Cyclosporine Plus Methotrexate or Cyclosporine Plus Mycophenolate Mofetil as Graft Versus Host Disease Prophylaxis in Acute Leukemia Transplant: Comparison of Toxicity, Engraftment Kinetics and Transplant Outcome

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Abstract We sought to compare two graft-versus-host disease (GVHD) prophylaxis regimen, cyclosporine and methotrexate (CsA+MTX) with CsA+mycophenolate mofetil (MMF) in 77 acute leukemia patients who underwent hematopoietic stem cell transplant (HSCT) between January 2008 and March 2013. Fifty-three patients received CsA+MTX while 24 received CsA+MMF. The incidence of grade 3–4 mucositis and grade 3–4 diarrhea was 74 and 6 % with CsA+MTX compared to 33 % and 21 % with CsA+MMF ($P = 0.001$ and 0.09 respectively). Forty-two (79 %) patients in CsA+MTX group required total parenteral nutrition compared to 14 (58 %) in CsA+MMF group ($P = 0.09$). The incidence of engraftment fever was 17 % with CsA+MTX and 41 % with CsA+MMF ($P = 0.02$). The median time to neutrophil and platelet engraftment was 14 days and 13 days with CsA+MTX compared to 12 days and 10 days with

CsA+MMF ($P = 0.003$ and 0.08 respectively). The incidence of any grade and grade II–IV acute GVHD was 45 and 13 % with CsA+MTX compared to 42 and 29 % with CsA+MMF ($P = \text{NS}$). Incidence of overall and extensive chronic GVHD was 57 and 38 % with CsA+MTX compared to 42 and 17 % with CsA+MMF ($P = \text{NS}$). Incidence of relapse was 38 % with CsA+MTX compared to 33 % with CsA+MMF ($P = \text{NS}$). TRM was 6 % with CsA+MTX and 21 % with CsA+MMF ($P = \text{NS}$). At 2 years, overall survival (OS) was 64 % in CsA+MTX group compared to 46 % in CsA+MMF group ($P = \text{NS}$). We conclude that CsA+MMF is associated with lesser toxicity, faster myeloid engraftment and similar rates of acute and chronic GVHD, TRM, relapse and OS compared to CsA+MTX in acute leukemia transplant.

Keywords Cyclosporine · Graft-versus-host disease · Hematopoietic stem cell transplantation · Methotrexate · Mycophenolate mofetil

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Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) remains an important and potentially curative treatment modality for patients with acute leukemia [1]. This favorable effect of HSCT in leukemia on long term survival is offset to an extent by transplant related mortality (TRM). Acute graft versus host disease (GVHD) is one of the commonest causes of increased morbidity and mortality in the post transplant period [2]. Strategies employed as GVHD prophylaxis include immunosuppressive agents and T cell depletion. Calcineurin inhibitor cyclosporine (CsA) has been one of the most widely used immunosuppressive drug in GVHD prophylaxis over last 4 decades [3]. CsA

combined with another immunosuppressive agent i.e. methotrexate (MTX) further decreased the incidence of severe acute GVHD and led to improved survival [4]. Over last 2 decades, another calcineurin inhibitor tacrolimus (TAC) has been compared with CsA, either alone or in combination with MTX [5–7]. Besides small differences in the incidence of acute GVHD, no combination drug prophylaxis has been proven to be superior to CsA and MTX in terms of overall survival (OS).

Mycophenolate mofetil (MMF), a pro-drug of mycophenolic acid, inhibit T cell activation and proliferation and has been used in combination with CsA for GVHD prophylaxis [8–10]. Compared to MTX, MMF has been shown to be less toxic when used in combination with CsA for GVHD prophylaxis [8]. However, these studies included a heterogeneous group of patients who underwent HSCT for a variety of hematological malignancies. None of the studies have compared MMF and calcineurin inhibitor based GVHD prophylaxis with MTX and calcineurin inhibitor based GVHD prophylaxis exclusively in acute leukemia patients. Also, non-significant differences in the incidence of acute GVHD, relapse and OS seen between these 2 GVHD prophylaxis regimens require further confirmation in acute leukemia setting. In this study, we sought to compare CsA and MTX with CsA and MMF based GVHD prophylaxis in acute leukemia patients undergoing HSCT for differences in toxicity and various transplant related outcomes, including incidence and severity of acute and chronic GVHD, incidence of leukemia relapse, engraftment kinetics, TRM, relapse free survival (RFS) and OS.

Materials and Methods

Patients

All consecutive patients with acute leukemia who underwent HSCT between January 2008 and March 2013 were included in this retrospective study. Written informed consent was obtained from all patients who received CsA+MTX as per the institutional practice. CsA+MMF was used in patients who consented to participate in an ongoing MMF pharmacokinetic study [11]. The study has been approved by Institutional Ethics Committee and it conforms to the provisions of the Declaration of Helsinki. At the time of HSCT, patients were either in first complete remission (CR-1) or second complete remission (CR-2) or in relapsed/refractory (RR) state. Poor risk acute myeloid leukemia (AML) was characterized by at least one of the following: unfavorable cytogenetics [complex karyotype, monosomal karyotype, del 5, del 7, 5q-, 7q-, inv(3), t(3;3), t(6;9), 11q23 translocations], TLC > 100 × 10⁹/L at

baseline, not achieving CR after induction, disease stage ≥CR-2 or persistent disease at transplant. High risk acute lymphoblastic leukemia (ALL) was defined by at least one of the following characteristics-poor risk cytogenetics [t(9;22), t(1;19), t(4;11), hypodiploidy, complex karyotype], total leucocyte count (TLC) > 100 × 10⁹/L at baseline, not achieving CR after induction, disease stage ≥CR-2 or persistent disease at transplant. Patients underwent either matched related donor (MRD) transplant or matched unrelated donor (MUD) transplant or haplo-identical donor (HID) transplant depending on donor availability and human leucocyte antigen (HLA) matching. Low resolution HLA typing for HLA antigen loci A, B and DRB1 was done for all patients and siblings (if available) using polymerase chain reaction (PCR) with sequence specific primers (SSP). All cases with antigen mismatch at one locus were further tested for antigen match at HLA-C and HLA-DQB1. Patients with no sibling donor available and those with more than one antigen mismatch sibling donor underwent unrelated donor search. High resolution HLA typing for HLA-A, HLA-B, HLA-C, HLA-DRB1 and HLA-DQB1 was used to find out the most adequately MUD. Patients with no MRD or MUD available underwent HID transplant. Stem cell graft used was either T-cell replete granulocyte-colony stimulating factor (G-CSF) mobilized peripheral blood stem cells (PBSCs) or bone marrow harvest or cord blood derived stem cells. Ex vivo T-cell depletion was not done in any transplant.

Conditioning Regimen

Conditioning regimen used in patients who were in CR-1 or CR-2 was either full intensity (FI) i.e., total body irradiation with cyclophosphamide (TBI-Cy) or busulfan with cyclophosphamide (BuCy); or reduced intensity (RI) i.e., fludarabine based. TBI dose in FI conditioning regimen was 12–14.4 Gy given in 8 fractions over 4 days from day-7 to day-4 while cyclophosphamide dose was 60 mg/kg intravenously (iv) over 2 h on day-3 and day-2. Busulfan was given orally as 1 mg/(kg dose) every 6 hourly for 4 days from day-8 to day-5 with cyclophosphamide at 60 mg/kg iv over 2 h on day -3 and day-2. In RI conditioning, fludarabine was used iv at a dose of 30 mg/m²/day over 30 min from day-7 to day-3 in combination with one of the alkylating agents (melphalan-140 mg/m², oral busulfan-8–12 mg/kg, cyclophosphamide-120 mg/kg, treosulfan-42 g/m²) with or without 2 Gy TBI. Patients who were transplanted in RR state (i.e. in disease) received intensive multi-agent conditioning regimen comprising of cytarabine at 10 g/m², fludarabine at 150–180 mg/m², anthracyclin derivative (mitoxantraone 30 mg/m² or idarubicin 30 mg/m²) along with an alkylating agent (melphalan 140–180 mg/m², oral busulfan 12–16 mg/kg,

cyclophosphamide 120 mg/kg, treosulfan 42 g/m²). These were included in the FI group.

GVHD Prophylaxis

CsA+MTX was used in 53 (69 %) patients and CsA+MMF in 24 (31 %) patients. CsA was started on day-1 of transplant at 1.5 mg/kg/twice daily iv as 2 h infusion. Trough CsA blood level was measured twice a week starting from day +3 to day +5 of transplant by chemiluminiscence assay (Architect analyser, Abbott Healthcare-detection limit 25 ng/ml). CsA level monitoring was stopped at the start of CsA tapering. Every subsequent CsA dose was modified to achieve therapeutic level of 150–200 ng/ml if levels were out of range. In most cases, CsA was tapered from day +90 and stopped by day +180. In patients with high risk of relapse, CsA was tapered from day +60 and stopped by day +120. CsA was discontinued in patients who developed significant CsA related nephrotoxicity or neurotoxicity and was subsequently replaced by MMF. Intravenous CsA was replaced by oral CsA upon resolution of mucositis and diarrhea. MTX was given iv at 15 mg/m² on day +1 and 10 mg/m² on day +3, +6, +11. All doses of MTX were followed 24 h later with leucovorin rescue. MTX doses were omitted if patient developed grade IV oral mucositis. MMF was used in place of MTX in patients who participated in an ongoing MMF pharmacokinetic study [11]. MMF was started on day-1 of transplant and continued till day +30 at a dose of 600 mg/(m² twice) daily administered orally. It was continued beyond day +30 in patients who developed acute GVHD. Patients who underwent HID transplant received post transplant cyclophosphamide (50 mg/kg on day +3 and +4) in addition to CsA+MMF (started on day +5). In HID transplants MMF was used at a higher dose (15 mg/kg thrice daily) from day +5 till day +35 while CsA was used at same dose. Patients who underwent one antigen mismatched sibling donor transplant or MUD transplant also received rabbit anti-thymocyte globulin (Thymoglobulin) at a dose of 2.5 mg/(kg dose) on day-2 (1 dose) or day-2 and day-1 (2 doses). All patients received azole anti-fungal prophylaxis which was continued till day +100 of transplant or longer if patient was on steroids for treatment of GVHD.

Definitions

Acute GVHD was defined as GVHD developing in first 100 days of transplant. Diagnosis and grading of aGVHD was done according to standard criteria [12] and treatment was initiated with methylprednisolone 1–2 mg/kg/day given intra-venously, followed by tapering upon clinical improvement. Histological confirmation was done only in patients with suspected upper gut and skin GVHD wherever possible. Diagnosis of lower gut GVHD was made clinically.

Chronic GVHD was defined as GVHD developing after 100 days of transplant and was classified as limited or extensive stage according to standard criteria [13]. Treatment of cGVHD was initiated with local steroids with or without addition of systemic steroids depending on the site of cGVHD and response to local steroids.

Engraftment: Time to myeloid engraftment was defined as first of 3 consecutive days with an absolute neutrophil count of $\geq 0.5 \times 10^9/L$ [17]. Time to platelet engraftment was defined as first of 7 consecutive days when the platelet count is maintained $\geq 20 \times 10^9/L$ without platelet transfusion. Failure to engraft by day +30 was considered primary engraftment failure.

Engraftment fever was defined as fever ($\geq 38.5^\circ C$) occurring in the peri-engraftment period for which no infectious cause was ascertained and resolution of fever with use of systemic steroids.

Severity of oral mucositis and diarrhea was graded as per Common Terminology Criteria for Adverse Events (CTCAE)—Version 3.0.

TRM was defined as death occurring in the post-transplant period not related to relapse of leukemia.

RFS was defined as the time interval between date of transplant and date of relapse or death or last follow-up if in CR.

OS was defined as the time interval between date of transplant and date of death or last follow-up.

Statistical Considerations

For analysis, patients were divided into two groups depending on the GVHD prophylaxis regimen used—Group A (CsA+MTX) and Group B (CsA+MMF). Primary objective was to determine and compare the toxicity profile of two GVHD prophylaxis regimens. Secondary objectives were to determine and compare the following transplant outcomes between the two groups: incidence and severity of acute GVHD, incidence and severity of chronic GVHD, engraftment kinetics, TRM, incidence of relapse, RFS and OS. Comparisons were done between two groups for discrete variables using χ^2 test while continuous variables were compared by Mann–Whitney test. Survival analysis was done by Kaplan–Meier method and compared using Log-Rank test.

Results

Patient and Transplant Characteristics

Seventy-seven patients (52 males and 25 females) with acute leukemia who underwent HSCT during the study period were included. The diagnosis was AML in 52 (68 %)

patients, ALL in 23 (30 %) patients and biphenotypic acute leukemia in 2 (2 %) patients. The median age was 30 years (range 6–51). Forty two (54 %) patients were in CR1, 20 (26 %) in CR2 and 15 (20 %) in RR state at the time of HSCT. Poor risk disease was present in 22 (42 %) patients with AML. High risk disease was present in 17 (74 %) patients with ALL. Sixty five (84 %) patients underwent MRD transplant, 10 (13 %) underwent MUD transplant and 2 (3 %) underwent HID transplant. Hematopoietic stem cell graft was PBSCs in 70 (91 %) transplants, bone marrow harvest in 5 (7 %) and cord blood in 2 (2 %) transplants. Forty (52 %) patients underwent gender mismatched transplants which included female donor to male recipient in 23 (30 %) cases. FI conditioning regimen was used in 42 (55 %) transplants and RI conditioning regimen was used in 35 (45 %) transplants (Table 1).

Fifty-three (69 %) patients received CsA+MTX (Group A) while 24 (31 %) patients received CsA+MMF (Group B) as GVHD prophylaxis. Thymoglobulin was used in 12 (16 %) transplants (MUD transplants and one antigen mismatched sibling donor transplants) while post-transplant cyclophosphamide was used in two HID transplants. There were no significant differences between the two groups with respect to age, gender, diagnosis, ABO and gender mismatch, intensity of conditioning regimen used and median dose of CD34 + /CD3 + cells infused (Table 2). Thirty-seven (70 %) patients in Group A were in CR-1 compared to 5 (21 %) in group B ($P < 0.01$), while 5 (10 %) patients in group A had RR disease compared to 10 (42 %) in group B ($P < 0.01$). Forty-nine (92 %) patients in group A underwent MRD transplant compared to 16 (67 %) in group B ($P < 0.01$). Four (8 %) patients in group A underwent MUD transplant compared to 6 (24 %) in group B ($P < 0.01$).

Comparison of Toxicity Between Two Groups

The incidence of grade 3–4 mucositis and grade 3–4 diarrhea was 74 and 6 % in group A compared to 33 and 21 % in group B ($P = 0.001$ and 0.09 respectively). Forty-two (79 %) patients in group A required total parenteral nutrition (TPN) compared to 14 (58 %) in group B ($P = 0.09$). Median duration of TPN was 11 days in group A compared to 8.5 days in group B. The incidence of engraftment fever was 17 % in group A and 41 % in group B ($P = 0.02$). The incidences of nephrotoxicity, neurotoxicity and hypertension were similar in both groups (Table 3).

Comparison of Transplant Related Outcomes Between Two Groups

The median time to neutrophil and platelet engraftment was 14 days and 13 days in group A compared to 12 days

Table 1 Baseline characteristics (n = 77)

	n	%
Age at transplant		
Median	30 years	
Range	6–51 years	
Male/Female	52/25	67/33
ABO mismatch transplants	32	42
Gender mismatched transplants	40	52
Female donor to male patient transplants	23	30
Diagnosis		
Acute lymphoid leukemia (ALL)	23	30
Acute myeloid leukemia (AML)	52	68
Biphenotypic leukemia	02	2
Baseline risk score		
ALL—standard risk	1	4
High risk	17	74
Not known	5	22
AML—good risk	5	10
Intermediate risk	21	40
Poor Risk	22	42
Not known	4	8
Disease status at transplant		
Complete remission—1	42	55
Complete remission—2	20	26
Refractory/relapsed (in disease)	15	20
Stem cell source		
Bone Marrow	05	6
Umbilical cord	02	3
Peripheral blood stem cells	70	91
Type of transplant		
Matched related transplant	65	84
Matched unrelated transplant	10	13
Haplo-identical transplant	02	3
Type of conditioning regimen		
Full intensity	42	55
Reduced intensity	35	45
Graft versus host disease prophylaxis		
Cyclosporine+methotrexate	53	69
Cyclosporine+mycophenolate mofetil	22	29
Cyclosporine+mycophenolate+cyclophosphamide	02	2
Antifungal prophylaxis		
Voriconazole	59	77
Posaconazole	14	18
Itraconazole	03	4
Fluconazole	01	1

and 10 days in group B ($P = 0.003$ and 0.08 respectively). Thirty four (44 %) patients developed acute GVHD and median time to onset was 30 days. Fourteen (18 %) patients developed grade II–IV acute GVHD while 20

Table 2 Comparison of patient and transplant characteristics between Group A (CSA+MTX) and Group B (CSA+MMF)

	Group A—CSA+MTX (n = 53)	Group B—CSA+MMF (n = 24)	P value
Median age at transplant	28	35	0.613
Males, n (%)	39 (74)	13 (54)	0.092
ABO mismatch transplants, n (%)	22 (42)	10 (42)	0.990
Gender mismatched transplants, n (%)	27 (51)	13 (54)	0.793
Female donor to male patient transplants, n (%)	18 (34)	5 (21)	0.244
Diagnosis and baseline risk, n (%)			
Acute lymphoid leukemia (ALL)			0.645
Standard risk	1 (2)	0 (0)	
Poor risk	14 (26)	3 (13)	
Not known	4 (8)	1 (4)	
Acute myeloid leukemia (AML)			0.274
Good risk	3 (6)	2 (8)	
Intermediate risk	17 (32)	4 (17)	
Poor risk	13 (25)	9 (38)	
Not known	1 (2)	3 (13)	
Biphenotypic leukemia	0 (0)	2 (8)	
Disease status at transplant, n (%)			
ALL			0.004
Complete remission—1	15 (28)	0 (0)	
Complete remission—2	4 (8)	4 (17)	
Relapsed refractory	0 (0)	0 (0)	
AML			0.029
Complete remission—1	22 (42)	5 (21)	
Complete remission—2	7 (13)	4 (17)	
Relapsed refractory	5 (10)	9 (38)	
Biphenotypic leukemia			—
Complete remission—1	0 (0)	0 (0)	
Complete remission—2	0 (0)	1 (4)	
Relapsed refractory	0 (0)	1 (4)	
Stem cell source, n (%)			0.032
Bone marrow	2 (4)	3 (13)	
Umbilical cord	0 (0)	2 (8)	
Peripheral blood stem cells	51 (96)	19 (79)	
Type of transplant, n (%)			0.008
Matched related transplant	49 (93)	16 (67)	
Matched unrelated transplant	4 (8)	6 (25)	
Haplo-identical transplant	0 (0)	2 (8)	
Type of conditioning regimen, n (%)			0.590
Full intensity	30 (57)	12 (50)	
Reduced intensity	23 (43)	12 (50)	
ATG used, n (%)	7 (13)	5 (21)	0.393
Antifungal prophylaxis, n (%)			0.0002
Voriconazole	47 (89)	12 (50)	
Posaconazole	3 (6)	11 (46)	
Itraconazole	3 (6)	0 (0)	
Fluconazole	0 (0)	1 (4)	
Baseline presenting median WBC count	16.55	14.35	0.868
Median pre-transplant hemoglobin	10.10	9.50	0.327

Table 2 continued

	Group A—CSA+MTX (n = 53)	Group B—CSA+MMF (n = 24)	P value
Median pre-transplant serum albumin	3.90	3.80	0.225
Median pre-transplant serum creatinine	0.90	0.86	0.678
Median pre-transplant serum bilirubin	0.50	0.44	0.641
Median pre-transplant SAP	110	135	0.062
Median pre-transplant body mass index	21.78	22.64	0.322
Median pre-transplant GFR	87.07	78.16	0.25
Median cell dose infused per kg patient body weight			
TNC ($\times 10^8$)	7.18	6.77	0.180
CD34 ($\times 10^6$)	5.13	5.03	0.162
CD3 ($\times 10^6$)	169.15	137.75	0.211

Table 3 Comparison of transplant related outcomes between Group A (CSA+MTX) and Group B (CSA+MMF)

	Group A—CSA+MTX (n = 53)	Group B—CSA+MMF (n = 24)	P value
Median days to platelet engraftment	13	10	0.081
Median days to myeloid engraftment	14	12	0.003
Incidence of acute GVHD (all grades), n (%)	24 (45)	10 (42)	0.767
Incidence of acute GVHD (grade II–IV), n (%)	7 (13)	7 (29)	0.172
Incidence of chronic GVHD, n (%)			0.136
Overall	30 (57)	10 (42)	
Limited stage	10 (19)	6 (25)	
Extensive stage	20 (38)	4 (17)	
Incidence of relapse, n (%)	20 (38)	8 (33)	0.801
Slippage of chimerism, n (%)	14 (26)	8 (33)	0.287
Transplant related mortality, n (%)	3 (6)	5 (21)	0.10
Overall survival at 2 years, n (%)	34 (64)	11 (46)	0.144
Incidence of engraftment fever, n (%)	9 (17)	10 (42)	0.02
Toxicity of cyclosporine, n (%)			0.925
Nephrotoxicity	14 (26.4)	7 (29.2)	
Hypertension	22 (41.5)	10 (41.7)	
Neurotoxicity	3 (5.7)	2 (8.3)	
Other drug toxicities			
Mucositis, all grades, n (%)	50 (94.3)	14 (58.3)	0.0001
Mucositis, grade 3–4, n (%)	39 (73.6)	8 (33.3)	
Median grade of mucositis	4	1.5	
Diarrhoea, all grades, n (%)	27 (51.0)	15 (62.5)	0.473
Diarrhoea, grade 3–4, n (%)	3 (5.7)	5 (20.8)	
Median grade of diarrhea	1	2	
TPN required	42 (79)	14 (58)	0.09
Median no of days of TPN	11	8.5	

(26 %) patients developed grade 1 acute GVHD. Systemic steroids were used in 28 patients, of which 26 patients (93 %) responded. Median duration of steroid usage was 10 weeks. The incidence of any grade and grade II–IV acute GVHD was 45 and 13 % in group A compared to 42

and 29 % in group B (P = NS). Incidence of overall and extensive chronic GVHD was 57 and 38 % in group A compared to 42 and 17 % in group B (P = NS). Incidence of relapse was 38 % in group A compared to 33 % in group B (P = NS). At 2 years of median follow up, 32 (41 %)

patients have died. Twenty-four (31 %) patients died of leukemia relapse while 8 (10 %) patients died of transplant related causes (2 due to engraftment failure and neutropenic sepsis; 6 due to complications of GVHD). There were 19 (35 %) deaths in CsA+MTX group compared to 13 (54 %) in CsA+MMF group ($P = \text{NS}$). Three patients died of transplant related causes (complications of GVHD) while 16 died of disease relapse in CsA+MTX group. Five patients died of transplant related causes (3- complications of GVHD and 2-engraftment failure/neutropenic sepsis) while 8 died of disease relapse in CsA+MMF group. Overall, TRM was 6 % in CsA+MTX group and 21 % in CsA+MMF group ($P = \text{NS}$). OS at 2 years was 64 % in CsA+MTX group compared to 46 % in CsA+MMF group ($P = \text{NS}$).

Discussion

Multiple efforts have been made in last two decades to effectively suppress severe acute GVHD and at the same time exploit the anti-leukemic potential of graft versus leukemia (GVL) effect in HSCT for acute leukemia. These include use of immunosuppressive drugs singly and in combination [14, 15], tailoring the dose of CsA depending on blood levels [16, 17] and evaluation of newer immunosuppressive agents in comparison with standard agents [5–10]. CsA combined with either MTX or MMF is the most widely used immunosuppressive drug [5, 6, 8]. Recently, the European Group for Blood and Marrow Transplantation (EBMT) and European Leukemia Net have proposed recommendations for GVHD prophylaxis in allogeneic HSCT [18]. They recommend CsA+MTX as GVHD prophylaxis for patients receiving myeloablative conditioning and CsA+MMF for patients receiving reduced intensity conditioning. However, these recommendations are based on expert consensus and a Delphi-like approach as no combination GVHD prophylaxis regimen has been shown conclusively to be superior to other. One prospective study [8] and two retrospective studies [9, 10] have compared CsA+MTX with CsA+MMF for regimen related toxicity and survival outcomes. These studies have reported decreased mucositis and faster engraftment with MMF containing GVHD prophylaxis regimen compared to MTX containing regimen with no impact on incidence of acute and chronic GVHD and survival. However, the drawback of these studies was that they included heterogeneous group of patients with respect to diagnoses. Another limitation of these studies was that the study arms were not matched with respect to known risk factors for development of acute GVHD. Also, the study arms were not well balanced for factors prognosticating for TRM, relapse and survival. Thus, there is

evidence which suggests that CsA+MMF might be superior to CsA+MTX in terms of decreased toxicity but it still remains to be determined which regimen is superior in terms of engraftment kinetics, prevention of severe forms of acute GVHD and OS. This becomes even more important in acute leukemia setting given the fact that exploitation of GVL effect is of utmost importance in post transplant period. We have tried to address few of these issues within the limitations of a retrospective study.

Mucositis developing in the immediate post transplant period is associated with increased incidence of infection, increased TPN requirement and longer hospital stay [19, 20]. Conditioning regimen related mucosal injury, TBI induced mucosal damage and short course MTX used in GVHD prophylaxis all contribute to the development of mucositis. MTX plays a significant role in the development of severe mucositis, so much so that omission of fourth dose of MTX given on day +11 has been tried. However, omission of fourth dose of MTX has resulted in variable outcomes with one study reporting no impact on long term sequelae [21] while another reporting increased incidence of severe acute GVHD [22]. In our study the incidence of grade 3–4 oral mucositis was significantly less in patients who received CsA+MMF (33 %) compared to those who received CsA+MTX (74 %). This finding is in concordance with other similar studies comparing these regimens (Table 4). There was no significant difference with respect to incidence of diarrhea between two groups. More number of patients in CsA+MTX group (79 %) required TPN compared to CsA+MMF group (58 %) ($P = 0.09$). The incidence of CsA related toxicity including nephrotoxicity, neurotoxicity and hypertension was similar between the two groups.

Use of CsA in GVHD prophylaxis has been associated with increased incidence of engraftment fever compared to TAC based GVHD prophylaxis regimen [23]. In our study we found increased incidence of engraftment fever when CsA was combined with MMF (42 %) compared to when it was combined with MTX (17 %). The reason behind this finding largely remains unexplained, however it can be postulated at this point that CsA+MMF produced less intense immunosuppression compared to CsA+MTX or MMF had inherent pro-engraftment characteristics which resulted in increased incidence of engraftment fever. Kinetics of both myeloid and platelet engraftment have been shown to be influenced by GVHD prophylaxis regimen. Two studies reported significantly shorter median time to myeloid engraftment in patients who received CsA+MMF compared to those who received CsA+MTX [8, 9]. Another study reported no difference in median time to myeloid engraftment between two groups [10]. Compared to these studies, we found significantly shorter time to myeloid engraftment in

Table 4 Summary of studies comparing CsA+MTX with CsA+MMF as GVHD prophylaxis

	Bolwell et al. [8]		Neumann et al. [9]		Pinana et al. [10]		Our study	
Study type	Prospective		Retrospective		Retrospective		Retrospective	
No. of patients	40		93		145		77	
Underlying disease	Acute leukemia, NHL, MDS, CML, CLL, HD		Acute leukemia, MDS, CML		Acute leukemia, MDS, Myeloproliferative disease, Lymphoproliferative disease, Multiple myeloma		Acute leukemia	
	CsA+MTX	CsA+MMF	CsA+MTX	CsA+MMF	CsA+MTX	CsA+MMF	CsA+MTX	CsA+MMF
No. of patients	19	21	67	26	93	52	53	24
Grade 3–4 mucositis (%)	65	21	–	–	57	23	74	33
P value	0.008		–		0.001		<0.001	
Median days to myeloid engraftment	18	11	18	12	15	15	14	12
P value	<0.001		<0.001		0.8		0.003	
Median days to platelet engraftment	23	19	–	–	13	11	13	10
P value	0.008		–		0.8		0.081	
Incidence of Grade II–IV acute GVHD (%)	37	48	61	38	33	38	13	29
P value	0.49		0.11		0.5		0.172	
Incidence of chronic GVHD (%)	64	63	45	50	68	71	57	42
P value	NS		0.81		0.7		0.136	
Incidence of relapse (%)	47	38	21	10	21	29	38	33
P value	0.81		0.17		0.3		0.801	
OS (%)	68	52	55	76	51	52	64	46
OS time point	6 months		2 years		2 years		2 years	
P value	0.23		0.21		0.7		0.144	

CsA+MMF group (12 days) compared to CsA+MTX group (14 days) (Table 4). This is explained by myelo-suppressive effect of MTX which has been shown to be associated with delayed engraftment [15, 24]. Median time to platelet engraftment was less in CsA+MMF group (10 days) compared to CsA+MTX group (13 days), however this was not statistically significant. This compares well with two similar studies, one of which [8] reported significantly shorter duration of platelet engraftment with CsA+MMF compared to CsA+MTX while other [10] reported no difference in median time to platelet engraftment between the two regimens (Table 4).

We found no difference in the incidences and severity of both acute and chronic GVHD between patients who received CsA+MTX and CsA+MMF. This is in concordance with the findings reported in other similar studies (Table 4). This further reiterates the fact that both the regimens are equally efficacious in preventing all grades of acute and chronic GVHD. However, two patients in CsA+MMF group who underwent haplo-identical transplant received post transplant cyclophosphamide which

might have influenced acute GVHD in these two patients. There was no significant difference in the incidence of relapse, slippage of chimerism and TRM between the two groups.

Limitations of this retrospective study were higher proportion of patients with relapsed refractory disease and a higher proportion of MUD/HID transplants in CsA+MMF group. We believe that this limitation might have influenced survival outcome seen in this study, even though difference in survival between two groups was not statistically significant. We conclude that CsA combined with either MTX or MMF remains an effective GVHD prophylaxis regimen in acute leukemia patients undergoing HSCT. Use of MTX is associated with higher incidence of grade 3–4 mucositis and increased TPN requirement. Use of MMF is associated with higher incidence of engraftment fever and shorter time to myeloid engraftment compared to MTX. Both regimens were equally effective in preventing acute and chronic GVHD. There was no impact of GVHD prophylaxis regimen on the incidence of leukemia relapse, TRM and OS.

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Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

References

- Kolb HJ (2008) Graft-versus-leukemia effects of transplantation and donor lymphocytes. *Blood* 112:4371–4383
- Mohty M, Gaugler B (2008) Inflammatory cytokines and dendritic cells in acute graft-versus-host disease after allogeneic stem cell transplantation. *Cytokine Growth Factor Rev* 19:53–63
- Powles RL, Clink HM, Spence D, Morgenstern G, Watson JG, Selby PJ et al (1980) Cyclosporin A to prevent graft-versus-host disease in man after allogeneic bone-marrow transplantation. *Lancet* 16:327–329
- Storb R, Deeg HJ, Pepe M, Appelbaum F, Anasetti C, Beatty P et al (1989) Methotrexate and cyclosporine versus cyclosporine alone for prophylaxis of graft-versus-host disease in patients given HLA-identical marrow grafts for leukaemia: long-term follow up of a controlled trial. *Blood* 73:1729–1734
- Ratanatharathorn V, Nash RA, Przepiorka D, Devine SM, Klein JL, Weisdorf D et al (1998) Phase III study comparing methotrexate and tacrolimus (prograf, FK506) with methotrexate and cyclosporine for graft-versus-host disease prophylaxis after HLA-identical sibling bone marrow transplantation. *Blood* 92:2303–2314
- Nash RA, Antin JA, Karanes C, Fay JW, Avalos BR, Yeager AM et al (2000) Phase 3 study comparing methotrexate and tacrolimus with methotrexate and cyclosporine for prophylaxis of acute graft-versus-host disease after marrow transplantation. *Blood* 96:2062–2068
- Hiraoka A, Ohashi Y, Okamoto S, Moriyama Y, Nagao T, Koda Y et al (2001) Phase III study comparing tacrolimus (FK506) with cyclosporine for graft-versus-host disease prophylaxis after allogeneic bone marrow transplantation. *Bone Marrow Transplant* 28:181–185
- Bolwell B, Sobecks R, Pohlman B, Andresen S, Rybicki L, Kuczkowski E et al (2004) A prospective randomized trial comparing cyclosporine and short course methotrexate with cyclosporine and mycophenolate mofetil for GVHD prophylaxis in myeloablative allogeneic bone marrow transplantation. *Bone Marrow Transplant* 34:621–625
- Neumann F, Graef T, Tappich C, Vaupel M, Steidl U, Germing U et al (2005) Cyclosporine A and mycophenolate mofetil vs cyclosporine A and methotrexate for graft-versus-host disease prophylaxis after stem cell transplantation from HLA-identical siblings. *Bone Marrow Transplant* 35:1089–1093
- Piñana JL, Valcárcel D, Fernández-Avilés F et al (2010) MTX or mycophenolate mofetil with CsA as GVHD prophylaxis after reduced-intensity conditioning PB SCT from HLA-identical siblings. *Bone Marrow Transplant* 45:1449–1456
- Gota V, Pathak K, Mittal S et al (2014) A limited sampling strategy for therapeutic drug monitoring of mycophenolate mofetil for prophylaxis of acute graft versus host disease in allogeneic stem cell transplantation. *Bone Marrow Transplant* 49:S295
- Glucksberg H, Storb R, Fefer A, Buckner CD, Neiman PE, Clift RA et al (1974) Clinical manifestations of graft-versus-host disease in human recipients of marrow from HLA-matched sibling donors. *Transplantation* 18:295–304
- Shulman HM, Sullivan KM, Weiden PL, McDonald GB, Striker GE, Sale GE et al (1980) Chronic graft versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. *Am J Med* 69:204–217
- Storb R, Deeg HJ, Pepe M et al (1989) Graft-versus-host disease prevention by methotrexate combined with cyclosporine compared to methotrexate alone in patients given marrow grafts for severe aplastic anaemia: long-term follow-up of a controlled trial. *Br J Haematol* 72:567–572
- Locatelli F, Bruno B, Zecca M et al (2000) Cyclosporine A and short-term methotrexate versus cyclosporine A as graft versus host disease prophylaxis in patients with severe aplastic anemia given allogeneic bone marrow transplantation from HLA identical sibling: results of a GITMO/EBMT randomized trial. *Blood* 96:1690–1697
- Malard F, Szydlo RM, Brissot E, Chevallier P, Guillaume T, Delaunay J et al (2010) Impact of cyclosporine-A concentration on the incidence of severe acute graft-versus-host disease after allogeneic stem cell transplantation. *Biol Blood Marrow Transplant* 16:28–34
- García Cadenas I, Valcarcel D, Martino R, Piñana JL, Barba P, Novelli S, et al. Impact of cyclosporine levels on the development of acute graft versus host disease after reduced intensity conditioning allogeneic stem cell transplantation. *Mediators of Inflamm* 2014. <http://dx.doi.org/10.1155/2014/620682>
- Ruutu T, Gratwohl A, de Witte T, Afanasyev B, Apperley J, Bacigalupo A et al (2014) Prophylaxis and treatment of GVHD: EBMT-ELN working group recommendations for a standardized practice. *Bone Marrow Transplant* 49:168–173
- Ruescher TJ, Sodeifi A, Scrivani SJ et al (1998) The impact of mucositis on α -hemolytic streptococcal infection in patients undergoing autologous bone marrow transplantation for hematological malignancies. *Cancer* 82:2275–2281
- Bolwell BJ, Kalaycio M, Sobecks R et al (2002) A multivariable analysis of factors influencing mucositis after autologous progenitor cell transplantation. *Bone Marrow Transplant* 30:587–591
- Atkinson K, Downs K (1995) Omission of day 11 methotrexate does not appear to influence the incidence of moderate to severe acute graft-versus-host disease, chronic graft-versus-host disease, relapse rate or survival after HLA-identical sibling bone marrow transplantation. *Bone Marrow Transplant* 16:755–758
- Kumar S, Wolf RC, Chen MG et al (2002) Omission of day +11 methotrexate after allogeneic bone marrow transplantation is associated with increased risk of severe acute graft-versus-host disease. *Bone Marrow Transplant* 30:161–165
- Kanda J, Kaynar L, Kanda Y et al (2013) Pre-engraftment syndrome after myeloablative dual umbilical cord blood transplantation: risk factors and response to treatment. *Bone Marrow Transplant* 48:926–931
- Gondo H, Marada M, Taniguchi S et al (1993) Cyclosporine combined with methylprednisolone or methotrexate in prophylaxis of moderate to severe acute graft-versus-host disease. *Bone Marrow Transplant* 12:437–441