Risk Factors, Pattern and Clinical Outcome of Acute Graft Versus Host Disease in Acute Leukemia Patients Undergoing Allogeneic Stem Cell Transplant

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Received: 24 September 2014 / Accepted: 30 December 2014 / Published online: 7 January 2015
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Abstract We sought to determine risk factors, pattern and outcome of acute graft versus host disease (aGVHD) in seventy-seven acute leukemia patients who underwent allogeneic stem cell transplant at our centre from January 2008 to March 2013. GVHD prophylaxis with cyclosporine-methotrexate or cyclosporine-mycophenolate mofetil was used. Patients were divided in 2 groups, grade II-IV aGVHD (group A) and grade 0-I aGVHD (group B). Incidence of any grade and grade II-IV aGVHD was 44 and 18 %, respectively. The most common site of aGVHD was gastro-intestinal tract (65 %) followed by skin (35 %). Higher total nucleated cell (TNC) dose infused was associated with increased incidence of grade II-IV aGVHD.

Incidence of relapse and incidence of slippage of chimerism was 21 and 36 % in group A while 37 and 27 % in group B respectively. Transplant related mortality (TRM) was 21 % in group A and 13 % in group B. Probability of OS and RFS at 4 years was 63 and 34 % in group A compared with 40 and 38 % in group B, respectively. We conclude that higher TNC dose infused is a risk factor for grade II-IV aGVHD with gut being the commonest site. Grade II-IV aGVHD did not have a significant impact on incidence of relapse, TRM and OS.

Keywords Acute graft-versus-host disease · Acute leukemia · Allogeneic stem cell transplantation · GVHD prophylaxis · Transplant outcome

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Introduction

Allogeneic stem cell transplant (ASCT) is an important and effective treatment modality for treating patients with acute leukemia. However, the benefits of ASCT are offset to an extent by increased morbidity and mortality associated with GVHD [1]. Established acute graft versus host disease (aGVHD) is difficult to treat, with systemic steroids being the mainstay of therapy [2, 3]. However, aGVHD can be effectively prevented to some extent by use of immunosuppressive drugs [4]. CsA combined with either methotrexate (MTX) or mycophenolate mofetil (MMF) remain the most common immunosuppressive drugs used for GVHD prophylaxis [5–7]. But, excessive immunosuppression to decrease the risk of GVHD leads to increased risk of relapse [decreased graft versus leukemia effect (GVL)], graft rejection and delayed immune reconstitution predisposing to infections [8–10]. Therefore, it is required to carefully titrate the doses of immunosuppressive agents

in order to maintain just adequate immunosuppression which prevents GVHD and also does not hamper GVL effect. Such a strategy would lead to best optimization of transplant outcomes in acute leukemia.

Several factors including patient-donor related factors, disease related factors, conditioning regimen and GVHD prophylaxis used can influence the incidence and severity of aGVHD [11–17]. However, variation is seen across different studies with respect to risk factors for development of aGVHD as well as the incidence, severity and pattern of organ involvement in aGVHD. One potential reason for these differences could be the fact that these studies included patients who were transplanted for a spectrum of malignant and non-malignant hematological disorders. Therefore, this study was done with the aim to determine risk factors, severity and pattern of aGVHD in acute leukemia patients undergoing ASCT. We also sought to determine if the development of severe aGVHD had an impact on post-transplant outcomes including transplant related mortality (TRM), leukemia relapse and survival.

Materials and Methods

Patients

All patients diagnosed with acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL) and acute leukemia of ambiguous lineage (ABL) who underwent ASCT from January 2008 to March 2013 were included in this retrospective study. Patients were either in first (CR-1) or second (CR-2) remission or in relapsed/refractory (RR) state at the time of transplant. High risk 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) $>100 \times 10^9/L$ at baseline, not achieving CR after induction, disease stage $\geq$ CR-2 or persistent disease at transplant. Poor risk 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 $>100 \times 110^9/L$ at baseline, not achieving CR after induction, disease stage $\geq$ CR-2 or persistent disease at transplant. Depending on availability of human leucocyte antigen (HLA) matched donor, patients underwent either matched related donor (MRD) transplant, MRD transplant or haplo-identical donor (HID) transplant. Hematopoietic stem cell graft was either T cell replete granulocyte-colony stimulating factor (G-CSF) mobilized peripheral blood stem cells (PBSCs), bone marrow harvest or cord blood derived stem cells.

Conditioning Regimen and GVHD Prophylaxis

Conditioning regimen was full intensity (FI) i.e. Total Body Irradiation with cyclophosphamide (TBI-Cy) or busulfan with cyclophosphamide (BuCy) in 27 (35 %) transplants and reduced intensity (RI) i.e. fludarabine based in 50 (65 %) transplants. TBI dose in FI conditioning regimen was 12–14.4 Gy in 8 fractions over 4 days from day-7 to day-4 while cyclophosphamide was given as 60 mg/kg intravenously(iv) over 2 h on day-3 and day-2. Busulfan was given oral as 4 mg/kg/day in 4 divided doses for 4 days from day-8 to day-5 with cyclophosphamide as 60 mg/kg intravenous over 2 h on day -3 and day-2. In RI conditioning regimen fludarabine was used iv at a dose of 30 mg/m² 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. GVHD prophylaxis was CsA + MTX in 53 (68.8 %) patients and CsA + MMF in 24 (31.2 %) patients. CsA and MMF was started on day-1 of transplant at 1.5 mg/kg/twice daily iv as 2 h infusion and 600 mg/m²/twice daily orally, respectively. Trough CsA blood level was measured twice weekly by chemiluminiscence assay (Architect analyser, Abbott Healthcare - detection limit 25 ng/ml) starting from day +3 to day +5 of transplant and continued till the start of CsA tapering. CsA dose was modified to achieve therapeutic level of 150–200 ng/ml. CsA dose and duration was also modified depending on the risk of relapse, development of GVHD and toxicity. CsA was tapered from day +90 and stopped by day +180. In high risk patients, CsA was tapered from day +60 and stopped by day +120. In patients who developed significant CsA related nephrotoxicity or neurotoxicity, CsA was replaced by MMF. Intravenous CsA was replaced by oral CsA upon resolution of mucositis and diarrhea. MMF was administered from day -1 to day +30. MTX was given iv at 15 mg/m² on day +1 and 10 mg/m² on day +3, +6, +11. MTX doses were omitted if patient developed grade IV oral mucositis. Two 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 patients the dose of MMF was 15 mg/kg thrice daily till day +35 if there were no signs of acute GVHD. In addition to standard GVHD prophylaxis, patients who underwent one antigen mismatched sibling donor transplant or MUD transplant received rabbit anti-thymocyte globulin (Thymoglobulin) at a dose of 2.5 mg/kg/dose on day-2 (1 dose) or day-2 to day-1(2 doses). Azole antifungal prophylaxis was used in all patients and continued till day +100 of transplant or longer if patient was on steroids for treatment of GVHD.

Definitions

Acute GVHD

GVHD developing in first 100 days of transplant. The diagnosis and grading of aGVHD was done clinically according to standard criteria [18, 19]. Histological confirmation was done only in patients with suspected upper gut and skin GVHD. Patients with suspected lower gut GVHD did not undergo colonoscopy and biopsy. Diagnosis of lower gut GVHD was made clinically. Treatment of aGVHD was started with methylprednisolone 1–2 mg/kg/day given intra-venously, followed by tapering upon clinical improvement [19].

Chronic GVHD

GVHD developing after 100 days of transplant. cGVHD was classified as limited stage or extensive stage according to standard criteria [20]. 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 $\geq 0.5 \times 10^9/\text{L}$ [21]. Time to platelet engraftment was defined as first of 7 consecutive days when the platelet count is maintained $\geq 20 \times 10^9/\text{L}$ without platelet transfusion.

TRM

Death occurring in the post-transplant period not related to relapse of leukemia.

Relapse Free Survival (RFS)

The time interval between date of transplant and date of relapse or death or last follow-up if in CR.

Overall Survival (OS)

The time interval between date of transplant and date of death or last follow-up.

Statistical Considerations

For analysis patients were divided in 2 groups, grade II-IV aGVHD (group A) and no-grade I aGVHD (group B). To determine factors which might pre-dispose to grade II-IV

aGVHD, univariate comparisons were done between group A and group B for discrete variables by Chi square test and for continuous variables by Mann–Whitney Test. Potential factors analyzed for increased risk of grade II-IV aGVHD were gender; diagnosis (AML, ALL); disease status at transplant (CR1, CR2, RR); ABO blood group and gender mismatch; conditioning regimen (FI, RI); GVHD prophylaxis used (CsA + MTX, CsA + MMF); type of donor (MRD, MUD, HID); source of stem cells (PBSCs, bone marrow, cord blood); cell dose infused (TNC, CD34 + cells, CD3 + cells). Two groups were also compared to determine the impact of severe aGVHD on transplant related outcomes including incidence of leukemia relapse, TRM, slippage of chimerism, RFS and OS. Survival analysis was done by Kaplan–Meier method and outcomes were compared using log-rank test.

Results

Seventy-seven transplants (52 males and 25 females) were done which included 52 (67.5 %) patients with AML, 23 (29.9 %) with ALL and 2 (2.6 %) with ABL. The median age was 30 years (range 6–51). Forty-two (54.5 %) patients were in first complete remission (CR1), 20 (26 %) in second complete remission (CR2) and 15 (19.5 %) in relapsed/refractory state (RR) at the time of ASCT. Seventeen (73.9 %) patients with ALL had high risk disease at transplant. Twenty two (42.3 %) patients with AML had poor risk disease. Sixty five (84.4 %) patients underwent MRD transplant, 10 (13 %) MUD transplant and 2 (2.6 %) HID transplant. Hematopoietic stem cell graft was PBSCs in 70 (90.9 %) transplants, bone marrow harvest in 5 (6.5 %) and cord blood in 2 (2.6 %) transplants (Table 1).

Incidence, Severity and Risk Factors for aGVHD

Thirty four (44 %) patients developed aGVHD and median time to onset was 30 days. Fourteen (18 %) patients developed grade II-IV aGVHD while 20 (26 %) patients developed grade I aGVHD. Higher TNC infused was the only factor found significantly associated with increased incidence of grade II-IV aGVHD (Table 1). Median TNC dose infused per kg body weight of patient was found to be 9.9×10^8 in group A compared to 6.6×10^8 in group B ($P = 0.041$). However, as 5 and 2 patients in no GVHD/grade I GVHD had marrow and cord grafts respectively, we excluded these 7 patients and compared the TNC of the 2 groups with only PBSC grafts. When only PBSC grafts were evaluated, there was no significant difference in the infused TNC (9.88×10^8 vs $7.09 \times 10^8/\text{kg}$, $p = 0.12$). There was no impact of gender, diagnosis (AML, ALL), disease status at transplant (CR1, CR2, RR), ABO blood

Table 1 Analysis of patient and transplant related potential risk factors for Grade II-IV aGVHD

	Total N = 77(%)	Grade II-IV aGVHD N = 14(%)	No-grade I aGVHD N = 63(%)	P value
Male	52 (67.5)	10 (71.4)	42 (66.7)	0.731
ABO mismatched transplants	32 (41.6)	8 (57.1)	24 (38.1)	0.191
Gender mismatched transplants	40 (51.9)	8 (57.1)	32 (50.8)	0.667
Diagnosis	23 (29.9)	5 (35.7)	18 (28.6)	0.4
Acute lymphoid leukemia (ALL)	52 (67.5)	8 (57.1)	44 (69.8)	
Acute myeloid leukemia (AML)	02 (02.6)	1 (7.1)	1 (1.6)	
Biphenotypic leukemia				
Disease status at transplant	42 (54.5)	6 (42.9)	36 (57.1)	0.821
Complete remission–1	20 (26.0)	5 (35.7)	15 (23.8)	
Complete remission–2	15 (19.5)	3 (21.4)	12 (19.1)	
Refractory/Relapse (in disease)				
Stem cell source	05 (06.5)	0	5 (7.9)	0.425
Bone marrow	02 (02.6)	0	2 (3.2)	
Umbilical cord	70 (90.9)	14 (100)	56 (88.9)	
Peripheral blood stem cells				
Type of transplant	65 (84.4)	12 (85.7)	53 (84.1)	0.791
Matched related transplant	10 (13.0)	2 (14.3)	8 (12.7)	
Matched unrelated transplant	02 (02.6)	0	2 (3.2)	
Haplo-identical transplant				
Type of conditioning regimen	27 (35)	6 (42.8)	21 (33.3)	0.545
Full intensity	50 (65)	8 (57.2)	42 (66.67)	
Reduced intensity				
Graft versus host disease prophylaxis	53 (68.8)	8 (57.1)	45 (71.4)	0.297
Cyclosporine + Methotrexate	24 (31.2)	6 (42.9)	18 (28.6)	
Cyclosporine + Mycophenolate mofetil				
Median (range) cell dose infused per kg body weight of patient (all patients)	7.6 (0.98–20.96)	9.9 (4.06–17.62)	6.6 (0.98–20.96)	0.041
	5.12 (0.26–18.70)	5.03 (1.74–9.30)	5.12 (0.26–18.70)	0.853
TNC ($\times 10^8$)	163.3 (12.2–569.6)	166.6 (47.0–362.4)	160.1 (12.2–569.6)	0.738
CD34 ($\times 10^6$)				
CD3 ($\times 10^6$)				
Median (range) cell dose infused per kg body weight of patient (only PBSC grafts, n = 70)	7.93 (3.68–20.96)	9.88 (5.07–17.62)	7.09 (3.68–20.96)	0.120
	5.20 (2.43–18.70)	5.03 (3.71–9.30)	5.25 (2.43–18.70)	0.386
TNC ($\times 10^8$)	171.30 (47.0–569.6)	166.6 (47.0–362.4)	174.7 (48.8–569.6)	0.731
CD34 ($\times 10^6$)				
CD3 ($\times 10^6$)				
Median pre-transplant hemoglobin (gm/dl)	9.9	9.8	10.0	0.526
Median pre-transplant serum albumin (gm/dl)	3.9	3.85	3.9	0.850
Median pre-transplant serum creatinine (mg/dl)	0.9	0.9	0.87	0.890
Median pre-transplant serum bilirubin (mg/dl)	0.45	0.46	0.45	0.809
Median pre-transplant SAP (IU/ml)	119	120	119	0.809

group and gender mismatch, conditioning regimen (FI, RI), GVHD prophylaxis used (CsA + MTX, CsA + MMF), type of donor (MRD, MUD, HID), source of stem cells (PBSCs, bone marrow, cord blood), CD3 and CD34 cell dose infused on the incidence of severe aGVHD (Table 1).

Pattern of Organ Involvement in aGVHD

The most common site was gastro-intestinal tract, seen in 22 (65 %) patients. Nineteen (56 %) patients developed lower gut aGVHD, 2 (6 %) developed upper gut aGVHD

while 1 (3 %) developed both upper and lower gut aGVHD. Among those with lower gut involvement (20 patients), 9 (45 %) patients had stage ≥ 2 acute gut GVHD. Skin GVHD was seen in 12 (35 %) patients (six with stage 1 and 6 with stage 2). None of the patients had both gut and skin involvement. No patient developed associated liver GVHD.

Transplant Outcome

Systemic steroids were used in 28 patients, of which 26 patients (93 %) responded. Median duration of steroid usage was 10 weeks. Incidence of relapse and incidence of slippage of chimerism was 21 % and 36 % in group A while 37 % and 27 % in group B, respectively ($P = \text{NS}$). TRM was 21 % in group A and 13 % in group B ($P = \text{NS}$). Probability of OS and RFS at 4 years was 63 % and 34 % in group A compared with 40 % and 38 % in group B, respectively ($P = \text{NS}$) (Table 2).

Discussion

Acute GVHD remains a common and one of the most morbid complication of allogeneic transplantation for hematological disorders. Over the past thirty years, few studies have made an attempt to identify factors associated with increased risk of aGVHD [11, 17]. However, due to significant variation in transplant practices, variation in underlying diagnoses, variation in condition regimens used, and variation in GVHD prophylaxis used, there exists variability in the risk factors most significantly implicated in aGVHD across these studies. Among the non-modifiable risk factors, gender mismatched transplants and in particular female donor-male recipient transplants have been found to be associated with increased risk of aGVHD [14]. In our study, we found that gender mismatched transplant did not increase the risk of severe aGVHD. Greater degree of HLA mismatch between patient and donor is associated with increased risk of aGVHD [11, 22]. There was concern that unrelated donor transplant might be associated with increased aGVHD compared to sibling donor transplant because of expected increase in degree of major and minor histo-compatibility mismatch. However, now there is

evidence suggesting that outcome of 10/10 HLA matched unrelated donor transplant is similar to HLA identical sibling donor transplant with respect to incidence of aGVHD [23]. In our study also, we did not find any significant difference in the incidence of severe aGVHD with respect to the type of transplant (MRD, MUD, HID). Few studies have reported that use of graft source as PBSCs has been associated with increased risk of cGVHD and less frequently, with aGVHD [12, 24–26]. Others have reported no impact of stem cell source on aGVHD [27]. In our study, patients who received bone marrow graft (8 %) were too small to make this comparison. However, we did not see any difference in incidence of severe aGVHD with respect to stem cell source.

Few studies have also evaluated the impact of conditioning regimen (FI, RI) and use of TBI on the incidence of aGVHD [28–30]. These studies reported increased incidence and severity of aGVHD in patients receiving FI conditioning regimen. In our study, 71 % of patients received FI conditioning in group A (grade II–IV aGVHD) compared to 51 % in group B (No-grade I aGVHD). However, this association between FI conditioning regimen and severe aGVHD was not found statistically significant. Another important risk factor for the development of aGVHD is the type of GVHD prophylaxis used. CsA combined with either MTX or MMF remain two of the most widely accepted GVHD prophylaxis regimens [19]. Few studies have reported decreased incidence of severe aGVHD with tacrolimus based prophylaxis regimens without any impact on survival [5, 6]. We used CsA + MTX (69 %) and CsA + MMF (31 %) as GVHD prophylaxis and found no significant difference in the incidence of severe aGVHD. Any influence of diagnoses on the incidence of aGVHD was limited in our study as only patients transplanted for acute leukemia were included.

Association of cell dose infused with aGVHD, cGVHD, relapse and survival is complex and has varied significantly between studies. One study reported increased risk of severe aGVHD with higher CD3 + and CD34 + cell doses in patients receiving ASCT [31]. Another study has reported decreased risk of relapse and no impact on aGVHD/cGVHD with higher nucleated marrow cell dose infused [32]. On the contrary, few others have highlighted increased risk of extensive cGVHD with higher TNC dose infused [33]. One

Table 2 Impact of Grade II–IV aGVHD on transplant related outcomes

	Grade II–IV aGVHD	No-grade I aGVHD	P value
Incidence of relapse, n (%)	3 (21.4)	23 (36.5)	0.281
Slippage of chimerism, n (%)	5 (35.7)	17 (27)	0.513
Transplant related mortality, n (%)	3 (21.4)	8 (12.7)	0.397
Probability of relapse free survival at 4 years	34 %	38 %	0.569
Probability of overall survival at 4 years	63 %	40 %	0.417

recent study has reported higher TNC dose to be an independent factor associated with increased incidence of cGVHD, decreased relapse and improved overall survival [34]. However, none of these studies found an association between TNC dose and incidence of severe aGVHD. Contrary to this we found higher TNC to be significantly associated with increased incidence of grade II-IV aGVHD. This may be partly explained by the fact that increased TNC results in increase in the donor derived antigen presenting cells which take part in initiation and propagation of aGVHD [35]. However, this also could partly be attributed to the fact that none of the patients with bone marrow or cord grafts developed acute GVHD. It is well known that bone marrow and cord grafts contain fewer nucleated cells compared to PBSC grafts. In keeping with this, when only PBSC grafts were evaluated, no significant difference was found in our study between the two groups.

Gastrointestinal tract, skin and liver are the principal organs involved in patients with aGVHD. GVHD involving lower gastrointestinal tract manifests as large volume watery diarrhea which may become bloody, abdominal cramps, nausea and vomiting [36]. GVHD involving upper gastrointestinal tract manifests as anorexia, dyspepsia, food intolerance, nausea, and vomiting [37, 38]. Skin GVHD manifests as maculopapular rash that starting with palm and sole involvement. It can become pruritic, progressively involves other areas, can result in generalized erythroderma and skin exfoliation. Liver GVHD manifests as jaundice from hyperbilirubinemia and increased serum alkaline phosphatase due to cholestasis. The pattern, frequency and severity of organ involvement in aGVHD have varied across studies. One large study reported 33 % incidence of grade II to IV aGVHD with gastrointestinal tract, skin and liver involvement seen in 74, 70 and 44 percent, respectively [5]. A study from India done in children transplanted for thalassemia major reported 28 % incidence of grade II-IV aGVHD [39]. In our study the incidence of grade II-IV aGVHD was 18 %. Overall, gastrointestinal tract was the commonest site (65 %) followed by skin (35 %). Thus, our findings are similar to those reported previously. An interesting and as yet unexplained finding in our study was that no patient developed acute liver GVHD. Also, none of the patients developed aGVHD with simultaneous involvement of two organ systems. This is in contrast to the findings reported in earlier studies, where as much as 24 % patients with aGVHD had involvement of all three organs systems [5]. In our study, complete resolution of aGVHD was seen in 93 % patients who received systemic steroids. This compares well with other similar studies which have reported more than 90 % resolution rates of aGVHD with systemic steroids [37, 39].

Severe aGVHD is associated with increased morbidity and mortality in ASCT setting [1, 40]. GVHD prophylaxis

can effectively prevent aGVHD in majority of cases. However, few studies have reported increased risk of relapse associated with decreased incidence of GVHD secondary to GVHD prophylaxis [8, 9]. Higher doses of immunosuppressive agents used in GVHD prophylaxis also correlates with increased leukemia relapse [10]. Thus, both development of severe aGVHD (associated with increased TRM) as well as profound suppression of aGVHD (associated with increased leukemia relapse) might have negative impact on the final transplant related outcome i.e. OS. This protective effect of GVHD on leukemia relapse has largely been explained by the GVL effect mediated by donor derived T-cells. One study also highlighted the beneficial effect of cGVHD in decreasing leukemia relapse and increasing overall survival [41]. Similar beneficial effect on overall survival has not been demonstrated in terms of aGVHD [40, 42]. In our study also, development of any grade and grade II-IV aGVHD did not have a significant impact on post-transplant outcomes namely TRM, leukemia relapse, RFS and OS. Though the incidence of leukemia relapse was less in patients who developed severe aGVHD (21 %) compared to those who did not (37 %), it was not statistically significant. Similarly, there was a trend towards increased TRM in patients who developed severe aGVHD (21 %) compared to those who did not (13 %), it was not statistically significant. This non-significant increase in TRM in patients with severe aGVHD was compensated by non-significant decrease in relapse incidence resulting in similar probability of RFS and OS at four years (Figs. 1, 2).

We included only acute leukemia patients in this study in order to eliminate the confounding factor of multiple diagnoses. This resulted in a relatively small sample size, which is probably one of the limitations of this study.

Fig. 1 Impact of Grade II-IV acute GVHD on overall survival (OS)

Fig. 2 Impact of Grade II-IV acute GVHD on relapse free survival (RFS)

Increase in TRM, decrease in leukemia relapse and increase in probability of OS at 4 years seen in patients with severe aGVHD might have not reached statistical significance because of the sample size. A larger sample size may have demonstrated more convincingly the correlation of severe aGVHD with TRM and leukemia relapse. However, we believe that even with a larger sample size it would have been difficult to draw any conclusion on the association of aGVHD with long term survival. Larger studies with much longer follow up are probably required to answer this question. In conclusion, gastro-intestinal tract was the commonest site of aGVHD in our patients with acute leukemia who underwent ASCT. No patient developed acute liver GVHD. Development of grade II-IV aGVHD did not have an impact on transplant related outcomes namely TRM, leukemia relapse, RFS and OS.

Acknowledgment The authors declare that they have not received support in the form of grants and/or equipment and drugs from any source.

Conflict of interest The authors declare no conflict of interest.

References

1. 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
2. Van Lint MT, Uderzo C, Locasciulli A, Majolino I, Scimé R, Locatelli F, Giorgiani G, Arcese W, Iori AP, Falda M, Bosi A, Miniero R, Alessandrino P, Dini G, Rotoli B, Bacigalupo A (1998) Early treatment of acute graft-versus-host disease with high- or low-dose 6-methylprednisolone: a multicenter randomized trial from the Italian group for bone marrow transplantation. *Blood* 92:2288–2293
3. Van Lint MT, Milone G, Leotta S, Uderzo C, Scime R, Dallorso S, Locasciulli A, Guidi S, Mordini N, Sica S, Cudillo L, Fagioli

- F, Selleri C, Bruno B, Arcese W, Bacigalupo A (2006) Treatment of acute graft-versus-host disease with prednisolone: significant survival advantage for day p5 responders and no advantage for nonresponders receiving antithymocyte globulin. *Blood* 107:4177–4181
4. Storb R, Deeg HJ, Whitehead J, Appelbaum F, Beatty P, Bensinger W, Buckner CD, Clift R, Doney K, Farewell V, Hansen J, Hill R, Lum L, Martin P, McGuffin R, Sanders J, Stewart P, Sullivan K, Witherspoon R, Yee G, Thomas ED (1986) Methotrexate and cyclosporine compared with cyclosporine alone for prophylaxis of acute graft versus host disease after marrow transplantation for leukemia. *N Engl J Med* 314:729–735
5. Ratanatharathorn V, Nash RA, Przepiorka D, Devine SM, Klein JL, Weisdorf D, Fay JW, Nademanee A, Antin JH, Christiansen NP, van der Jagt R, Herzig RH, Litzow MR, Wolff SN, Longo WL, Petersen FB, Karanes C, Avalos B, Storb R, Buell DN, Maher RM, Fitzsimmons WE, Wingard JR (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
6. Nash RA, Antin JA, Karanes C, Fay JW, Avalos BR, Yeager AM, Przepiorka D, Davies S, Petersen FB, Bartels P, Buell D, Fitzsimmons W, Anasetti C, Storb R, Ratanatharathorn V (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
7. Bolwell B, Sobecks R, Pohlman B, Andresen S, Rybicki L, Kuczkowski E, Kalaycio M (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
8. Patterson J, Prentice HG, Brenner MK, Gilmore M, Janossy G, Ivory K, Skeggs D, Morgan H, Lord J, Blacklock HA, Hoffbrand AV, Apperley JF, Goldman JM, Burnett A, Gribben J, Alcorn M, Pearson C, Mcvickei I, Hann IM, Reid C, Wardle D, Gravett PJ, Bacigalupo A, Robertson AAG (1986) Graft rejection following HLA matched T-lymphocyte depleted bone marrow transplantation. *Br J Haematol* 63:221–230
9. Goldman JM, Gale RP, Horowitz MM, Biggs JC, Champlin RE, Gluckman E, Hoffmann RG, Jacobsen SJ, Marmont AM, McGlave PB, Messner HA, Rimm AA, Rozman C, Speck B, Tura S, Weiner RS, Bortin MM (1988) Bone marrow transplantation for chronic myelogenous leukemia in chronic phase. Increased risk for relapse associated with T-cell depletion. *Ann Intern Med* 108:806–812
10. Bacigalupo A, Van Lint MT, Occhini D, Guolandi F, Lamparelli T, Sogno G, Tedone E, Frasson F, Tong J, Marmont AM (1991) Increased risk of leukemia relapse with high-dose cyclosporine A after allogeneic marrow transplantation for acute leukemia. *Blood* 77:1423–1428
11. Anasetti C, Beatty PG, Storb R, Martin PJ, Mori M, Sanders JE, Thomas ED, Hansen JA (1990) Effect of HLA incompatibility on graft-versus-host disease, relapse, and survival after marrow transplantation for patients with leukemia or lymphoma. *Hum Immunol* 29(2):79–91
12. Champlin RE, Schmitz N, Horowitz MM, Chappuis B, Chopra R, Cornelissen JJ, Gale RP, Goldman JM, Loberiza FR Jr, Herstein B, Klein JP, Montserrat E, Zhang MJ, Ringden O, To many SC, Rowlings PA, Van Hoef ME, Gratwohl A (2000) Blood stem cells compared with bone marrow as a source of hematopoietic cells for allogeneic transplantation. IBMTR Histocompatibility and Stem Cell Sources Working Committee and the European Group for Blood and Marrow Transplantation (EBMT). *Blood* 95(12): 3702–3709

13. Eisner MD, August CS (1995) Impact of donor and recipient characteristics on the development of acute and chronic graft-versus-host disease following pediatric bone marrow transplantation. *Bone Marrow Transplant* 15(5):663–668
14. Gale RP, Bortin MM, van Bekkum DW, Biggs JC, Dicke KA, Gluckman E, Good RA, Hoffmann RG, Kay HE, Kersey JH (1987) Risk factors for acute graft-versus-host disease. *Br J Haematol* 67(4):397–406
15. Häggglund H, Bostrom L, Remberger M, Ljungman P, Nilsson B, Ringden O (1995) Risk factors for acute graft-versus-host disease in 291 consecutive HLA-identical bone marrow transplant recipients. *Bone Marrow Transplant* 16(6):747–753
16. Izumi N, Furukawa T, Sato N, Okazuka K, Tsukada N, Abe T, Yano T, Kurasaki T, Masuko M, Toba K, Takahashi M, Aizawa Y (2007) Risk factors for acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation: retrospective analysis of 73 patients who received cyclosporin A. *Bone Marrow Transplant* 40(9):875–880
17. Jagasia M, Arora M, Flowers ME, Chao NJ, McCarthy PL, Cutler CS, Urbano-Ispizua A, Pavletic SZ, Haagenson MD, Zhang MJ, Antin JH, Bolwell BJ, Bredeson C, Cahn JY, Cairo M, Gale RP, Gupta V, Lee SJ, Litzow M, Weisdorf DJ, Horowitz MM, Hahn T (2012) Risk factors for acute GVHD and survival after hematopoietic cell transplantation. *Blood* 119(1):296–307
18. Glucksberg H, Storb R, Fefer A, Buckner CD, Neiman PE, Clift RA, Lerner KG, Thomas ED (1974) Clinical manifestations of graft-versus-host disease in human recipients of marrow from HLA-matched sibling donors. *Transplantation* 18:295–304
19. Ruutu T, Gratwohl A, de Witte T, Afanasyev B, Apperley J, Bacigalupo A, Dazzi F, Dreger P, Duarte R, Finke J, Garderet L, Greinix H, Holler E, Kröger N, Lawitschka A, Mohty M, Nagler A, Passweg J, Ringden O, Socié G, Sierra J, Sureda A, Wiktor-Jedrzejczak W, Madrigal A, Niederwieser D (2014) Prophylaxis and treatment of GVHD: EBMT-ELN working group recommendations for a standardized practice. *Bone Marrow Transplant* 49(2):168–173
20. Shulman HM, Sullivan KM, Weiden PL, McDonald GB, Striker GE, Sale GE, Hackman R, Tsoi MS, Storb R, Thomas ED (1980) Chronic graft versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. *Am J Med* 69(2):204–217
21. Ali MY, Oyama Y, Monreal J, Winter J, Tallman M, Gordon LI, Williams S, Singhal S, Mehta J (2002) Reassessing the definition of myeloid engraftment after autotransplantation: it is not necessary to see $0.5 \times 10^9/l$ neutrophils on 3 consecutive days to define myeloid recovery. *Bone Marrow Transplant* 30:749–752
22. Arora M, Weisdorf DJ, Spellman SR, Haagenson MD, Klein JP, Hurley CK, Selby GB, Antin JH, Kernan NA, Kollman C, Nadeem A, McGlave P, Horowitz MM, Petersdorf EW (2009) HLA identical sibling compared with 8/8 matched and mismatched unrelated donor bone marrow transplant for chronic phase chronic myeloid leukemia. *J Clin Oncol* 27(10):1644–1652
23. Yakoub-Agha I, Mesnil F, Kuentz M, Boiron JM, Ifrah N, Milpied N, Chehata S, Esperou H, Vernant JP, Michallet M, Buzyn A, Gratecos N, Cahn JY, Bourhis JH, Chir Z, Raffoux C, Socié G, Golmard JL, Jouet JP (2006) Allogeneic marrow stem-cell transplantation from human leukocyte antigen-identical siblings versus human leukocyte antigen-allelic-matched unrelated donors (10/10) in patients with standard-risk hematologic malignancy: a prospective study from the French Society of Bone Marrow Transplantation and Cell Therapy. *J Clin Oncol* 24(36):5695–5702
24. Bensinger WI, Martin PJ, Storer B, Clift R, Forman SJ, Negrin R, Kashyap A, Flowers ME, Lilleby K, Chauncey TR, Storb R, Appelbaum FR (2001) Transplantation of bone marrow as compared with peripheral-blood cells from HLA-identical relatives in patients with hematologic cancers. *N Engl J Med* 344(3):175–181
25. Hahn T, McCarthy PL Jr, Zhang MJ, Wang D, Arora M, Frangoul H, Gale RP, Hale GA, Horan J, Isola L, Maziarz RT, van Rood JJ, Gupta V, Halter J, Reddy V, Tiberghien P, Litzow M, Anasetti C, Pavletic S, Ringden O (2008) Risk factors for acute graft-versus-host disease after human leukocyte antigen-identical sibling transplants for adults with leukemia. *J Clin Oncol* 26(35):5728–5734
26. Schrezenmeier H, Passweg JR, Marsh JC, Bacigalupo A, Bredeson CN, Bullorsky E, Camitta BM, Champlin RE, Gale RP, Fuhrer M, Klein JP, Locasciulli A, Oneto R, Schattenberg AV, Socie G, Eapen M (2007) Worse outcome and more chronic GVHD with peripheral blood progenitor cells than bone marrow in HLA-matched sibling donor transplants for young patients with severe acquired aplastic anemia. *Blood* 110(4):1397–1400
27. Remberger M, Ringden O, Blau IW, Ottinger H, Kremens B, Kiehl MG, Aschan J, Beelen DW, Basara N, Kumlien G, Fauser AA, Runde V (2001) No difference in graft-versus-host disease, relapse, and survival comparing peripheral stem cells to bone marrow using unrelated donors. *Blood* 98(6):1739–1745
28. Couriel DR, Saliba RM, Giralt S, Khouri I, Andersson B, de Lima M, Hosing C, Anderlini P, Donato M, Cleary K, Gajewski J, Neumann J, Ippoliti C, Rondon G, Cohen A, Champlin R (2004) Acute and chronic graft-versus-host disease after ablative and nonmyeloablative conditioning for allogeneic hematopoietic transplantation. *Biol Blood Marrow Transplant* 10(3):178–185
29. Mielcarek M, Martin PJ, Leisenring W, Flowers ME, Maloney DG, Sandmaier BM, Maris MB, Storb R (2003) Graft-versus-host disease after nonmyeloablative versus conventional hematopoietic stem cell transplantation. *Blood* 102(2):756–762
30. Sorrow ML, Maris MB, Storer B, Sandmaier BM, Diaconescu R, Flowers C, Maloney DG, Storb R (2004) Comparing morbidity and mortality of HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative and myeloablative conditioning: influence of pretransplantation comorbidities. *Blood* 104(4):961–968
31. Gaziev J, Isgrò A, Marziani M, Daniele N, Gallucci C, Sodani P, Simone MD, Adorno G, Paciaroni K, Andreani M, Lanti A, Del Proposto G, Testi M, De Angelis G, Roveda A, Alfieri C, Saltarelli F, Lucarelli G (2012) Higher CD3(+) and CD34(+) cell doses in the graft increase the incidence of acute GVHD in children receiving BMT for thalassemia. *Bone Marrow Transplant* 47(1):107–114
32. Barrett AJ, Ringden O, Zhang MJ, Bashey A, Cahn JY, Cairo MS, Gale RP, Gratwohl A, Locatelli F, Martino R, Schultz KR, Tiberghien P (2000) Effect of nucleated marrow cell dose on relapse and survival in identical twin bone marrow transplants for leukemia. *Blood* 95(11):3323–3327
33. Przepiorka D, Anderlini P, Saliba R, Cleary K, Mehra R, Khouri I, Huh YO, Giralt S, Braunschweig I, van Besien K, Champlin R (2001) Chronic graft-versus-host disease after allogeneic blood stem cell transplantation. *Blood* 98(6):1695–1700
34. Martin P, Li S, Alyea EP, Ho VT, Cutler CS, Armand P, Koreth J, Luckey CJ, Kao G, Ritz J, Antin JH, Soiffer RJ (2013) Impact of total nucleated cell and cd34 + cell dose on transplant related outcomes in reduced intensity conditioning allogeneic hematopoietic cell transplants. *Blood* 122(21):2048
35. Socié G, Blazar BR (2009) Acute graft-versus-host disease: from the bench to the bedside. *Blood* 114(20):4327–4336
36. Martin PJ, McDonald GB, Sanders JE, Anasetti C, Appelbaum FR, Deeg HJ, Nash RA, Petersdorf EW, Hansen JA, Storb R (2004) Increasingly frequent diagnosis of acute gastrointestinal graft-versus-host disease after allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant* 10(5):320–327
37. Weisdorf DJ, Snover DC, Haake R, Miller WJ, McGlave PB, Blazar B, Ramsay NK, Kersey JH, Filipovich A (1990) Acute upper gastrointestinal graft-versus-host disease: clinical

- significance and response to immunosuppressive therapy. *Blood* 76(3):624–629
38. Wakui M, Okamoto S, Ishida A, Kobayashi H, Watanabe R, Yajima T, Iwao Y, Hisamatsu T, Hibi T, Ikeda Y (1999) Prospective evaluation for upper gastrointestinal tract acute graft-versus-host disease after hematopoietic stem cell transplantation. *Bone Marrow Transplant* 23(6):573–578
 39. George B, Pn N, Mathews V, Lakshmi K, Viswabandya A, Abraham A, Ganapule A, Srivastava A (2014) Acute graft versus host disease following sibling donor transplantation for thalassemia major. *Biol Blood Marrow Transplant* 20(2 Suppl): S169
 40. García Cadenas I, Valcarcel D, Martino R, Piñana JL, Barba P, Novelli S, Esquirol A, Garrido A, Saavedra S, Granell M, Moreno C, Briones J, Brunet S, Sierra J (2014) Impact of cyclosporine levels on the development of acute graft versus host disease after reduced intensity conditioning allogeneic stem cell transplantation. *Mediators Inflamm*. doi:[10.1155/2014/620682](https://doi.org/10.1155/2014/620682)
 41. Valcárcel D, Martino R, Caballero D, Martín J, Ferra C, Nieto JB, Sampol A, Bernal MT, Piñana JL, Vazquez L, Ribera JM, Besalduch J, Moraleda JM, Carrera D, Brunet MS, Perez-Simón JA, Sierra J (2008) Sustained remissions of high-risk acute myeloid leukemia and myelodysplastic syndrome after reduced-intensity conditioning allogeneic hematopoietic transplantation: chronic graft-versus-host disease is the strongest factor improving survival. *J Clin Oncol* 26(4):577–584
 42. Malard F, Szydlo RM, Brissot E, Chevallier P, Guillaume T, Delaunay J, Ayari S, Dubruille V, Le Gouill S, Mahe B, Gastinne T, Blin N, Saulquin B, Harousseau JL, Moreau P, Mohty M (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(1):28–34