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ORIGINAL ARTICLE

The effect of cyclosporine initiation time on the outcome of matched allogeneic stem-cell transplantation following fludarabine-based conditioning

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We have no conflicts of interest.

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Summary

Cyclosporine (CSA) is the most commonly used medication for GVHD prophylaxis. The initiation time varies from day −4 to day 0. Initially, we gave CSA starting on day −1. However, since 2003 we have changed CSA initiation timing policy in most of our protocols to day −4, to achieve stable and controlled pretransplant CSA levels. Here, we assessed if initiation time impact the outcome of allogeneic stem-cell transplantation (allo-SCT). Data of 261 patients who underwent allo-SCT for hematological malignancies from a fully matched donor, treated with CSA as a single agent for GVHD prophylaxis were prospectively collected. Patients were divided according to CSA initiation time and analyzed for outcome. The acute GVHD severity, cGVHD extent, GVHD-associated mortality were significantly lower in the CSA −4 group. There was no difference in the rate and timing of acute or chronic GVHD. Overall survival did not differ between the groups. We conclude that the initiation of CSA at day −4 reduced the severity of aGVHD, extent of cGVHD, and GVHD-associated mortality without impact on overall survival.

Introduction

Graft versus host disease (GVHD) is the major cause of allo-SCT-related morbidity and mortality [1]. The balance between GVHD prevention and graft versus leukemia/lymphoma (GVL) is always delicate. The option of T-cell depletion reduces GVHD risk significantly, but also results in higher relapse rate [2]. The introduction of cyclosporine (CSA) is the 1980s for GVHD prophylaxis has made a tremendous change in GVHD-related mortality and morbidity [3]. More than 20 years ago the Seattle group demonstrated that low levels of CSA could result in increased risk of GVHD and that CSA levels should be carefully monitored [4]. Later on, the same group reported a relative risk of 0.7 for every 100 ng/ml increase in CSA levels [5,6]. With the significant improvement in the supportive care and HLA matching, a recent study also demonstrated the importance of adequate CSA trough levels on the prevention of Grade III–IV GVHD [7]. In this study, the reduction in severe GVHD did not translate into improvement in overall survival (OS). The traditional GVHD prophylaxis is short-term methotrexate (MTX) with long-term CSA, even though it is thought that this two drug combination results in higher relapse rates [8]. Therefore, we use CSA as a single agent for GVHD prophylaxis in fully matched allo-SCTs. In spite of the fact that CSA is a well-known GVHD prophylaxis regimen, the initiation time remains to be elucidated. In a prospective study in a pediatric group of patients, Lanino *et al.* [9], did not find any outcome difference between two groups of patients in which CSA was initiated on day −7 and day −1, respectively. Our practice was to initiate

CSA at day −1 in all our protocols. Since 2003, we changed our policy of initiation time to day −4, to achieve better therapeutic trough CSA levels on transplant day. In this study, we assessed the clinical outcome of this change.

Methods

Patients

Four hundred and seventy six patients' records, that underwent allo-SCT between the years 1996–2008, and were followed prospectively by our data management group, were reviewed. We identified 261 consecutive patients with hematological malignancies who were transplanted with T-cell repleted grafts from fully matched donors and in which CSA was used as a single agent for GVHD prophylaxis. All patients that matched these criteria were analyzed. GVHD prophylaxis consisted of short-term CSA 3 mg/kg intravenously, or 6 mg/kg orally daily in two divided doses, starting either from day −1 (137 patients – group 1) or day −4 (124 patients – group 2). Specific CSA trough levels were routinely measured twice weekly, and the dose was adjusted accordingly to achieve therapeutic levels around 150–200 ng/ml. The CSA dosage was tapered during the second-third month post transplant, according to chimeric status and GVHD evidence. The indications for donor lymphocyte infusions (DLI) were same for both groups, e.g. DLIs were given in case of mixed chimerism, relapse or as prophylaxis in high-risk patients and only following the cession of CSA.

All patients were conditioned with fludarabine-based regimens, 59 with myeloablative (fludarabine 30 mg/m^2/day × 6 and PO busulfan 4 mg/kg/day × 4 or IV busulfex 3.2 mg/kg/day × 4), and 202 with reduced intensity protocols (mostly fludarabine 30 mg/m^2/day × 6 and PO busulfan 4 mg/kg/day × 2 or IV busulfex 3.2 mg/kg/day × 2). Donors were fully HLA A, B, C, DRB1, and DQB1 matched siblings or matched unrelated donors (MUD). Patients received peripheral blood stem cells (PBSCs) or bone marrow stem-cells transfusion. Patients, donors, and transplants characteristics are summarized in Table 1.

Table 1. The patients' characteristics.

	Group 1 (n = 137)	Group 2 (n = 124)	P
Gender (n = 261)			
Female	46 (33.6%)	46 (37.1%)	0.6
Male	91 (66.4%)	78 (62.9%)	
Median age (n = 261)	39.1	39.1	NS
Induction regimen (n = 261)			
RIC	128 (93%)	74 (60%)	<0.001
Myeloablative	9 (7%)	50 (40%)	
Graft cell dose			
Median CD34+ cells/kg (n = 184)	9.54×10^6	9.88×10^6	0.76
Disease status (n = 254)			
Remission	39 (29.5%)	38 (31%)	0.89
Nonremission	93 (70.5%)	84 (69%)	
Diagnosis (n = 261)			
CML	28 (20.4%)	5 (4%)	<0.001
Other hematologic malignancy	111 (79.6%)	119 (96%)	
Donor relation (n = 261)			
Related	113 (82.5%)	88 (71%)	0.039
Unrelated	24 (17.5%)	36 (29%)	
Donation source (n = 261)			
Peripheral blood stem cells	119 (86.9%)	111 (89.5%)	0.6
Bone marrow	18 (13.1%)	13 (10.5%)	
aGVHD (n = 230)			
Yes	79 (68.1%)	66 (57.9%)	0.13
No	37 (31.9%)	48 (42.1%)	
cGVHD (n = 139)			
Yes	60 (83.3%)	50 (74.6%)	0.22
No	12 (16.7%)	17 (25.4%)	

aGVHD, acute graft versus host disease; CML, chronic myeloid leukemia, cGVHD, chronic graft versus host disease; RIC, reduced intensity conditioning.

Supportive care

Prior to transplantation, all patients received trimethoprim/sulfamethoxazole until day −2, acyclovir from the initiation of therapy to at least until day +120, and allopurinol until day −1. Trimethoprim/sulfamethoxazole was reinstituted after recovery from neutropenia for 6 months. Patients were not receiving any antibiotics prophylaxis. Febrile neutropenia was treated according to the hospital's protocols.

Starting with conditioning, cytomegalovirus (CMV) was monitored with a DNA-polymerase chain reaction (PCR) test or pp65 antigenemia on a weekly basis. CMV reactivation indicated replacing acyclovir with ganciclovir until a minimum of two negative tests was obtained. Patients were treated in reverse isolation HEPA-filtered rooms, and received a regular diet. Additional supportive measures, such as parenteral nutrition and blood component transfusion, were administered as necessary. As stated above, CSA was initiated either on day −1 or day −4 in a dose of 6 mg/kg orally or 3 mg/kg intravenously.

Acute and chronic GVHD (aGVHD, cGVHD) were graded according to the International Bone Marrow Transplantation Registry (IBMTR) severity indices [10]. Immediately upon the appearance of signs and symptoms of GVHD, i.v. methylprednisolone (2 mg/kg) was administered.

To assess engraftment, degree of chimerism, minimal residual disease, and early relapse, patients were monitored at regular intervals by cytogenetic analysis, by male/female amelogenine gene PCR bands [11,12], variable-number

tandem repeat or later by short tandem repeats PCR assays [13,14]. All patients or their guardians signed an approved informed consent prior to the procedure.

Definitions-statistics

Pre-engraftment deaths were excluded from the analysis of aGVHD and deaths before day 100 were excluded from analysis of cGVHD. Nonrelapse mortality was defined as death from any cause that is not associated with relapse of the original hematological malignancy. Overall survival was defined as the time from transplant to death or last follow-up. Time to relapse was defined as time from transplant to recurrence of the hematologic malignancy.

We used Chi-squared test and Fisher's exact test to compare the two different groups. Kaplan–Meier method was used to analyze survival, and for proportional hazard we used Cox regression model. Statistical analysis was done with SPSS PASW statistics software version 18 (IBM SPSS, Somers, NY, USA).

Results

Group 1 (CSA initiation on day −1) had 137 patients, 91 men and 46 women. The median age at transplant was 39.1 years, (range: 11 months–63.2 years). Group 2 (CSA initiation on day −4) had 124 patients, 78 men and 46 women. The median age at transplant was also 39.1 years, (range: 11 months–74.9 years).

Patient's characteristics are presented in Table 1. The median follow-up for all patients in groups 1 and 2 was 14.5 and 10.3 months, respectively. For the surviving patients, the median follow-up was 9.3 years in group 1 and 3.7 years in group 2. The two groups did not significantly differ in age, gender, graft source, and disease status at transplant (remission vs. active disease). There were significantly more ($P = 0.039$) related donors in group 1 (as expected from the fact that they were mostly transplanted in earlier years), as well as significantly more reduced intensity conditioning (RIC) cases in this group.

The distribution of the different diseases among patients is presented in Table 2. We have found no difference in the incidence of the different diseases between the groups, excluding CML. Group 1 had 28 cases of CML (20.4%), while group 2 had five (4%) ($P < 0.001$); this difference was expected as the approach to the treatment of CML has changed during these years.

All transplant induction regimens were fludarabine-based and mostly with busulfan/busulfex. In group 1, 128 patients (93%) had reduced RIC, while nine (7%) had myeloablative regimens. In group 2, 74 patients (60%) had RIC, while 50 (40%) had myeloablative protocols ($P < 0.001$). Median CD34+ cell count was 9.54 and 9.88×10^6/kg in group 1 and 2, respectively (NS).

Table 2. Distribution of indication for transplant in both groups.

Indication for transplant	Group 1	Group 2	*P*
AML	43	37	0.89
ALL	19	17	1
CML	28	5	<0.001
CLL	0	2	0.22
NHL	28	33	0.30
Hodgkin's lymphoma	6	2	0.28
Myelofibrosis	0	3	0.10
Multiple myeloma	4	10	0.097
JMML	0	3	0.10
MDS	9	12	0.25

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; JMML, juvenile myelomonocytic leukemia; MDS, myelodysplastic syndrome; NHL, nonHodgkin's lymphoma.

The median level of CSA at day 0 and day +4 was not significantly different between the groups (175 ± 29 and 196 ± 19 ng/ml at day 0 for groups 1 and 2, respectively; and 228 ± 16 and 186 ± 16 ng/ml at day +4 for groups 1 and 2, respectively; $P = 0.33$ and 0.31).

The absolute neutrophil count (ANC) recovery (ANC $>0.5 \times 10^9$/l) was at a median of 16 days (range: 4–42 days) in group 1 and 15 days (range: 2–73 days) in group 2. The median time to platelets (PLT) recovery (PLT $>20 \times 10^9$/l) was 12 days (range: 0–73) and 11 days (range: 1–111) for groups 1 and 2, respectively (NS). Graft Rejection occurred in four patients in group 1 and two patients in group 2 (NS).

Acute GVHD occurrence was evaluable in 230 of 261 patients. Seventy-nine of 116 evaluable cases (68%) in group 1 had aGVHD of any grade, and 66/114 (58%) of group 2 ($P = 0.13$). Acute GVHD grade data were available in 136 patients. In group 1, 31 of 72 (43%) cases of aGVHD grade 1–2, and 41/72 (57%) cases of aGVHD grade 3–4. In group 2, 40 of 64 (62.5%) cases had aGVHD grade 1–2, while 24/64 (38.5%) had aGVHD grade 3–4 (Fig. 1, $P = 0.026$).

Chronic GVHD occurrence was evaluable in 139 patients. Sixty of the 72 (83.3%) patients in group 1, and 50/67(74.6%) of patients in group 2 experienced cGVHD ($P = 0.22$). Chronic GVHD extent data were available in 86 patients. Extensive cGVHD was present in 23 of 37 patients (62.2%) from group 1 and 16/49 (32.7%) of group 2 patients, while limited cGVHD was present in 37.8% of group 1 and 67.3% of group 2 ($P = 0.009$).

Relapse rate was 39% in group 1 and 26.6% in group 2 ($P = 0.036$). Median time to relapse was 43.9 months for group 1 and was not reached for group 2 ($P = 0.15$).

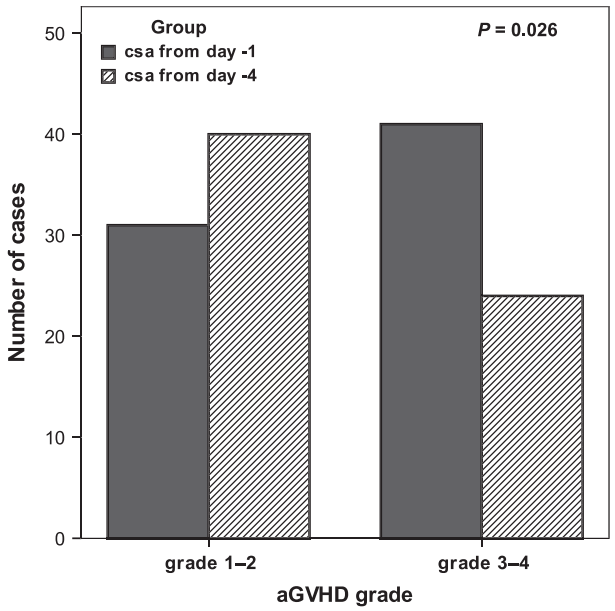

Figure 1 Acute GVHD incidence and grading in the two groups. There was no difference in the overall rate of acute GVHD. However, significantly more cases of grade 3–4 aGVHD occurred in the group of CSA −1 (group 1) ($P = 0.026$).

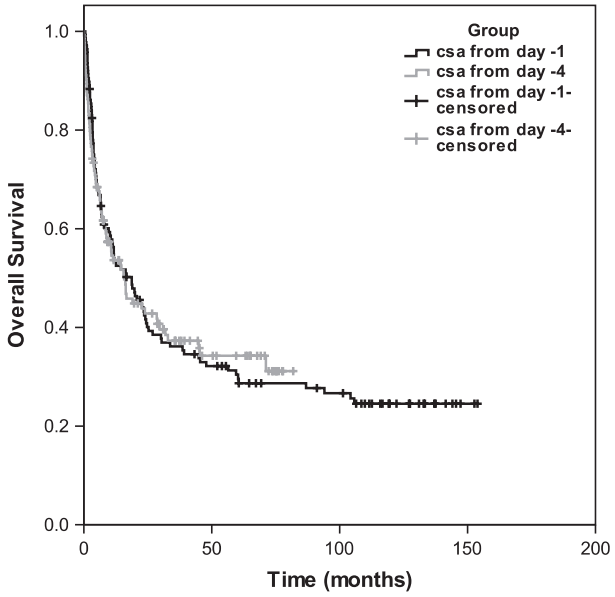

Figure 2 Overall survival for group 1 and group 2, demonstrating no difference between the groups.

Median OS was 16.2 months for all patients and did not differ between the two groups [Fig. 2]. Relapse-related mortality was 28% in group 1 and 18% in group 2, while nonrelapse mortality was 42% and 43%, respectively (NS). GVHD-associated mortality (combined acute and chronic) was significantly lower in group 2 ($P = 0.012$) [Fig. 3].

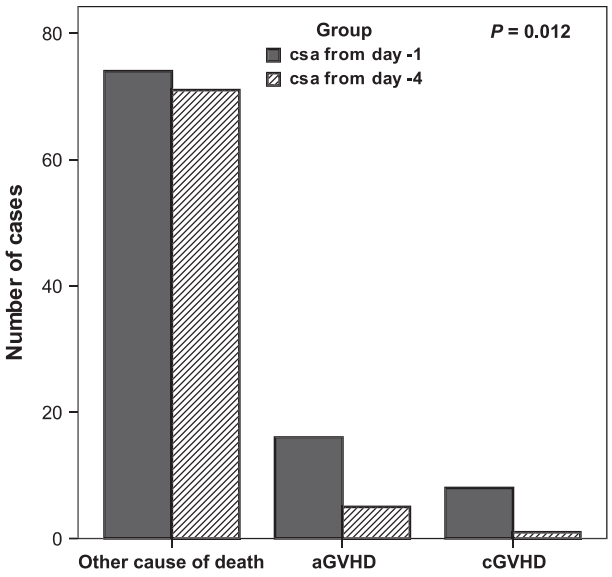

Figure 3 Death cause analysis in the two groups reveals GVHD (combined acute and chronic) as significantly more frequent cause of mortality in the group treated with CSA from day −1 ($P = 0.012$) (group 1).

There was no significant correlation between aGVHD peak score ($P = 0.3$), cGVHD extent ($P = 0.59$), GVHD associate death ($P = 0.27$) to the type of donor (related or unrelated).

In a multivariate analysis for OS, including group, protocol type (RIC vs. myeloablative), basic disease and disease status at transplantation (active vs. in remission), we have found three predictors for better outcome: CML as the baseline disease ($P < 0.001$, HR: 0.22; CI: 0.11–0.42), no active disease at time of transplant ($P = 0.022$, HR: 0.56; CI: 0.37–0.985) and myeloablative induction protocol ($P = 0.022$, HR: 0.6; CI: 0.39–0.92). As mentioned before, CML was significantly more common as transplantation indication in group 1 (pre-Imatinib era). We have repeated the analysis of OS for the same groups excluding CML patients. In this subset we were, again, able to demonstrate significant difference between the groups in terms of aGVHD severity ($P = 0.046$) but did not inspect any OS difference.

The RIC was also more frequent in group 1. Analysis of the RIC cases only in both groups ($n = 202$), revealed no OS difference between the groups and statistically significant difference in the rate of aGVHD ($P = 0.02$), severity of aGVHD (1–2 vs. 3–4, $P = 0.04$), extent of cGVHD ($P = 0.009$), and GVHD-associated mortality ($P = 0.007$). Subset analysis of the non-CML cases in the RIC group ($n = 175$), demonstrated significant difference in aGVHD rate ($P = 0.04$), GVHD-associated mortality ($P = 0.014$) cGVHD extent ($P = 0.03$). Acute GVHD severity was not different ($P = 0.07$) between the groups.

Discussion

The importance of CSA level for allo-SCT transplant outcome is well established. It was shown that CSA levels in the first post-transplant week [7] or first 2 weeks [6] are crucial for GVHD prevention. Others have shown that 3rd week's levels are of significance [15]. The initiation time for CSA can be important in terms of achieving appropriate trough level on one hand and side effects of CSA on the other. It was also shown in a retrospective analysis of patients treated with allogeneic HSCT after nonmyeloablative conditioning using fludarabine and 2 Gy TBI, that higher post-transplant levels of CSA were associated with higher risk of rejection [16]. CSA is known to be nephrotoxic and neurotoxic, and its administration with other nephrotoxic agents, which are commonly given before and during allo-SCT, can be detrimental. It would therefore be rational, to initiate CSA as late as possible for toxicity reasons but early enough to effectively prevent aGVHD. The initiation time for CSA varies from day −4 to day 0 in different centers. Our attitude has changed over the years. Traditionally, we initiated CSA on day −1. Eight years ago, we changed CSA initiation time in most of our fludarabine-based protocols to day −4 to have stable, controlled therapeutic pretransplant CSA blood levels. Day −4 was chosen since CSA half-life (about 24 h), would enable us to reach compatible levels on day 0 on one hand, and would not cause too much early toxicity on the other. We found that early initiation of CSA does not affect aGVHD rate and timing, but does reduce the severity of aGVHD, extent of cGVHD as well as GVHD-associated mortality and has no influence on engraftment. This was found in spite of the significantly higher number of unrelated donors and myeloablative conditioning regimens used in this group (both shown to increase the rate of GVHD). This effect could have occurred through several mechanisms. The first is obviously better levels of CSA on the day of transplant and forward. However, the levels at transplant date and later were not significantly different between the groups. CSA has been shown to have a suppressive effect on antigen-presenting cells (APCs) [17,18]. This inhibitory effect may be relevant for the induction of GVHD [9] therefore the reduction of GVHD severity may be also explained by a longer pretransplant exposure of APCs to CSA. Other mechanisms involved in the induction of GVHD that occur during the pretransplant days and are influenced by CSA and thus may explain the observed effect may be: the blockage of free radical production [19], nitric oxide modulation and glutathione redox [20,21], and lipopolysaccharide production suppression [22].

The finding of higher relapse rate in group 1 can be, at least partially, explained by the higher severity of aGVHD and cGVHD. Severe aGVHD is not associated with strong enough anti-leukemic effect but naturally initiates the use of more immunosuppressants which is a risk factor for relapse [23]. We have been able to show that within the subset of RIC patients not only aGVHD severity and GVHD-associated mortality were lower, but also aGVHD rate was significantly reduced with initiation of CSA on day −4. In spite of the known relationship between aGVHD severity and mortality in allo-SCT [23], we did not find a significant OS difference between the groups, as Malard *et al.* did not [7]. One of the possible explanations to this would be that group 2 patients, who were transplanted later, were less favorable to begin with. We found no age or disease status difference between the groups. The significant demographic differences between the two groups were the indication for transplant (group 1 had significantly more CML patients, because of the introduction of imatinib that significantly reduced the referral of CML patients to transplant) and group 2 had significantly more patients transplanted from unrelated donors. Subset analysis of the non-CML patients failed to reveal OS difference. Another possible explanation for the lack of difference in OS between the groups is the conditioning protocols. Group 1 had significantly more RIC cases, which is associated with less transplant-related mortality. Analysis of the RIC cases excluding all the CML patients was still significant for GVHD-related mortality but not significant for OS.

Also need to be taken into consideration are the time differences between the groups. Even though we tried to look for specific dissimilarities which may have influenced outcome, there are many other factors which are not easily measurable and which are different between the two periods. One of the most important differences which are hard to measure, is the difference in supportive care. Improvement of supportive care, although, should not explain the difference in aGVHD severity in patients who treated with the same medication for GVHD prophylaxis. If we would have found a survival difference between the groups, if would have partially been explained by difference in supportive care. The disagreement between our results and the recently published paper by Lanino *et al.* [9] may be the consequence of more heterogenic group of patients in our case, which included mostly adults (median age 39.1 years compared to 9 years in Lanino *et al.*) and both MUD and related donor allogeneic stem-cell transplantations.

Many centers use both calcineurin inhibitor and T-cell anti-proliferative agent as GVHD prophylaxis rather than CSA alone. We have found an isolated effect of early initiation of CSA on outcome. Future studies will be needed to verify if this effect persists when double therapy is used.

Patients undergoing allo-SCT, like any other major medical intervention, have major concerns about quality of life issues. The growing success rate of allo-SCT resulted in large number of long-term survivors whose life quality is mainly dependent on their health status. Acute and especially cGVHD were shown to have a major impact on the quality of life of allo-SCT survivors [24,25]. Any intervention that may reduce the severity or rate of acute or cGVHD will be of benefit in this sense, especially if there is no negative influence on OS. Our results demonstrate that aGVHD severity, cGVHD extent, and GVHD-associated mortality were reduced in group 2 without an impact on OS. We therefore conclude that initiation of CSA in day −4 is better as it may improve the quality of life of the patients undergoing allogeneic SCT without changing the OS.

Authorship

MK: analyzed data and wrote the paper. LD: collected data and performed research. SG: collected data and analyzed data. IR: performed research, analyzed data, and wrote the paper. SP: performed research and analyzed data. AM: performed research. RO: designed research and analyzed data. MS: designed research, performed research, collected data, and wrote the paper.

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