Influence of cyclosporine A trough level on acute graft versus host disease prophylaxis in pediatric allo- hematopoietic stem cell transplantation

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Abstract

Purpose Cyclosporine A (CsA) is the cornerstone prophylactic drug for graft versus host disease (GVHD) in allogeneic hematopoietic stem cell transplantation (allo-HSCT); however, its optimal trough level is yet to be determined. Therefore, in this study, we focused on the CsA trough levels and estimated their association with acute GvHD (aGVHD) risk in a consecutive cohort of 72 pediatric patients receiving allo-HSCT.

Method The trough CsA level was monitored 3–4 times in a week via mass spectrometry analysis during medication. The occurrence of GVHD, the trough of CsA level before and after allo-HSCT and other clinical information were recorded.

Results The cumulative incidence of aGVHD at 100 days was 19.44% for grade I and 23.61% for grades II–IV. Multivariable Cox regression analysis revealed that the optimal trough CsA level for aGVHD prophylaxis was >119 ng/mL, 146–214.5g/mL, >123.25 ng/mL, and 100.2–166 ng/mL on the -3^{rd} day, 3^{rd} day, 1^{st} week, and 2^{nd} month after HSCT, respectively. None of the cutoff values for CsA were significantly associated with the survival outcome.

Conclusion Our findings indicate that adequate management of CsA levels during the engraftment period might improve the clinical outcomes for pediatric patients undergoing hematopoietic stem cell transplantation.

Clinical trial registration: China Clinical Trial Registration Center (ChiCTR2000034702). Registered 15 July 2020.

Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a potentially curative treatment for several hematological malignancies. Haploidentical HSCT (haplo-HSCT) is a common procedure in China, constituting 48% of all allo-HSCT cases owing to the shortage of HLA-identical siblings and unrelated donors [1]. The GIAC [2] ('G'CSF-stimulation of the donor; 'I'ntensified immunosuppression through post-transplantation CsA, mycophenolate mofetil, and short-course methotrexate; 'A'ntithymocyte globulin added to conditioning to help prevent GVHD and aid engraftment; and 'C'ombination of PBSC and bone-marrow allografts) and PTCy (post-transplant high-dose cyclophosphamide [Cy]) protocols are the two mainstream clinical practice models in which transplantation is performed without *in vitro* T-cell depletion. [3, 4]The GIAC protocol is myeloablative, and the allografts comprise colony-stimulating factor-primed (CSF) mobilized bone marrow (BM) and peripheral blood (PB) stem cells combined with intensive pharmacological immunosuppression, including antithymocyte lobulin (ATG). Over 50% of the haplo-HSCT performed worldwide follow this protocol [3]. Although the implementation of the GIAC protocol has resulted in acceptable nonrelapse mortality (NRM), reduced relapse rate, and favorable disease-free survival after allo-HSCT, it is associated with greater post-transplant complications, including acute graft-versus-host disease (aGVHD) and chronic GVHD (cGVHD), compared with the PTCy protocol. [4–6] While maintaining its advantage, the GIAC protocol has been fine-tuned and has progressed in the recent decade, thereby enabling a significant reduction prevalence for patients with aGVHD (23–43%). [5, 7, 8]One of the key challenges in successful transplantation across HLA barriers is to control the incidence of aGVHD and cGVHD. Cyclosporine (CsA) or tacrolimus-based regimens combined with methotrexate (MTX) or mycophenolate mofetil (MMF) are widely used globally and are currently considered the backbone of alloreactivity prophylaxis. [9]

A higher concentration of CsA, particularly in the early post-transplantation period, has been associated with reduced GVHD risk. [10–14] However, the coadministration of drugs in GVHD prophylactic regimens, targeted blood concentrations, starting dose, and duration of CsA employed in malignant or nonmalignant HCT show wide variations. The heterogeneity is also reflected in the differences between pediatric and adult allo-HSCT practices and the contradiction in published guidelines and real-life clinical practices. [15, 16] Hence, in this study, we aimed to capture the appropriate CsA levels for different pre- and early post-transplantation periods to reduce the incidence of GVHD in a cohort of Chinese pediatric patients receiving allo-HSCT with the GIAC protocol.

Materials And Methods

Cases

A total of 72 consecutive pediatric cases (age: 1–18 years) who received allo-HSCT at the Hebei Yanda Lu Daopei hospital in the Hebei Province of China between December 2017 to June 2018 were included in this assessment. The cases were followed longitudinally and involved 1-year cyclosporine concentration and 1 or 3 years of overall survival (OS) until death or loss to follow-up. The endpoint of the last follow-up for all surviving patients was August 1, 2020. This study was performed in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Yanda Lu Daopei Hospital.

Transplantation procedure and diagnosis of GVHD

All patients showing hematological malignancies underwent GIAC protocol-based myeloablative HSCT. Based on the preconditioning treatment, the patients were grouped as follows: (1) 27 patients received preconditioning treatment composed of cytarabine (ARA-C) (2–4 g/m^2/day i.v.) on days − 10 to -9 (days are expressed relative to transplantation), busulfan (3.2 mg/kg/day i.v.) on days − 9 to -6, Cy (500 mg/m^2/day i.v.) on days − 5 to -2, methyl chloride hexamethylene urea nitrate (Me-CCNU) (250 mg/m^2/day orally) once on day − 3, and a cumulative dose of ATG (rabbit ATG; 7.5

mg/kg for MUD and 3.5–5 mg/kg for matched sibling donors (MSD) for 4 days; Genzyme Polyclonals S.A.S, USA) on days − 5 to -2; (2) 38 patients received total body irradiation (TBI)-based conditioning regimen (1200 cGy total, 200 cGy Bid on days − 8 to -6, with Cy ATG ARA-C and Me-CCNU); (3) 7 aplastic anemia patients received treatment composed of busulfan (3.2 mg/kg/day i.v.) on days − 7 to-6, Cy (500 mg/m^2/day i.v.) on days − 5 to -2, FLU (40 mg/m^2/day i.v) on days − 5 to -2, and ATG (rabbit ATG; 10 mg/kg total for 4 days; Genzyme Polyclonals S.A.S) on days − 5 to -2. These patients received grafts of combined mobilized PB stem cells (PBSC) and BM or PBSC alone [2]. The GVHD prophylaxis regimen was composed of cyclosporine A (CsA), short-term MTX, and MMF [14, 20]. MTX was administered via i.v. at 15 mg/m^2 on day + 1 and 10 mg/m^2 on days + 3, +6, and + 11. MMF (15 mg/kg every 12 h) was administered orally from day − 9 until neutrophil engraftment.

We followed the consensus recommendations of China for monitoring, treating, and preventing leukemia relapse after allo-HSCT [17]. The characteristics and features of the patients, donors, and HSCT procedures are summarized in Table 1. OS was calculated from the date of HSCT until the date of last follow-up, the date of death from any cause, or to August 1, 2020 and 1 year after the diagnosis of HSCT acute GVHD in accordance with the revised Glucksberg criteria (grades 0–IV) [18] and chronic GVHD was diagnosed according to the NIH classification [19]. The time taken to neutrophil recovery was defined as the first of 3 consecutive days in which the absolute neutrophil count was > 0.5 × 10^9/L and platelet recovery as the first of 7 consecutive days with a platelet count of > 20 × 10^9/L without the need for platelet transfusion. Transplant-associated thrombotic microangiopathy (TA-TMA) was defined in accordance with the Cincinnati Children's Hospital Medical Center criteria [20].

CsA level monitoring and administration

The whole blood CsA level was measured by an in-house developed high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) protocol. The chromatograph adopts the Jasper HPLC (SCIEX, USA). The mass spectrometer used was the SCIEX 4500 tandem quadrupole MS (SCIEX), using an electrospray (ESI) ion source. The data were analyzed with the Analyst MD Version 1.6.2 (SCIEX).

CsA (1.5 mg/kg every 12 h i.v.) was started from day − 9. The trough CsA level was measured 3–4 times a week, and the dose was titrated with the target CsA level of 100–200 ng/mL, taking into account the toxic and side effects. CsA administration was switched to the oral route when the patient's bowel function returned to normal. After discharge from the hospital post-transplantation, the patients visited the outpatient clinic for follow-up, where their CsA blood levels were monitored. The laboratory assessments were performed 2–3 times every week during the first 6 months following HSCT, and once a week until 1 year thereafter. The blood samples were assessed 12 h after the prior dose, immediately before the morning dose. CsA was tapered at 6 months and gradually discontinued at 12 months after HSCT in patients with malignant disease who had a persistent negative minimal residual disease (MRD) and no aGVHD or cGVHD.

Considering that we focused on the occurrence of both cGVHD and aGVHD, we examined the drug levels for a more extended period (i.e., 3 days before HSCT and for 12 months after HSCT). Based on the different concentrations measured during a particular week and month, the mean weekly and monthly concentrations of CsA were calculated for each patient.

Statistical analyses

Variables were tested for normality with Shapiro–Wilk statistics, and categorical variables were described as the mean (SD) and percentages. Other continuous variables that were not Gaussian distributed were presented as median. The Kaplan–Meier method was applied to estimate the OS, while endpoints were subject to competing risks estimated using the cumulative incidence methods using death as a competing risk for the cGVHD endpoints. Multivariate analyses were performed by Cox regression analysis for aGVHD, cGVHD, and OS, considering the age at the time of transplantation, gender, donor type, source of cells, GVHD prophylaxis, type of conditioning regimen, cytomegalovirus infection at the time of transplant, type of disease, CD3, CD34, and the use of ATG. The factors significant at $P < 0.15$ in univariate analysis were considered as candidates in the models. The backward method was applied to select variables for the final models. The Cox proportional hazards model was adopted to estimate aGVHD, while the Fine and Gray (competitive) model was used to analyze cGVHD. Cut-off of continuous variables were grouped mainly using the following 2 cases: (1) median classification was adopted in a competitive model for the cGVHD and (2) Maximally Selected Rank and Statistics (R 3.5.2: packages maxstat) [21] for aGVHD. After correcting the results of the multivariate analysis, the cyclosporine concentration was introduced into the corresponding model. The results were reported as probability (%) and 95% confidence intervals (CI). *p*-values for time-to-event endpoints refer to HR analyses throughout the follow-up. Statistical analyses were performed with SAS (version 9.4; Cary, NC). CsA's smooth processing was adopted for the penalized spline (P-spline) function and implemented using the R software version 3.5.2: packages smooth HR. $p < 0.05$ was considered to indicate a statistically significant difference.

Results

Characteristics of donors and transplantations

In this study, 72 consecutive pediatric HSCTs were analyzed (median age, 8 years; range, 1–18 years), which included 44 male patients (61.11%). The diagnoses were acute myeloid leukemia (AML, n = 29, 40.28%), acute lymphoblastic leukemia (ALL, n = 24, 33.33%), and others (n = 19, 26.39%). Twenty-eight patients (38.89%) were treated with TBI. Forty-seven (65.28%) patients received haplo-HSCTs. Twelve pediatric patients underwent transplantation from unrelated donors (16.67%). The stem cell source was both BM and PBSC in 79.17% (n = 57) of the cases, PBSC alone in 16.67%

(n = 12) of the cases, and cord blood (CB) in 4.17% (n = 3) of the cases. The median times of neutrophil and platelet engraftment were 13 days (interquartile range[IQR] 12–17 days) and 13 days ([IQR] 12–17.68 days), respectively. Patient, donor, and transplant characteristics are listed in Table 1.

Multivariate analyses of aGVHD, cGVHD, and OS

aGVHD and cGVHD

The cumulative incidences of aGVHD at 100 days were 19.44% (95% CI, 11.06–30.47%) for grade I and 23.61% (95% CI, 14.40–35.09%) for grade II–IV. In multivariate analysis, three factors affected aGVHD, as follows: (1) Patient age > 3 years (n = 65) was associated with a significantly lower incidence of aGVHD ($p = 0.015$); (2) Donor's age > 31 years was linked to a significantly higher incidence of aGVHD ($p = 0.024$) and aGVHD II–IV ($p = 0.050$); (3) Elevated absolute lymphocyte and monocyte graft counts (ALC and AMC, respectively) were related to an increased incidence of aGVHD ($p = 0.007$) and aGVHD II–IV ($p = 0.004$) (Table 2).

The 1-year cumulative incidence of cGVHD was 31.94% (95% CI, 21.44–43.99%). An analysis of the incidence of cGVHD over time using the Kaplan–Meier curve and Cox regression analysis revealed that recipients with unrelated donors showed an incidence lower than those with HLA-identical sibling donors (HR: 8.702, CI: 1.148–65.983 $p = 0.036$) and similar to those with haploidentical donors (HR: 3.966, CI: 0.55–28.582 $p = 0.172$). Moreover, regardless of the donor type, the $CD3^+$ cell count of the recipient's weight demonstrated significance in multivariate analyses (Table 2). However, the cytomegalovirus status of the donor–recipient air, sex of the donor/patient, conditioning regimen, and graft type did not impact cGVHD incidence in our model.

Os

The 1-year probability of OS following HSCT was 87.5% (9 patients died), and the total OS was 79.17% (15 patients died) with the median follow-up of 28 months (range: 51–1291 days). For all patients who died, the median time from transplantation to death was 866 days after HSCT. Multivariate analysis indicated that the incidence of TMA (n = 6), $CD3^+$ graft content, and time for platelet engraftment were significantly associated with OS (Table 2). Further analysis suggested that the cutoff values for CsA were not significantly associated with the OS outcome ($p > 0.05$).

Trough CsA level and GVHD

Trough blood CsA level

In our study, all patients started CsA from day − 9, and CsA level was monitored 3–4 times a week in the early period. The median concentrations of CsA in the blood in the − 3 days, 1st, 2nd, 3rd, and 4th week and 1st, 2nd, 3rd, 3rd − 6th, and 6th − 12th month after HSCT were 97.34 (IQR, 60.75–131.5), 158.50 (IQR, 123.00–197.33), 161.67 (IQR, 115.00–202.67), 173.00 (IQR, 133.50–210.50), 162.13 (IQR, 128.50–202), 128.25 (IQR, 81.5–155.5), 133.13 (IQR, 89.33–174.5), 91.13 (IQR, 52.00–115.80), 67.68 (IQR, 47.10–99.95), and 81.21 (IQR, 26– 128.40) ng/mL respectively. Based on the predefined target range of 100– 200 ng/mL in the early period after HSCT, the distribution of CsA level during the different periods is shown in Fig. 1. The results implied that 38.33%, 66.67%, 59.15%, 62.32%, 64.52%, 47,5%, 60%, 42%, 25%, and 48.28% of the patients had a CsA level ranging from 100 to 200 ng/mL during the − 3 days, 1st, 2nd, 3rd, and 4th week and 1st, 2nd, 3rd, 3rd − 6th, and 6th − 12th month after HSCT. The median peak of CsA levels occurred in the third week after transplantation in all patients. The CsA dose was constantly adjusted according to the measured CsA levels; hence, most patients achieved the target concentration.

The occurrence of GVHD according to the CsA level

After adjusting for the donor's age and the graft ALC and AMC cells, the CsA level was introduced into the corresponding model. An analysis of the incidence of aGVHD over time using Cox regression analysis revealed the cumulative incidence of acute GVHD according to the blood CsA levels during the pre- and post-transplant period (Fig. 2). Maximally Selected Rank and Statistics [21] indicated that the CsA level as an accurate discriminator of the risk of I–IV aGVHD and grade II–IV aGVHD.

The cutoff values providing an alternative balance avoid the occurrence aGVHD were 119 ng/mL, 146–214.5 ng/mL, 123.25 ng/mL, and 100.2–166 ng/mL at − 3 days, 3rd and 1st week, and 2nd month after HSCT, respectively (Table 3, Fig. 2). Patients having the lowest CsA level in the 3 days preceding HSCT had a significantly higher risk of aGvHD, corresponding to an HR of 0.304 (95% CI: 0.124–0.747; $p = 0.010$), and those with the lowest value also had a risk of grade II–IV aGVHD in the 1st week after HSCT (HR: 0.273; 95% CI: 0.099–0.753; $p = 0.012$). Moreover, during the 3rd week following HSCT, low CsA level (≤ 146 ng/mL) and high (> 214.5 ng/mL) CsA level were the independent parameters significantly associated with an elevated risk of aGVHD. (HR: 3.541, 95% CI: 1.569–7.948; $p = 0.002$ and HR: 2.799, 95% CI: 1.052–7.441; $p = 0.039$, respectively). Meanwhile, during the 2nd month following HSCT, high (> 166 ng/mL) CsA level was the independent parameter significantly associated with an increased risk of grade II–IV aGVHD (HR: 25.661, 95% CI: 2.707–243.26; $p = 0.005$); low (≤ 100.2 ng/mL) CsA level showed the same trend but was not significant (HR: 4.943, 95% CI: 0.511–47.790; $p = 0.168$). In contrast, no other significance was found in multivariate analyses between CsA level in other periods and aGvHD. No cutoff values were detected for cGVHD.

Discussion

To the best of our knowledge, this study is the first to demonstrate the optimal trough CsA level in children during the different pre- and post-transplant periods for aGVHD and cGVHD prophylaxis based on the GIAC protocol. As children constitute a large proportion of the younger generation in China, haplo-HSCT cases in China account for nearly 48% of the total. [1] Accordingly, the GIAC protocol has been widely incorporated in most comprehensive HSCT centers in China. In addition, Handgretinger [22] estimates that more than half of all HLA haplotype-mismatched transplants will be performed worldwide following similar protocols. Previous studies at our center demonstrated that conditioning regimens based on GIAC achieved favorable engraftment, OS, and an acceptable morbidity of GVHD among allo-HSCT patients [23–29].

The optimal trough CsA level for prophylactic aGVHD is shown here after controlling other factors in multivariate analysis. Our research is the first one to report the optimal CsA level before transplantation. Early initiation of CsA before HSCT can achieve a high CsA trough blood concentration rapidly during the early post-HSCT period; hence, the drug was initiated − 9 days before HCT in our analysis, and statistical results showed that if the CsA level is > 119 ng/mL during the − 3 days, optimal prophylactic efficacy can be achieved. This is different from the PTCy protocol in which CsA is typically initiated late, usually at + 3 and + 4 day, because the early initiation of CsA before haplo-HCT is thought to abolish the PTCy effect [30]. Very early immunosuppressive treatment may reduce the efficacy of PTCy by preventing the activation and proliferation of alloreactive T-cells, which contributes to increased susceptibility to PTCy.

An Israel study on matched allogeneic stem-cell transplantation established that the initiation of CsA on day − 4 is better than that on day − 1 in terms of acute GVHD severity, cGVHD extent, and GVHD-associated mortality. [31] A review on kidney transplants also stated that the immune system before the transplantation is more conducive to the establishment of tolerance than that afterward[32]. Therefore, controlling the pretransplant CsA level is essential for the favorable outcome of HSCT.

Furthermore, the optimum CsA level in the 1st month shows significant age-based differences. Our findings suggest that a trough CsA level of > 123 ng/mL in the 1st week and 146–214 ng/mL in the 3rd week after allo-HSCT might be appropriate for prophylactic aGVHD. This finding is similar to that of a French pediatric study, which reported a cutoff value of > 120 ng/mL during the 1st 2 weeks [33]. Possibly, no significant differences exist in CsA tolerance across racial/ethnic subgroups in children. However, this result is different from those reported in other studies performed, which included adult patients. The findings indicated that > 301 ng/mL in the 1st week [34], 350–500 ng/mL in the 1st month [35], > 200 ng/mL in the 1st 2 weeks [14], and ≥ 348 ng/mL in the 1st week [11] post-transplant were the most significant parameters associated with a reduced risk of aGVHD. Drug metabolism with substantial variation based on age contributed to this difference.

Moreover, our result shows that a CsA level of 100–166 ng/mL in the 2nd month after HSCT is optimal for aGVHD prophylaxis. This value is similar to the European guideline, which is 100–200 ng/mL for the same period after HSCT [36, 37]. Most guidelines [15, 16, 36, 38] recommend two drugs for GVHD prophylaxis (CsA plus MTX or CsA plus MMF), but we combined three drugs. Early multidrug use can lower the concentration and achieve better efficacy, thereby reducing the side effects of high-dose monotherapy on normal tissues.

In our study, when compared with MSD, unrelated donor-matched unrelated donors (MUD) recipients exhibited significantly lower rates of cGVHD. This finding is not an undesired outcome for patients with HSCT. Several factors likely contributed to this result. One possible explanation for this anomalous trend in donor type is the difference in ATG levels between MSD and MUD. Most centers used additional ATG for MUD and mismatched donor HSCT but not for MSD. A recent study [16] of the European Society for Blood and Marrow Transplantation centers (26 countries) showed that 21% (16/74) of the centers used antithymocyte globulin (ATG) in MSD for GVHD prophylaxis. In comparison, almost all patients (94%, 68/72 including MSD patients) received ATG in the present study, and the ATG dose used in MUD was almost two-fold higher than that in MSD. According to the pharmacokinetic study of Markus G. [39] in pediatric patients, the increase in the serum level of ATG is proportionate to the infused dose (when not > 20 mg/kg). It is, therefore, reasonable to speculate that the ATG level in MUD was almost two-fold higher than that in MSD in our study. Multiple studies [40, 41] have suggested that a high serum level of ATG immediately before graft infusion for GVHD prophylaxis is safe and effective, thereby resulting in low rates of cGVHD.

Several factors are thought to have contributed to the low incidence of relapse (6.3%, 4/64) among patients with hematological malignancies in this study. First, the accurate monitoring of drug levels in the medium-term treatment regimen contributed to this result. The effector T cells are not only the major players in the pathogenesis of cGVHD but also in the graft-versus-leukemia (GVL) effect, both of which can be abrogated by intense immunosuppressive therapy. Tang et al. [42] have reported that mild and moderate cGVHD were associated with a significantly lower risk of 3-year relapse. Thus, the goal of optimizing the CsA levels is to acquire the benefit of minimizing GVHD and promoting GVL. In this study, 65% of the recipients underwent haploidentical HSCT compared with MSD, which amounted to only 18%. Greater histoincompatibility between donors and recipients is associated with augmented GvL and, hence, a lower risk of relapse.

In this study, no significant correlation was observed between CsA level and OS. This finding is consistent with data from several studies in recent years [11, 34, 35]. One probable reason is that increased transplant-related risks, such as NRM caused by GVHD, are offset by the reduction in the risk of relapse achieved by GVHD.

Several factors influence the CSA levels, such as the concurrent use of agents such as azoles [43], interindividual differences in the variability of drug delivery, uptake, and metabolism [44, 45], and the disease status of the patient. Thus, providing pharmaceutical care for CSA by adjusting CSA doses can achieve varying ranges, which was reported as optimal in this study at different times during allo-HSCT. Several factors, such as the donor and patient ages and the numbers of graft ALC and AMC cells, were considered in this multivariate analysis. Therefore, it was possible to more closely resemble the real-world optimal intervals of CSA after calibrating these variables.

In conclusion, this study has provided optimal CsA cutoff levels for different pre- and post-transplantation periods, which might help fine-tune the manipulation of the balance between GVHD and GVL. Furthermore, the success rate of allo-HSCT and the quality of life of patients undergoing the procedure can be enhanced.

Declarations

Ethical Approval

This study was performed in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Yanda Lu Daopei Hospital.

Competing interests

The authors declare no competing interests.

Author contribution

Zi-Yi Yang, Hong-Xing Liu, and Jian-Ping Zhang designed the research, performed research, analyzed data, and wrote the paper. Lei-Wang, Ying-Zhao, Shuyu Zhang, and Qian-Zhang contributed to data extraction, analyzed data, and wrote the paper.

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Availability of data and materials

See online supplementary material (if available) and just ask the corresponding author for it.

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Tables

Table 1. Selected clinical characteristics pre- and peri-HSCT in pediatric patients

characteristics	No(%),or Median(IQR)
Patient age, median(IQR),years	8(5-12)
Male patient gender, no.(%)	44(61.11)
Donor age, median (IQR), years	33(25.58-38)
Donor, no.(%)	
Unrelated	12(16.67)
identical	13(18.06)
Haploidentical	47(65.28)
Sex of donor/ patient, no.(%)	
Male / Female	16(22.22)
Female / Male	15(20.83)
Male / Male	29(40.28)
Female / Female	12(16.67)
CMV status patient, no.(%)	
Negative	43(59.72)
Positive	29(40.28)
Diagnoses, no. (%)	
Acute myeloid leukemia	29(40.28)
Acute lymphoblastic leukemia	24(33.33)
Aplastic anemia	7(9.72)
Myelodysplastic syndrome	2(2.78)
Other*	10(13.89)
Mononuclear cell×10^8/kg, median(IQR)	9.21(7.36-11.8)
$CD3^+$cell ×10^8/kg, median(IQR)	1.77(1.3-2.62)
$CD34^+$cell ×10^6/kg, median(IQR)	5.9(4.42-6.06)
Stem cell source, no.(%)	
BM+PBSC	57(79.17)
PBSC	12(16.67)
CB	3(4.17)
TMA after HSCT, no.(%)	
Yes	6(8.33)
No	66(91.67)
Conditioning regimen, no.(%)	
TBI (+)	28(38.89)
BU (+)	44(61.11)
ATG (+)	68(94.44)
ABO mismatch	34(47.22)

* congenital agranulocytosis, chronic myeloid leukemia, mixed cell leukemia. Abbreviations: ATG, antithymo-globulins; CMV, cytomegalovirus; TBI, total-body irradiation: BM, bone marrow; PB, peripheral blood; CB, cord blood HSCT, hematopoietic stem cell transplantation.

Table 2. Multivariate analysis for aGVHD, cGVHD and OS

	aGVHD		aGVHD II-IV			cGVHD			OS	
	HR(95%CI)	P	HR(95%)	P		HR(95%CI)	P		HR(95%CI)	P
Age					**Donor**			**TMA**		
<3(ref) (n=7)	1		-		Unrelated donor	1		No	1	
≥3 (n=65)	0.343(0.145-0.812)	0.015	-	-	HLA-identical sibling donor	8.702 1.148-65.983	0.036	Yes	5.132 1.223-21.54	0.025
Age of donor					Haploidentical donor	3.966 0.55-28.582	0.172	**$CD3^+$cell $\times10^8$/kg**		
<31(ref)	1		1		**Graft $CD3^+$cell $\times10^8$/kg**			≤2.67(ref)	1	
≥31	2.837(1.144-7.035)	0.024	3.530(1.002-12.429)	0.05	Continuous, effect for +1y	1.244 1.126-1.373	<0.001	>2.67	9.131(2.599-32.079)	0.001
Graft ALC and AMC cell$\times10^8$/kg								**Time for platelet engraftment**		
<8.07(ref)	1		-					≤15(ref)	1	
≥8.07	3.849(1.445-10.249)	0.007	-	-				>15	4.919(1.384-17.489)	0.014
<9.56(ref)	-		1							
≥9.56	-	-	6.374(1.802-22.551)	0.004						

ref, reference.

absolute lymphocyte and monocyte graft counts (ALC and AMC, respectively

Table 3. Multivariate analysis of aGVHD and CsA trough concentration

	aGVHD I- IV		aGVHD II-IV	
	HR(95%CI)	P	HR(95%CI)	P
-3 days				
≤119(ref)	1		-	
>119	0.304 0.124-0.747	0.01	-	-
3rd week		0.008		
≤146	3.541 1.569-7.984	0.002	-	-
146-214.5((ref))	1		-	
>214.5	2.799 1.052-7.441	0.039	-	-
1st week				
≤123.25(ref)	-		1	
>123.25	-	-	0.273 0.099-0.753	0.012
2nd month				0.008
≤100.2			4.943 0.511-47.790	0.168
100.2-166(ref)			1	
166			25.661 2.707-243.26	0.005

Figures

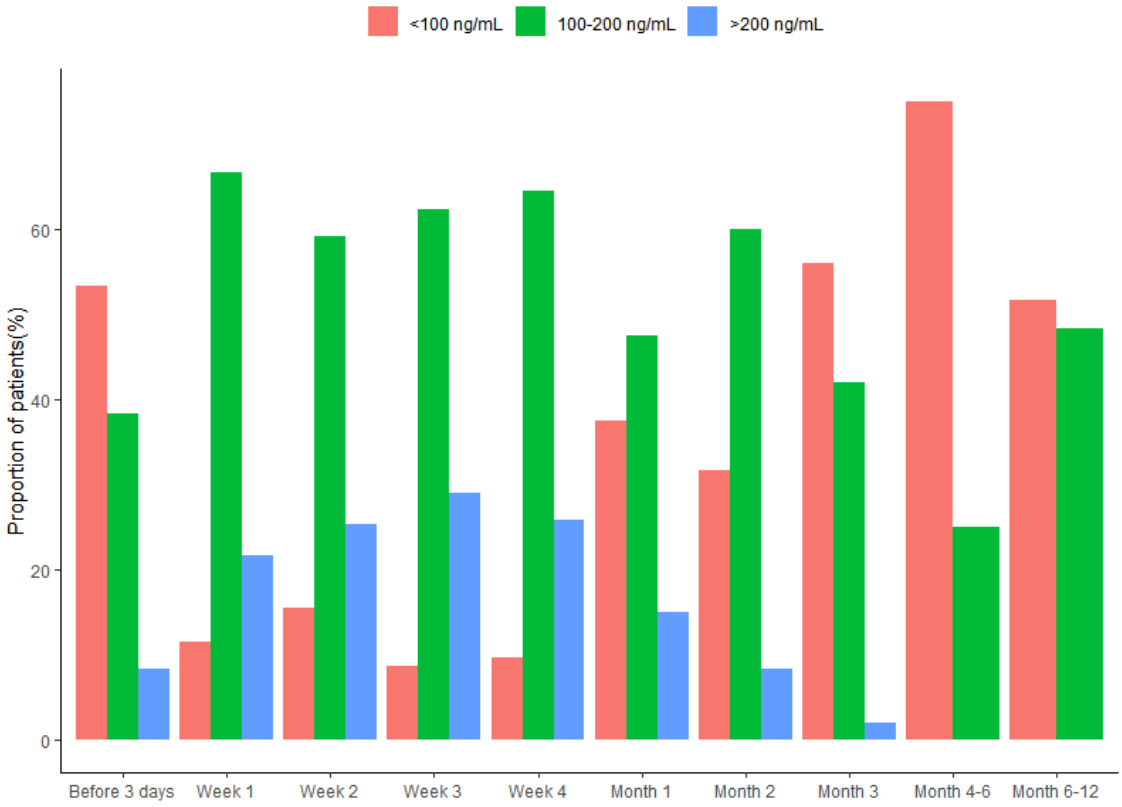

Figure 1

Distribution of patients in accordance with the CsA level during the 12-month of the study period. CsA, cyclosporine A.

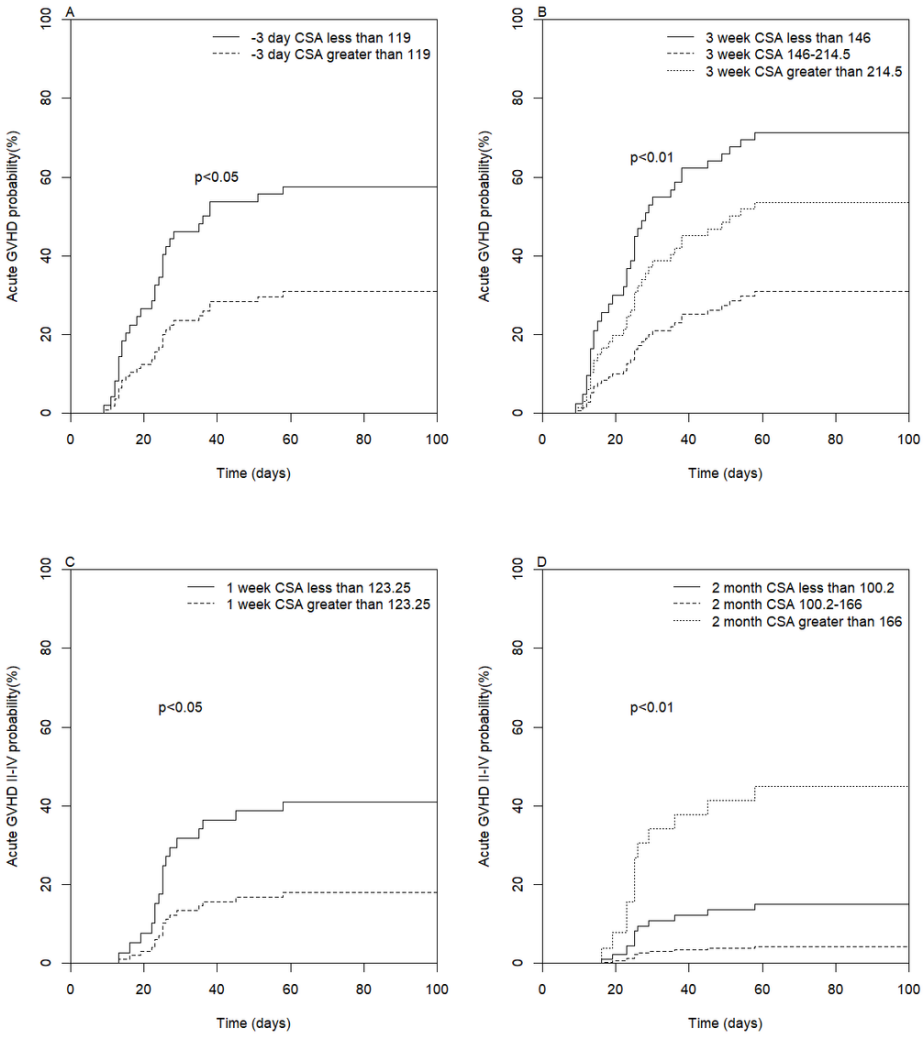

Figure 2

Cumulative incidence of aGvHD and grades II–IV aGvHD in accordance to the CsA level. The probability of chronic GVHD for the 3rd month, (A) chronic GVHD for 4th–6th month, (B) acute GVHD for -3 days, (C) acute GvHD for the third week, (D) grades II–IV aGvHD for the first week, (E) and grades II–IV aGvHD for the second month (F) according to the CsA level (different cutoff values) after HSCT. aGvHD, acute graft-vs-host disease; CsA, cyclosporine A

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- 21data.xlsx
- Risk1.csv
- Risk10.csv
- Risk2.csv
- Risk3.csv
- Risk4.csv
- Risk5.csv
- Risk6.csv
- Risk7.csv

- Risk8.csv
- Risk9.csv
- code1.sas
- data1.csv
- plot.r