD34+ cells (median: $8.29 \times 10^6/\text{kg}$)		
≤median	119	50.2
>median	118	49.8
Amount of CD34+ cells (quartile – cut-off value)		
Q1 ($5.01 \times 10^6/\text{kg}$)	60	25.3
Q2 ($8.29 \times 10^6/\text{kg}$)	59	24.9
Q3 ($12.09 \times 10^6/\text{kg}$)	60	25.3
Q4 ($51.85 \times 10^6/\text{kg}$)	58	24.5
Stem cells source		
peripheral blood	201	84.8
bone marrow	36	15.2
Conditioning regimen		
TBI-MAC	84	35.4
Bu-MAC	105	44.3
other (RTC/RIC)	48	20.3
Premature cessation of CsA		
yes	47	19.8
no	190	80.2
Risk of disease		
high	115	48.5
standard	122	51.5
Year of transplantation (median: 2009)		
≤2009	126	53.2
>2009	111	46.8
Year of transplantation (quartile)		
Q1 (2004–2006)	72	30.4
Q2 (2007–2009)	54	22.8
Q3 (2010–2012)	58	24.5
Q4 (2013–2015)	53	22.3

TBI-MAC – myeloablative conditioning regimen containing total body irradiation; Bu-MAC – myeloablative conditioning regimen containing busulfan; RTC – reduced-toxicity conditioning; RIC – reduced-intensity conditioning; CsA – cyclosporine.

Cw*, DRB1*, and DQB1* alleles. A matched donor was defined as 10/10 or 9/10 HLA-compatible donor. More than 1 mismatched allele was defined as a mismatched unrelated donor (MMUD). According to the ALL-SCT BFM International 2008 criteria, all 10 alleles were high-resolution types.

Graft-versus-host disease prophylaxis

Within the standard prophylaxis for aGvHD, all patients, since the day before HSCT received intravenous cyclosporine (CsA) in a unified initial dose of 1.5 mg/kg twice per day in 2-hour infusions. Further dosage of CsA was adjusted to the CsA trough level (target level: 100–200 µg/L), measured every second day in the majority of patients. Therefore, the dosage was modified to maintain the target level. Cyclosporine was switched into oral formulation when patient was able to tolerate oral intake. According to standard protocols, in patients without signs of GvHD, CsA administration was discontinued on day 120 in the majority of cases, preceded by gradual dose reduction. The 2nd prophylactic agent, methotrexate (MTX), was administered threefold in a standard dose of 10 mg/m² on days 1, 3 and 6 after HSCT. All patients were given in vivo T-cell depletion by either rabbit anti-thymocyte globulin (ATG) (n = 229) (ATG-Fresenius/Grafalon® (Neovii Biotech GmbH, Gräfelfing, Germany) – median: 45 mg/kg; or Thymoglobuline® (Genzyme Europe B.V., Naarden, the Netherlands) – median 7.5 mg/kg) or Campath-1H (Genzyme Europe BV) (n = 8) (median: 1 mg/kg).

Acute graft-versus-host disease diagnosis and staging

The diagnosis of aGvHD was based on the clinical findings and/or histopathological findings in the skin, gastrointestinal tract mucosa and liver biopsies.^{13–15} Grading and staging of aGvHD was performed using pediatric-specific criteria published by Jacobsohn.¹⁵ In the presented research, only grades II–IV were considered. Patients who presented symptoms of aGvHD after donor lymphocyte infusion were excluded from this study.

Risk factors and definitions

Potential risk factors for developing aGvHD were carefully analyzed and are listed in Table 2. The patients' risk status was defined by our own study-specific modification of the classification proposed by Meisel et al.¹⁶ Acute leukemia (AL) in 1st complete remission (1CR), chronic myeloblastic leukemia (CML) in chronic phase (CP), myelodysplastic syndrome-refractory cytopenia (MDS), and non-Hodgkin lymphoma (NHL) in complete remission (CR) was qualified as a standard risk. Acute leukemia in ≥2CR or non-remission, CML in equal to or greater than accelerating phase, MDS-refractory anemia with an excess of blasts, NHL in non-remission, and juvenile myelomonocytic leukemia (JMML) were defined as a high risk. Early termination of CsA administration was defined as termination before day 60 post-HSCT due to the toxicity of CsA. Cyclosporine-induced nephrotoxicity was diagnosed according to the following criteria: fall in baseline glomerular filtration rate – GFR >25% or doubling

the serum creatinine level.¹⁷ Neurotoxicity was defined as seizures, polyneuropathy, ataxy, impaired consciousness, or dizziness.¹⁸ Children with other obvious causes of neuro- and nephrotoxicity were excluded. Only patients who presented first symptoms of aGvHD after the cessation of CsA administration were considered under the influence of this risk factor.

Conditioning regimens

As many as 189 patients received myeloablative conditioning regimen (MAC) based either on myeloablative doses (12.8–19.6 mg/kg) of busulfan (Bu-MAC; n = 105), or on total body irradiation (TBI) at a median dose of 12 Gy (TBI-MAC; n = 84). Thirty-eight patients received reduced-toxicity conditioning (RTC), consisting mainly of fludarabine (160 mg/m²) and treosulfan (36–42 mg/kg) with thiotepa (10 mg/kg), melphalan (140 mg/m²) or cyclophosphamide (120 mg/kg). Ten patients received reduced-intensity conditioning (RIC) regimen, consisting of fludarabine with either melphalan or low, non-myeloablative doses of busulfan (2 mg/kg).^{19,20} In the statistical analysis, patients receiving RIC and RTC were considered collectively as 1 group.

Statistical analysis

The statistical analysis was performed using R statistical software (<https://www.r-project.org/>). Variables were analyzed in terms of their prognostic impact on aGvHD, OS and TRM. First, powered by the χ^2 test, we compared the baseline characteristics of patients with II–IV aGvHD and those with either no aGvHD or grade IaGvHD. Thereafter, patient data was entered into a competing risk regression model. We prosecuted 5 variants of regression models, containing either all or only the significant factors. Using the Bayesian Information Criterion (BIC) test, we selected the most adequate model. Acute graft-versus-host disease was defined by the abovementioned criteria, analyzed as time to event with death, relapse or rejection without aGvHD as a competing event. The p-values <0.05 were considered significant. The survival analysis was performed using the Kaplan–Meier estimation and the survival graphs were compared using the log-rank test. Since aGvHD is a time-dependent variable, we applied the landmark analysis at day 100 to the poor-prognosis group in order to avoid bias connected with autoselection. All patients who died or did not undergo a follow-up before day 100 were deleted from the survival analysis.

Results

A total of 237 patients underwent the 1st allo-HSCT from an UD. Two hundred thirty patients received stem cells from matched donor and 7 patients received stem cells

from MMUD (8/10). One hundred thirty-nine patients (58.6%) developed aGvHD stage II–IV within 100 days post-HSCT. The time of aGvHD diagnosis varied from 5 days post-HSCT up to 92 days post-HSCT (median: 18 days). Seventy-eight patients (32.9%) did not develop aGvHD or developed only stage I with no need for systemic treatment. Twenty (8.5%) of those who did not develop aGvHD encountered a competing event, such as graft rejection, relapse or death before day 100.

Forty-seven patients (19.8%) presented CsA-associated toxicity and required cessation of the therapy. The most common CsA toxicity observed in the study population was nephrotoxicity (n = 22) and neurotoxicity (n = 19).

Six patients presented other, more unspecific symptoms, such as allergic reactions (n = 5) and microangiopathy (n = 1). The beginning of CsA treatment discontinuation varied from 1 day post-HSCT up to 52 days post-HSCT (median: 15 days). Instead of CsA, the majority of patients (n = 45; 96%) received Mycophenolate Mofetil (MMF) intravenously at a standard prophylactic dose (20 mg/kg/day). Four of those patients received additional steroids at a standard dose of 1 mg/kg/day. Two (4%) patients received steroids alone.

Initially, we performed the univariate statistical analysis. In this analysis, 3 factors significantly increased the probability of aGvHD ($p < 0.05$): TBI-MAC ($p = 0.0272$),

Table 2. Univariate analysis of risk factors for acute graft-versus-host disease (aGvHD)

Risk factors	aGvHD			p-value
	yes n [%]	no (without a competing event) n [%]	no (with a competing event) n [%]	
Patient sex				
male	87 (62.6)	53 (67.9)	8 (40)	0.0705
female	52 (37.4)	25 (32.1)	12 (60)	
Sex mismatch				
matched	85 (61.2)	48 (61.6)	9 (45)	0.1221
male to female	27 (19.4)	15 (19.2)	2 (10)	
female to male	27 (19.4)	15 (19.2)	9 (45)	
Patient age [years]				
≤5	33 (23.7)	13 (16.7)	7 (35)	0.4084
>5 and <15	76 (54.7)	45 (57.7)	8 (40)	
≥15	30 (21.6)	20 (25.6)	5 (25)	
Donor age [years] (median: 31)				
≤median	74 (53.2)	41 (52.6)	8 (40)	0.5358
>median	65 (46.8)	37 (47.4)	12 (60)	
Amount of CD34+ cells (median: $8.29 \times 10^6/\text{kg}$)				
≤median	65 (46.8)	42 (53.8)	12 (60)	0.3985
>median	74 (53.2)	36 (46.2)	8 (40)	
Amount of CD34+ cells (quartile – cut-off value)				
Q1 ($5.01 \times 10^6/\text{kg}$)	32 (23)	22 (28.2)	6 (30)	0.7970
Q2 ($8.29 \times 10^6/\text{kg}$)	33 (23.7)	20 (25.6)	6 (30)	
Q3 ($12.09 \times 10^6/\text{kg}$)	35 (25.2)	21 (26.9)	4 (20)	
Q4 ($51.85 \times 10^6/\text{kg}$)	39 (28.1)	15 (19.3)	4 (20)	
Stem cells source				
peripheral blood	115 (82.7)	68 (87.2)	18 (90)	0.5425
bone marrow	24 (17.3)	10 (12.8)	2 (10)	
Conditioning regimen				
TBI-MAC	59 (42.4)	39 (50)	7 (35)	0.0272
Bu-MAC	59 (42.4)	19 (24.4)	6 (30)	
other (RTC/RIC)	21 (15.2)	20 (25.6)	7 (35)	
Premature discontinuation of CsA				
yes	36 (25.9)	7 (9)	2 (10)	0.0201
no	103 (74.1)	71 (91)	18 (90)	
Risk of disease				
high	71 (51.1)	36 (46.2)	8 (40)	0.5711
standard	68 (48.9)	42 (53.8)	12 (60)	
Year of transplantation (median: 2009)				
≤2009	88 (63.3)	33 (42.3)	5 (25)	0.0004
>2009	51 (36.7)	45 (57.7)	15 (75)	
Year of transplantation (quartile)				
Q1 (2004–2006)	54 (38.8)	17 (21.8)	1 (5)	0.0003
Q2 (2007–2009)	34 (24.5)	16 (20.5)	4 (20)	
Q3 (2010–2012)	32 (23)	17 (21.8)	9 (45)	
Q4 (2013–2015)	19 (13.7)	28 (35.9)	6 (30)	

preemptive discontinuation of CsA ($p = 0.0201$) and transplantation performed before year 2009 ($p = 0.0004$). Donor and recipient age, patient sex, donor–recipient sex mismatch, amount of infused CD34+ cells, stem cell source, and underlying disease stage seemed to be insignificant as risk factors for aGvHD (Table 2).

The same factors as in the univariate analysis proved to be significant in the multivariate analysis. The myeloablative conditioning regimen containing total body irradiation puts patients in a greater risk of aGvHD (RR (relative risk): 1.69; 95% confidence interval (95% CI) = 1.047–2.74; $p = 0.0320$). Premature discontinuation of CsA significantly increased the risk of aGvHD (RR = 1.99; 95% CI = 1.369–2.89; $p = 0.0003$). Transplantation performed before 2009 enhanced the possibility of aGvHD (RR = 1.97; 95% CI = 1.400–2.78; $p = 0.0001$). The relative risk regression model containing all significant factors is presented in Table 3.

Table 3. Results of multivariate analysis, competing risk regression model

Risk factors	RR (95% CI)	p-value
Conditioning regimen other (RTC/RIC)	1	–
Bu-MAC	1.11 (0.673–1.85)	0.67
TBI-MAC	1.69 (1.047–2.74)	0.0320
Early cessation of CsA no	1	–
yes	1.99 (1.369–2.89)	0.0003
Year of transplantation (median: 2009) >median	1	–
≤median	1.97 (1.400–2.78)	0.0001

RR – relative risk; CI – confidence interval.

The median follow-up in the study cohort, which was 2.3 years after HSCT, revealed that 138 patients remained alive. The general outcome in the aGvHD cohort was noticeably worse (TRM = 22.5%; OS = 60.8%) compared to aGvHD-free patients (TRM = 12%; OS = 74.1%). The difference between OS ($p = 0.08$) and TRM in both groups was at the limit of statistical significance ($p = 0.08$). Transplantation performed before 2009 contributed significantly to a worse survival ($p = 0.019$) and increased TRM ($p = 0.049$) (Fig. 1,2). Other factors, including those which statistically impact the incidence of aGvHD, did not influence OS and TRM (data not shown). Complete OS and TRM statistics are presented in Table 4.

Fig. 1. Comparison of overall survival (OS) for patients receiving hematopoietic stem cell transplantation (HSCT) before and after 2009

Fig. 2. Comparison of transplant-related mortality (TRM) for patients receiving hematopoietic stem cell transplantation (HSCT) before and after 2009

CIF – cumulative incidence function.

Discussion

Acute graft-versus-host disease as a major factor affecting morbidity and mortality in the early period post-HSCT still remains a challenge for clinicians and researchers worldwide. There is a great number of published studies attributing both the incidence of aGvHD and the general outcome to the influence of potential risk factors. Repeatedly, those studies demonstrate different or even contradictory results. However, it should be emphasized that most of the studies were performed on adult patients and lack of great cohort research conducted on the pediatric population is unquestionable.

Table 4. Comparison of overall survival (OS) and transplant-related mortality (TRM) in patients with or without acute graft-versus-host disease (aGvHD)

Time post HSCT	OS [%]		p-value	TRM [%]		p-value
	aGvHD	no aGvHD		aGvHD	no aGvHD	
1 year	87.0	90.3	0.084	13.8	8.7	0.145
3 years	72.2	85.3		18.6	12.2	
5 years	60.4	74.1		25.9	12.8	

The incidence of aGvHD in our study (58.6%) is very similar to that presented in other studies, but there are also some reports referring to a much higher frequency of this complication, reaching the level of 80%.^{1,2,9,10,23,24} Such discrepancies may be due to meaningful differences in the studied cohorts. In our study, patient selection was defined by strict criteria; therefore, the analyzed cohort was relatively homogenous.

A comparison of conditioning regimens Bu-MAC vs TBI-MAC vs other (RTC & RIC) shows TBI as a major risk factor for aGvHD in the pediatric population.^{21,22} Tissue damage, particularly in the gastrointestinal tract, undoubtedly exacerbated by TBI, is thought to be the 1st stage of inflammatory reaction through the activation of host antigen-presenting cells (APCs) and cytokine production. This may initiate an inflammatory cascade, leading to the development of aGvHD.²⁸ Lack of significant differences between the Bu-MAC patients and the group receiving RTC and RIC may suggest that chemo-based myeloablative regimens would not influence aGvHD development.^{29,30} This finding only highlights the contribution of TBI to aGvHD. There are also some studies suggesting that in younger recipients, a low dose of TBI may have a beneficial effect without increasing the risk for aGvHD. This indicates that reduced-toxicity regimens, containing low doses of TBI, could be further investigated also in pediatric HSCT recipients. However, in our study, the best survival rate was noted in the group of patients with Bu-MAC (data not shown). This finding may suggest that chemo-based MAC could be a golden standard for pediatric patients.³¹

Premature discontinuation of CsA increased the probability of aGvHD. There are a lot of reports in the literature about the essential role of calcineurin inhibitors in aGvHD prophylaxis. A large part of these reports focus on the superiority of one of them (CsA or tacrolimus) over the other. Another great part of the literature proves that a low plasma concentration level of CsA, especially in the early period post-HSCT (i.e., 2–3 weeks post-HSCT) is associated with a much higher risk of aGvHD.^{26,32–35} To our knowledge, this is the only study providing results that refer to the influence of early cessation of CsA on the occurrence of aGvHD. There is only one reference regarding premature discontinuation of CsA – the research conducted by Cadenas et al.³² Although this study was performed on a completely different population of patients, it contains a brief suggestion that there is no connection between discontinuation of CsA and aGvHD. The quoted study primarily focused on low CsA plasma concentration levels as a risk factor for aGvHD. To date, the optimal therapeutic approach for CsA therapy as aGvHD prophylaxis after HSCT remains unclear in terms of doses as well as the monitoring strategy.^{36,37} However, our results indicate that calcineurin inhibitors as part of aGvHD prophylaxis may be irreplaceable.

Hematopoietic stem cells transplantation performed before 2009 proved to be a crucial risk factor for

the development of aGvHD and contributed to a worse general outcome. This finding is a reflection of all the improvements in donor selection, transplantation regimens and supportive care that were made during the analyzed period of time. Furthermore, improved OS and reduced TRM after 2009 result from relevant advances in aGvHD therapy that were made during these years. Among all implemented GvHD treatment strategies, extracorporeal photopheresis seems to be promising both in our center (data not shown) and worldwide.

The CD34+ cell dose is one of the most ambiguous factors that have an impact on aGvHD and survival after HSCT. The results of our study showed no correlation between a greater amount of transplanted CD34+ cells and a higher incidence of aGvHD. In parallel, we found no interdependence between the CD34+ cells dose and either OS or TRM. Several studies, performed in both the adult and pediatric population of patients, confirm our findings. Previous research conducted in our center by Kałwak et al. also proves that there is no correlation between the CD34+ cell dose and aGvHD; notwithstanding, a greater amount of transplanted CD34+ cells contributed to better OS and general outcome.⁹ Similar results were reported by Pulsipher et al. in a multi-center study performed on a great cohort of adult and pediatric patients.³⁸ Another study demonstrating no correlation between CD34+ and aGvHD was carried out by Tsirigotis et al.³⁹ It also reports that the number of infused CD34+ cells has no influence on the general outcome. On the contrary, there are some reports suggesting that a higher number of infused CD34 cells may be a risk factor for aGvHD.^{5,40} The extremely discrepant findings concerning the impact of the CD34+ cell dose on aGvHD show that this variable should be analyzed individually, following the patient requirements. This knowledge can be beneficial, allowing clinicians to tailor the composition of the graft in accordance with the patient status and other peri-transplantation variables.

Other analyzed variables, such as donor and recipient age as well as donor–recipient sex mismatch, are well-known risk factors for aGvHD in the adult population. In children, however, the data is sparse. In our study, female donor to male recipient did not prove to be a risk factor for aGvHD, which was, however, noted as a risk factor in our population of male matched sibling donor recipients, when no ATG was given (data not shown).

Conclusions

To conclude, conditioning regimen and adequate immunosuppressive prophylaxis, including continuous CsA use, remain major factors affecting the incidence of aGvHD in children with malignant disorders undergoing UD-HSCT. However, to date, it is not possible to provide strict guidelines for minimizing the risk of aGvHD. According to our results,

the decision to stop CsA administration should be carefully considered, and avoided if possible. Choosing an approach may be discussed if CsA needs to be discontinued for some reason. There is no clear answer. Both MMF and steroids could be useful, but in our belief, MMF seems to be the most appropriate option, with less toxicity.

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