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ORIGINAL ARTICLE

Cyclosporine levels > 195 μg/L on day 10 post-transplant was associated with significantly reduced acute graft-versus-host disease following allogeneic hematopoietic stem cell transplantation

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Abstract

Acute graft-versus-host disease (aGvHD) remains a major complication of allogeneic hematopoietic stem cell transplantation (allo-HSCT). Prophylaxis with cyclosporine A (CsA) is the backbone of GvHD prevention. In a retrospective analysis of patients treated with allo-HSCT, we correlated CsA levels on the day of transplantation (day 0) and on day + 10 with the incidence of acute and chronic GvHD. We assessed 660 patients with either AML $n = 248$, lymphoma/myeloma $n = 127$, MDS/MPN $n = 124$, ALL $n = 79$, CLL $n = 36$, CML $n = 23$, or bone marrow failure $n = 22$. In patients with clinically relevant aGvHD grade ≥ 2, mean CsA levels was lower on day 0 and day + 10 (142 ± 88 μg/L and 183 ± 64 μg/L, respectively) compared to patients without aGvHD (156 ± 81 μg/L and 207 ± 67 μg/L, respectively; day 0: $p = 0.003$; day + 10: $p = 7.57 \times 10^{-9}$). In patients with CsA level < 200 μg/L, the incidence of aGvHD was significantly more frequent compared to patients with CsA levels > 200 μg/L [(234/356 (66%) versus 91/248 (37%); $p = 1.34 \times 10^{-12}$]. In patients with cGvHD, there was no significant difference between CsA levels < 200 μg/L (128/330) compared to CsA levels > 200 μg/L (96/233; $p = 0.312$). The optimal CsA cutoff level for the prevention (i.e., roughly 50% incidence reduction) of aGvHD was > 201 μg/L at day 0 and > 195 μg/L at day + 10. In a competing risk analysis, time to aGvHD grade ≥ 2 (using death of other causes as competing risk) was associated with CsA levels > 200 μg/L on day 0 and on day 10, unrelated donors, myeloablative conditioning (MAC), and for the diagnosis lymphoma/myeloma. Our data support close monitoring with active adjustments of CsA dosing to maintain therapeutic CsA levels above 195 μg/L in the first 10 days of allo-HCST to reduce aGvHD.

Keywords Cyclosporine A · Graft-versus-host disease · Allogeneic hematopoietic stem cell transplantation

Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is used in patients with hematological diseases as curative therapy [1]. However, the success of allo-HSCT is limited by early and late complications such as acute and chronic graft-versus-host disease (GvHD) [2, 3]. Acute (a)GvHD, an often early complication after allo-HSCT, is mediated by host-reactive, donor-derived T cells [2]. Steroid refractoriness in aGvHD is observed in ~ 50% of patients and is a major contributor of mortality and morbidity [4–6]. Endothelial injury is involved in aGvHD, particularly in steroid refractory aGvHD [4–6]. HLA mismatch between donor and recipient, age, cytomegalovirus (CMV) reactivation/end-organ disease, donor-recipient gender mismatch, AB0 incompatibility, still active disease status, stem cell source, donor type, conditioning regimen, and type of GvHD prophylaxis have been shown to be risk factors for developing aGvHD [2, 7, 8]. The pathophysiology of chronic (c)GvHD is much more complex and still remains to be determined [3]. Current approaches for the prevention and treatment of aGvHD involve direct blockade of T cell function [9, 10]. Cyclosporine (CsA), a calcineurin inhibitor (CNI), is one of the most commonly used pharmacologic agents for the prevention of GvHD [11]. Despite its

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widespread use in clinical practice, the dose, target blood level of CsA, and schedule of administration vary among protocols [11–14].

In particular, there is uncertainty about timing; many protocols start CsA on day − 1, others on day − 3 of the HSCT. Whether adequate blood levels of CsA on the day of HSCT or shortly thereafter are of importance for adequate GvHD prophylaxis has not been studied well.

In addition, despite of close monitoring, it is not always easy to keep the CsA levels within the desired range. There are multiple pharmacological interactions such as inhibition of CsA metabolism by, e.g., some of the imidazole antifungal drugs.

Based on these findings, there have been interesting reports conferring impact of CsA levels on the development of acute and chronic GvHD after allo-HSCT, in which higher CsA levels during early post-transplant period contributed to lower risk of both GvHD types [11, 12, 15, 16]. The aim of or study was the correlation of early CsA levels with the incidence of acute and chronic GvHD in patients undergoing allo-HSCT.

Patients and methods

Patient population and study design

This retrospective single-center cohort study at the Department of Hematology of the University Hospital Basel was performed according to the regulations of the local ethics committee. We included all patients (1) with hematological disease, (2) who received an allo-HSCT at our institution between January 2006 and March 2016, (3) with available CsA levels at day 0 (± 1 day) and day + 10 (± 1 day), and (4) who had chart data available for review of acute and chronic GvHD, resulting in a cohort of 660 patients. Patients with a haploidentical HSCT ($n = 36$; 5.5%) and syngeneic HSCT ($n = 11$; 1.7%) were excluded from further analysis. Using our electronic database, we extracted relevant parameters, including sex, age, donor type (HLA match/mismatch, genetically related/unrelated), stem cell source (bone marrow, peripheral blood, cord blood), and allo-HSCT characteristics (e.g., CMV status match of donors/recipients, specific GvHD prophylaxis). Serum creatinine measurements were performed before the start of conditioning. Approximation of glomerular filtration rates was based on CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equations [17], based on serum creatinine. Following the calculation of baseline eGFR, patients were divided into four separate groups: ≥ 90 mL/min/1.73 m^2, group 1; 60–89, group 2; 30–59, group 3; < 15–29, group 4 (following the cutoffs of CKD classification of the Kidney Disease Improving Global Outcomes (KDIGO) 2012 Guideline for the Evaluation and Management of CKD [18].

Conditioning regimens and graft-versus-host disease prophylaxis

Myeloablative conditioning (MAC) regimens included cyclophosphamide in combination with busulfan, cyclophosphamide and total body irradiation (TBI) ≥ 8 Gy, cytarabine, carmustine, etoposide, melphalan, and fludarabine (BEAM-fludarabine), and other protocols. Reduced-intensity conditioning (RIC) regimens consisted of fludarabine with low-dose TBI < 6 Gy, fludarabine combined with busulfan or melphalan, and other protocols. Reasons for RIC were advanced age or relevant comorbidities. GvHD prophylaxis administered along with the MAC was CsA (3 mg/kg bw/day i.v.; starting day − 3 over 6 h) and methotrexate (15 mg/m^2 i.v. day + 1; 10 mg/m^2 day + 3 and day + 6) as well as anti-T cell globulins (ATGs) in cases of unrelated donors and in matched related donors ≥ 40 years, as published [19]. In patients with RIC, GvHD prophylaxis consisted of CsA, methotrexate, and ATGs in cases of unrelated donors and in matched related donors ≥ 40 years, according to institutional standards (if RIC was fludarabine/busulfan), or cyclosporine A [1.5 mg/kg twice daily i.v. over 1 h or 3 mg/kg twice daily p.o. from day − 1 and mycophenolate mofetil (MMF) p.o.; 15 mg/kg twice daily, starting day 0] if RIC was fludarabine/low-dose TBI or BEAM-fludarabine.

The blood concentration of CsA was measured by high-performance liquid chromatography (HPLC) and was the same test about the whole study period. CsA trough levels were measured at least once weekly and additionally after dose adjustments in all patients, with a target level of 150–200 μg/L, although adjustments for levels above target were performed only in the case of toxicity (for example, renal toxicity or hypertension). Blood samples were collected 12 h after the prior dose, just before the morning dose. MMF levels were not measured systematically.

The route of CsA administration was switched to oral at a ratio of 1:2 when patients showed acceptable oral intake. In the absence of aGvHD, CsA was tapered and discontinued by day + 180. MMF was given until day 28 for patients with related donors and was tapered and stopped by day + 56 for those with unrelated donors.

GvHD diagnosis and therapy

Acute and chronic GvHD were diagnosed based on clinical symptoms and/or skin, oral, mucosal, liver, or gut biopsies and were graded using consensus criteria for acute and chronic GvHD [20, 21]. Corticosteroid treatment (methylprednisolone, i.v., 2 mg/kg/day) was started at diagnosis of clinically relevant aGvHD grade ≥ 2. CsA was continued in patients with aGvHD. Corticosteroid resistance was defined as no response after 7 days of treatment or clear progression after 5 days [22]. In cases of steroid-refractory aGvHD, second-

line therapies were alemtuzumab, ruxolitinib, or intra-arterial catheter-guided steroid administration for the treatment of intestinal GvHD.

Statistical analysis

Continuous variables are expressed as mean (± standard deviation [SD]) or median (with interquartile range [IQR]), as appropriate. Categorical variables are shown as rates/proportions. Differences in demographic, clinical, and transplantation parameters were assessed using the chi-squared or Fisher's exact test for categorical variables, and Student's *t* and Mann-Whitney *U* test for continuous variables, depending on data distributions. Because differences in the CsA levels were hypothesized to exist between patients with aGvHD and those without GvHD, we postulated clinically relevant GvHD to be the defining set variable in a receiver operating characteristic (ROC) curve model plotting CsA levels' sensitivity versus 1 − specificity to discriminate between patients with and without GvHD with special consideration of the respective area under the ROC (AUROC). The AUROC values illustrate the strength of a discriminating marker; that is, the better a diagnostic marker can discriminate between two conditions, the closer its AUROC value is to 1. The optimal cutoff point for CsA levels to discriminate for clinically revenant aGvHD was calculated using Youden's index (*Y*)—*Y* = sensitivity + specificity − 1—because this method can be applied to find the optimal cutoff value with the highest sensitivity and specificity when there is no particular requirement for sensitivity and/or specificity [23]. The association of time to aGvHD grade ≥ 2 with CsA levels on day 0 and day 10 adjusted for age, donor type, and conditioning regimen (RIC or MAC), and diagnosis was analyzed by means of a Fine and Gray competing risk regression model for multivariate analysis using death of other causes as competing risk [24]. All *p* values were two-sided. The above computations relied on standard software (SPSS Statistics v22; IBM, Chicago, IL, USA and Stata SE v15; StataCorp LLC, College Station, TX, USA), setting significance at $p < 0.05$.

Results

Patient characteristics

Our study included the analysis of 660 patient treated with an allo-HSCT. Their median age was 47 years (range 19–71); of these, 393 were male (59.5%) and 267 were female (40.5%), and included patients receiving transplants from identical siblings (41.2%), matched related (0.3%), mismatched related (4.2%), syngeneic (1.7%), and unrelated donors (52.6%). Stem cell source was peripheral blood (89.4%), bone marrow (8.3%), or cord blood (2.3%). Most of the patients were transplanted with 10/10 HLA-identical donor (529/660; 80.2%). One antigen mismatch, 9/10 donor, had 94/660 (14.2%) of patients. The main hematologic diagnoses are summarized in Table 1. Conditioning regimens were largely myeloablative (78.6%), as opposed to reduced intensity (21.4%). TBI was used in 260 patients (39.4%). Mean eGFR values via CKD-EPI equations were 91 mL/min/1.73 m^2, corresponding to a CKD stage 1 stage in 590/660 (89%) of patients, stage 2 in 53/660 (8%) of patients, and stage 3 in 17/660 (3%) of patients. The starting dose of CsA was the same, independent from renal function. Adjustments for dose levels above the target range were only performed in the case of renal toxicity or worsening of pre-existing renal impairment. We found no correlation between baseline renal function and CsA levels on day 0 and day + 10, possibly because we had only very few patients with a higher grade of renal impairment.

Acute and chronic GvHD and CsA blood levels

As shown in Table 1, clinically relevant aGvHD (grade ≥ 2) occurred in 344/660 patients (52.1%) and became steroid-refractory in 46/344 patients (13.4%) and 46/660 patients of the whole cohort (7%), respectively. The median time interval from allo-HSCT to diagnosis of aGvHD was 21 days (range 13–42). cGvHD occurred in 235 (35.6%) of patients [limited cGvHD in 139 (21.1%) and extensive cGvHD in 96 (14.5%) of patients].

In patients with clinically relevant aGvHD grade ≥ 2, mean CsA levels was lower at day 0 and day + 10 (142 ± 88 μg/L and 183 ± 64 μg/L, respectively) compared to patients without aGvHD (156 ± 81 μg/L and 207 ± 67 μg/L, respectively; day 0: $p = 0.003$; day + 10: $p < 0.0001$).

In patients with CsA level < 200 μg/L, the incidence of aGvHD grade ≥ 2 was higher than in patients with CsA levels > 200 μg/L [234/356 (66%) versus 91/248 (37%); $p < 0.0001$]. In patients with cGvHD, there was no significant difference between such with CsA levels < 200 μg/L (128/330) compared to those with CsA levels > 200 μg/L (96/233; $p = 0.312$).

In a competing risk analysis, time to aGvHD grade ≥ 2 (using death of other causes as competing risk) was associated with CsA levels > 200 μg/L on day 0 and on day 10 (Table 2), unrelated donors (relative risk [RR] 1.62; 95% CI 1.25–2.11; $p < 0.0001$ on day 0; and RR 1.60; 95% CI 1.20–2.03 on day 10; $p = 0.001$), MAC (RR 0.66; 95% CI 0.49–0.90 on day 0, $p = 0.010$; and RR 0.75; 95% CI 0.55–1.02; $p = 0.067$ on day 10), and for the diagnosis of lymphoma/myeloma (RR 0.68; 95% CI 0.46–0.99; $p = 0.049$).

As suggested by ROC and the AUROC as well as the respective Youden's index, the optimal CsA cutoff level for the prevention (i.e., roughly 50% incidence reduction) of aGvHD was > 201 μg/L at day 0 (AUROC 0.571, 95% CI 0.525–0.618,

Table 1 Patients' baseline characteristics

		All $n = 660$ (100%)
Median age (years) (range)		47 (19–71)
Sex; male		393 (59.5%)
Diagnosis	AML	248 (37.6%)
	ALL	79 (12%)
	CML	23 (3.5%)
	CLL	36 (5.5%)
	Lymphoma/myeloma	127 (19.2%)
	MDS/MPN	124 (18.8%)
	Bone marrow failure	22 (3.3%)
Conditioning regimen	RIC	141 (21.4%)
	MAC	519 (78.6%)
TBI	No	397 (60.2%)
	Yes	260 (39.4%)
Stem cell source	BM	55 (8.3%)
	CB	15 (2.3%)
	PBSC	590 (89.4%)
Donor	Identical sibling	272 (41.2%)
	Matched related	2 (0.3%)
	Mismatched related	28 (4.2%)
	Syngeneic[b]	11 (1.7%)
	Unrelated	347 (52.6%)
HLA-identical		529 (80.2%)
1-Antigen HLA mismatch		94 (14.2%)
2-Antigen HLA mismatch[b]		36 (5.5%)
GvHD prophylaxis		
	CsA	13 (1.1%)
	CsA + MTX	430 (65.2%)
	CsA + MMF	171 (25.9%)
	Other	46 (6.9%)
	ATG used (in all patients)	198 (30%)
aGvHD grade ≥ 2		344 (52.1%)
Time interval from HSCT to aGvHD diagnosis (days) (range)		21 (13–42)
aGvHD steroid-refractory[a]		46 (13.4%)
Chronic GvHD		235 (35.6%)
	Limited	139 (21.1%)
	Extensive	96 (14.5%)
CMV reactivation/end-organ disease		158 (23.9%)

Data shown from evaluable patients for aGvHD

aGvHD acute graft-versus-host disease, *ALL* acute lymphoid leukemia, *AML* acute myeloid leukemia, *ATG* anti-T cell globulins, *BM* bone marrow, *CB* cord blood, *CLL* chronic lymphocytic leukemia, *CML* chronic myeloid leukemia, *CMV* cytomegalovirus, *CsA* cyclosporine A, *MAC* myeloablative conditioning, *MDS* myelodysplastic syndrome, *MMF* mycophenolate mofetil, *MPN* myeloproliferative neoplasm, *MTX* methotrexate, *PBSC* peripheral blood stem cells, *RIC* reduced intensity conditioning, *TBI* total body irradiation

[a] In patients with clinically relevant aGvHD grade ≥ 2

[b] Patients excluded from analysis

$p = 0.003$) and > 195 μg/L at day + 10 (AUROC 0.638, 95% CI 0.594–0.683, $p = 7.59 \times 10^{-9}$) (Fig. 1). Achieving or not a CsA level > 201 μg/L on day 0 was strongly linked to whether RIC (38% of patients reached the level) or MAC (only 15% reached the level, $p = 4.11 \times 10^{-8}$) has been applied and whether TBI was given (31% of the irradiated patients reached the level vs. 13% of those not being irradiated, $p < 0.0001$) and was higher in patients transplanted for lymphomas/myelomas and bone

Table 2 Competing risk regression for multivariate analysis of time to aGvHD grade ≥ 2 using death of other causes as competing risk

Variable	Day 0 HR (95% CI)	*p* value	Day 10 HR (95% CI)	*p* value
CsA level > 200 μg/L	0.43 (0.30–0.63)	< 0.0001	0.50 (0.38–0.65	< 0.0001
Sibling or 1-antigen HLA mismatch donor	1.24 (0.62–2.48)	0.542	1.31 (0.66–2.62)	0.442
Unrelated donor	1.62 (1.24–2.11)	< 0.0001	1.60 (1.20–2.03)	0.001
Myeloablative conditioning	0.66 (0.49–0.90)	0.010	0.75 (0.55–1.02)	0.067

Data shown from evaluable patients for aGvHD

aGvHD acute graft-versus-host disease, *CI* confidence interval, *CsA* cyclosporine A, *HLA* human leukocyte antigen, *HR* hazard ratio

marrow failures ($p = 0.005$), but was not linked to sex, CMV reactivation, or age. Achieving or not CsA level > 195 μg/L at the—considering aGvHD—more relevant day + 10 was slightly linked to the type of underlying disease, the proportion of patients with higher levels being highest in bone marrow failures (i.e., 65%) and lowest in CLL (i.e., 22%), the proportion for all other diagnoses being fluctuating between 33 and 51% ($p = 0.013$), but was not linked to sex, conditioning, TBI, CMV reactivation, or age.

Discussion

GvHD remains one of the main life-threatening complications after allo-HSCT [2, 3]. The standard GvHD prophylaxis strategy is mostly based on the use of CNI in combination with either MTX or MMF [22, 25]. In our study, we found a higher incidence of aGvHD in patients with CsA levels < 200 μg/L early after allo-HSCT on day 0 and day + 10, but no difference for cGvHD, regardless of the donor type or intensity of the conditioning regimen. This sheds light on the issue of timing of GvHD prophylaxis initiation and on the importance of achieving therapeutic drug levels early in the transplant course.

We initiate CsA prophylaxis i.v. on day − 3 or on day − 1 (when using fludarabine/low-dose TBI or BEAM-fludarabine protocols) with the goal of achieving target levels by the time of the infusion of the allograft. To reach this goal before the graft infusion, we measure the CsA level and if the level is < 150 μg/L, we adjust the dosage. The rationale for this strategy

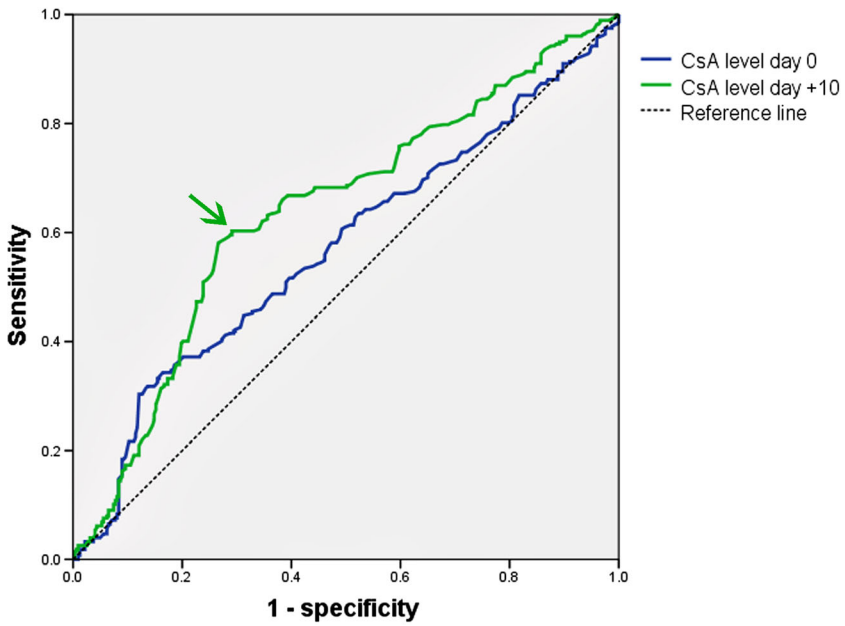

Fig. 1 Receiver operating characteristic (ROC) curve of cyclosporine A (CsA) levels on day 0 (blue) and day + 10 (green) with "no development of aGvHD grade ≥ 2" a state variable. The reference diagonal dotted reference line corresponds to a hypothetical action without any capability of influencing the risk to develop aGvHD. Note that the deviation of the ROC curves and the areas under the ROC (AUROC) as well as their 95% CI (see "Results" section) of CsA levels are to the upper left corner and > 0.5, respectively, indicating that any exposition to CsA decreases the chance of aGvHD development. The AUROC under the green curve (day + 10) is considerably larger than that under the blue one (day 0), pointing towards the more important relevance of day + 10's CsA levels in preventing aGvHD than those on day 0. The green arrow designates the point corresponding to the CsA level (i.e., > 195 μg/L) with highest both specificity and sensitivity to prevent aGvHD development and is at the same time the furthest away point to the dotted reference line

is that the activation and expansion of alloreactive T cells occur early after the graft infusion. In a GvHD mouse model, allo-reactive $CD4^+$ and $CD8^+$ T cells localized early in GvHD target organs like skin, liver, and gastrointestinal tract after transfusion [26]. Higher CsA level resulted in improved survival in this mouse GvHD model [27]. Thus as also supported by our current study, achieving therapeutic levels of CsA early after allo-HSCT would be potentially protective against aGvHD. Our results are in line with Rogosheske et al., who also found that higher CsA levels early post-transplantation contributed to lower risk of aGvHD, but not of cGvHD [12]. In contrast, a study by Park et al. showed that CsA levels between 200 and 250 μg/L during the first month after transplantation were significantly associated with the decreased risk of moderate to severe cGvHD [11]. The reason could be that they measured CsA levels over a longer time period of 3 months after allo-HSCT and considered the average monthly CsA level.

In our study, only a small proportion of patients reached CsA level > 201 μg/L on day 0. A study by Kedmi et al. showed that an early initiation of CsA at day − 4 reduced the severity of aGvHD, extent of cGvHD, and GvHD-associated mortality without impact on overall survival [16].

Our data confirmed the data of another study on the importance of CsA levels measured close to the development of aGvHD describing an association between CsA level < 200 μg/L and a higher risk of developing aGvHD in the following week [15].

In contrast to our study, a study by Ram et al. showed that higher calcineurin inhibitor (CNI) (CsA or tacrolimus) levels in the first 2 weeks after allo-HSCT were associated with a lower risk of aGvHD in non-myeloablative, but not in myeloablative transplants in a large cohort of 1181 patients [28]. In contrast to our study (we start CsA on day − 3 in MAC and on day − 1 in RIC), they started CsA on day − 1 in MAC and on day − 3 in RIC. Further, we applied methotrexate as GvHD prophylaxis on days + 1, + 3, and + 6 compared to four applications in the study by Ram et al. (on days + 1, + 3, + 6, and + 11).

The different starting points of CNIs in RIC and MAC and four MTX applications compared to three could explain the differences in GvHD incidence in both studies.

A further difference was that in study by Ram et al., CNI levels were obtained daily when applied intravenously. In our study, we routinely measured CsA levels once weekly. But in the case the target level is not reached, we performed CsA dose adjustments and controlled the CsA levels after 48 h. In a competing risk analysis, time to aGvHD grade ≥ 2 (using death of other causes as competing risk) was associated with CsA levels > 200 μg/L, and for the diagnosis lymphoma/myeloma (RR 0.68; 95% CI 0.46–0.99; $p = 0.049$). The reason could be that more patients with lymphoma/myeloma achieved CsA level > 201 μg/L on day 0 compared to other diagnosis.

We acknowledge that there are substantial limitations to the current study. We had a heterogeneous study population with different diseases, different remission states before allo-HSCT, conditioning regimens, GvHD prophylaxis, and use of ATG, but therefore representing a real-world practice. Consequently, heterogeneous distribution of treatment procedures, especially with regard to whether or not patients were treated with ATG because it could have affected T cell activity as well, might possibly have caused confusion in the interpretation of the results that we can neither exclude nor address. Additionally, the study is limited by its retrospective nature. Further, the starting time points of CNI in RIC and MAC conditioning regimen and how much MTX was applied for GvHD prophylaxis can influence GvHD incidence. Further, we have the influence on renal function on CsA levels. In our cohort, 11% of patients had renal insufficiency before start of conditioning (stage 2 in 53/660 (8%) of patients and stage 3 in 17/660 (3%) of patients). But in case of renal toxicity or decreasing renal function in pre-existing renal damage, CsA levels were adapted accordingly to avoid further renal toxicity.

In a current study, we evaluate the incidence of these proposed CsA levels in patients with an increased CsA starting dose on outcome. In conclusion, our data highlight that close monitoring with active adjustments of CsA dosing to maintain therapeutic CsA levels above 200 μg/L in the first 10 days of allo-HCST could reduce the incidence of aGvHD. In addition, results of this study advocate starting CsA administration early, e.g., day − 3 to assure adequate drug levels on the day of HSC infusion and the early transplant period thereafter.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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