Chronic Graft Versus Host Disease in Acute Leukemia Patients Undergoing Allogeneic Hematopoietic Stem Cell Transplant: Analysis of Risk Factors, Pattern and Long Term Outcome

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Received: 4 September 2014 / Accepted: 9 January 2015 / Published online: 20 January 2015
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Abstract Chronic graft versus host disease (cGVHD) is a common late complication of allogeneic hematopoietic stem cell transplant (HSCT). We analyzed risk factors, pattern and long term transplant outcomes of cGVHD at a tertiary cancer centre. Seventy-seven consecutive patients who underwent HSCT for acute leukemia were included. Forty (52 %) patients developed cGVHD; 24 (60 %) extensive stage while 16 (40 %) limited stage. Oral cavity was the commonest site of involvement (25 patients) followed by liver, skin and lung. We found that female donor to male recipient transplant and diagnosis of acute lymphoblastic leukemia (ALL) were the only factors associated with increased risk of cGVHD. The incidence of leukemia relapse was 18 % in patients who developed cGVHD compared to 51 % in those who did not ($P = 0.002$). Four year overall survival and relapse free survival (RFS) were 62 and 46 % in patients who developed cGVHD compared to 29 % ($P < 0.001$) and 29 % ($P < 0.001$) in patients who did not develop cGVHD, respectively. We conclude that cGVHD is more common in male patients with female donors and in patients transplanted for ALL. Oral cavity is the commonest site of cGVHD in our patients and transplant related survival outcomes are superior in patients who develop cGVHD.

Keywords Acute leukemia · Chronic graft-versus-host disease · Allogeneic stem cell transplantation · Outcome · Risk factors

Introduction

Chronic graft versus host disease (cGVHD) is a late complication of allogeneic hematopoietic stem cell transplantation (HSCT) and is a major cause of morbidity and mortality [1]. The incidence of cGVHD has varied from 30 to 70 % across studies [2, 3]. However, these studies are quite heterogeneous with respect to factors like diagnoses, patient-donor characteristics, type of conditioning regimen, stem cell source and GVHD prophylaxis used [2, 4, 5]. Development of cGVHD is associated with improvement in relapse free survival [2, 5, 6]. However, the impact on overall survival is less clear [2, 5]. There is limited data regarding risk factors and transplant related outcomes of cGVHD in patients with acute leukemia. In this study, we sought to determine the incidence, pattern and the risk factors for cGVHD in acute leukemia patients undergoing HSCT. We also aimed to assess the impact of cGVHD on transplant related outcomes including incidence of leukemia relapse, incidence of infective complications, relapse free survival (RFS) and overall survival (OS).

Patients and Methods

Patient and Transplant Characteristics

All patients who underwent allogeneic HSCT for acute leukemia from January 2008 to March 2013 were included in this retrospective study. Relevant data was collected from patient case records and electronic medical records. Acute myeloid leukemia (AML) patients were transplanted

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either in remission (Complete remission [CR]-1 or CR-2) or in disease. However, patients with ALL were transplanted only in complete remission. 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], total leukocyte count (TLC) $>100 \times 10^9/L$ at baseline, not achieving CR after induction, disease stage $\geq$ 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], TLC $>100 \times 10^9/L$ at baseline, not achieving CR after induction, disease stage $\geq$ CR-2 or persistent disease at transplant. Human leucocyte antigen (HLA) typing for all patients and donors was done by polymerase chain reaction with sequence specific primers (SSP). Matching was done at HLA-A, HLA-B and HLA-DRB1 for all patient and donor pairs. Those with less than full match or those with unrelated donors underwent additional testing for HLA-C and HLA-DQB1. Depending on donor availability and HLA matching, patients underwent either matched related donor (MRD) transplant or matched unrelated donor (MUD) transplant or haplo-identical donor (HID) transplant. All patients received conditioning chemotherapy with either full intensity regimens (busulfan–cyclophosphamide or total body irradiation–cyclophosphamide) or with reduced intensity regimens (Fludarabine based). Reduced intensity regimens were used in those patients who had significant comorbidities or those with borderline performance status. Rabbit anti thymocyte globulin (ATG) was used in matched unrelated donor transplants and in those with one antigen mismatched related donor transplants. Stem cell graft was either T cell replete peripheral blood stem cells (PBSCs) or bone marrow harvest or cord blood derived stem cells.

GVHD Prophylaxis and Treatment

GVHD prophylaxis consisted of either cyclosporine (CsA) with methotrexate (MTX) or CsA with mycophenolate mofetil (MMF). MMF was used in patients who participated in an ongoing MMF pharmacokinetic study [7]. CsA was given at a starting dose of 1.5 mg/kg twice daily intravenously (iv) from day-1. MTX was given iv at 15 mg/m² on D1 and 10 mg/m² on D3, D6 and D11. Leucovorin rescue was given 24 h after methotrexate. MMF was given at a dose of 600 mg/m² twice a day from day-1 to day +30. GVHD prophylaxis in patients undergoing haplo-identical transplant consisted of CsA+MMF (started on day +5 with MMF dose being 15 mg/kg thrice daily till day +35) along with post transplant cyclophosphamide (50 mg/kg on day +3 and day +4). CsA was

tapered by 10 % per week from day +90. However, in patients with high risk of relapse, CsA tapering was started early at day +45 to +60. Diagnosis of cGVHD was made clinically and defined as extensive stage disease or limited stage disease according to standard criteria [8]. Topical steroids were used for treatment of limited stage cGVHD involving skin, oral cavity and upper gut. Systemic steroids were used in patients with visceral GVHD and in those who did not respond to topical steroids. Additionally, for lung GVHD, we used azithromycin for its immune modulatory properties, imatinib as anti-fibrosis agent and montelukast as mast cell stabilizer. CsA or MMF were used as steroid sparing agents or as second line drugs in patients whose GVHD was steroid refractory or when the disease flared up as steroids were being tapered.

Anti-infective Prophylaxis

All patients received acyclovir 400 mg thrice daily for 6 months, levofloxacin 500 mg once daily or ciprofloxacin 500 mg twice daily for 3 months, cotrimoxazole (160 mg/800 mg) twice daily on Saturdays and Sundays for 1 year and IVIG 200 mg/kg on day +1, +21, +42, +63, +84. These prophylactic medications were started along with the start of conditioning regimen. Voriconazole or posaconazole was used as antifungal prophylaxis for 3 months post transplant. Patients who developed acute or chronic GVHD and were on steroids continued with acyclovir, fluoroquinolone, cotrimoxazole and antifungal prophylaxis till they remained on steroids beyond the above time periods.

Statistical Analysis

Patients were divided in 2 groups depending on development of cGVHD. Group A included those who developed cGVHD (both limited and extensive stage) while group B included those who did not develop cGVHD. In this analysis, we also included patients not surviving till day +100 post HSCT since chronic GVHD is known to occur prior to day +100 [9]. We analyzed patient and donor age, diagnosis, gender mismatch, disease status at transplant, stem cell source, use of ATG, GVHD prophylaxis, degree of HLA match, CD3 and CD34 cells infused and acute GVHD as potential risk factors for cGVHD. Incidence of relapse, slippage of chimerism, RFS and OS were evaluated as outcome measures. Development of viral and fungal infections was also analyzed. Categorical data was analyzed using Chi square test and continuous data with Mann–Whitney test. Multivariate analysis was done using logistic regression analysis. All P values were 2 sided. Survival analysis was done with Kaplan–Meier method. SPSS version 18 was used for statistical analyses.

Results

Patient and Transplant Characteristics

Seventy seven patients underwent HSCT for acute leukemia during the study period. The baseline patient and transplant characteristics are summarized in Table 1. The median follow up time for the entire cohort of patients was 26 months.

Incidence and Pattern of Chronic GVHD

Forty (52 %) developed chronic GVHD; 60 % of these were extensive stage (Table 2). The median time to the onset of chronic GVHD was 150 days (range 35–456 days). Chronic GVHD was de novo in 19 patients. Thrombocytopenia at onset was present in six patients. Oral cavity was the commonest site involved (25 patients) followed by liver, skin and lung (Fig. 1). Twenty three patients (58 %) required systemic steroids and median duration of use of systemic steroids was 116 days.

Risk Factors for Chronic GVHD

Several patient, donor and transplant related factors were evaluated as potential risk factors for chronic GVHD (Table 3). Three factors were found to be significantly associated with increased risk of cGVHD on univariate analysis—diagnosis of ALL, female donor to male recipient transplants and lower CD3 cell dose infused. On multivariate analysis, female donor to male recipient transplant ($P = 0.015$) and diagnosis of ALL ($P = 0.05$) were the only factors associated with increased risk of cGVHD (Table 4).

Transplant Related Outcomes

There was no difference in the median time to neutrophil and platelet engraftment between the 2 groups ($P = \text{NS}$). Incidence of relapse was 18 % among patients with cGVHD and 51 % among patients without cGVHD ($p = 0.002$). Similarly incidence of slippage of chimerism was also significant lower in patients who developed cGVHD ($p = 0.006$). Transplant related mortality was similar in both the groups ($P = 0.63$). Median OS, 4 year OS, median RFS and 4 year RFS were all significantly greater ($P < 0.001$) in patients who developed cGVHD compared to those who did not (Table 5, Figs. 2, 3). Among patients with chronic GVHD, the 4 year RFS was similar for patients of AML vs ALL (39 vs 84 %, $p = 0.45$). Similarly, no significant difference was found in overall survival according to diagnosis of AML versus ALL (4 year OS 45 vs 84 %, $p = 0.19$). Similar findings were seen among patients without chronic GVHD.

Table 1 Patient and transplant characteristics

Characteristics	Number	%
No of patients	77	100
Median age at transplant	30	–
Males	52	68
Diagnosis		
Acute lymphoid leukemia (ALL)	23	30
Acute myeloid leukemia (AML)	52	68
Biphenotypic leukemia	2	2
Baseline risk		
ALL		
Standard risk	1	1
Poor risk	17	22
Not known	5	7
AML		
Good risk	5	7
Intermediate risk	21	27
Poor risk	22	29
Not known	4	5
Biphenotypic leukemia	2	2
Disease status at transplant		
ALL		
Complete remission-1	15	20
Complete remission-2	8	10
Primary refractory/relapsed	0	0
AML		
Complete remission-1	27	35
Complete remission-2	11	14
Primary refractory/relapsed	14	18
Biphenotypic leukemia		
Complete remission-1	0	0
Complete remission-2	1	1
Primary refractory/relapsed	1	1
Type of transplant		
Matched related transplant	65	84
Matched unrelated transplant	10	13
Haplo-identical transplant	2	2
ABO mismatch transplants,	32	42
Gender mismatched transplants,	40	52
Female to male transplants,	23	30
Stem cell source		
Bone marrow	5	7
Umbilical cord	2	2
Peripheral blood stem cells	70	91
Degree of HLA matching		
BM or PBSC		
Full matched (6/6 or 10/10)	61	79
1 antigen mismatch	9	12
Haplo-identical transplant	5	7
Cord transplants	2	2

Table 1 continued

Characteristics	Number	%
Type of conditioning regimen		
Full intensity	27	35
Reduced intensity	50	65
ATG used	12	16
Graft versus host disease prophylaxis		
CsA+MTX	53	69
CsA+MMF+/-cyclophosphamide	24	31

Table 2 Analysis of pattern and severity of cGVHD (n = 40)

Parameter	Number	%
Overall incidence of cGVHD (n = 77)	40	52
Subtypes		
Extensive	24	60
Limited	16	40
Median time from transplant to onset of chronic GVHD, days	150	–
Thrombocytopenia at onset	6	15
De novo chronic GVHD	19	47
Organ involvement		
Oral cavity	25	63
Gut	8	20
Liver	17	43
Lung	12	30
Eye	11	28
Skin	16	40
Others	1	3
Use of systemic steroids, n (%)	23	58
Median duration of systemic steroids (n = 23)	116 days	–

Infective Complications

The incidence of cytomegalovirus (CMV), Epstein Barr Virus, adenovirus and BK virus reactivation were similar in both groups. The median number of episodes of CMV reactivation was also similar. Additionally, incidence of fungal infections was also similar in patients with or without GVHD (Table 6).

Discussion

Allogenic HSCT has resulted in long term cure of a significant fraction of patients with acute leukemia including relapsed and refractory cases. Chronic GVHD remain a significant cause of morbidity in the post-transplant period. It usually has onset after 100 days, but can manifest even earlier [9]. Overlap GVHD syndromes with features of

Fig. 1 Pattern of organ involvement in cGVHD

both acute and chronic GVHD have been described. Hence, clinical manifestations rather than the time of onset are considered more important in classifying GVHD [9].

In the last 2 decades, few studies have looked into the risk factors, transplant outcomes and infective complications in patients with chronic GVHD. Various studies have shown that older patient age, female donor to male recipient transplant, mismatched or unrelated donor, PBSCs as the source of stem cells and development of acute GVHD are associated with the development of chronic GVHD. Few studies also found that CMV reactivation, higher infused CD34 cell dose and lack of MTX in GVHD prophylaxis are also risk factors for cGVHD [10]. In our study, we found a strong correlation between female donor to male recipient transplant and the development of cGVHD. Lesser number of unrelated donor transplants and mismatched transplants in our study probably explain why these risk factors did not turn out significant. Similarly, more than 90 % patients had PBSCs as the source of stem cell graft; this could explain the lack of effect of source of stem cells on the development of cGVHD. In contrast to what has been reported earlier [2], we did not find a correlation between prior acute GVHD and increased risk for cGVHD. This could be due to the fact that a higher proportion of patients (65 %) in our study received reduced intensity conditioning regimen. Studies have shown that in patients undergoing reduced intensity transplantation, acute GVHD may not predict for the development of cGVHD [5]. Additionally, we found diagnosis of ALL to be associated with the development of chronic GVHD. The exact reason behind this remains unclear. Infused CD3 cell count was significant on univariate analysis. Analysis of infused CD3 count as risk factor for chronic GVHD has yielded variable results between studies [11, 12].

The pattern of organ involvement in cGVHD seen in our study is similar to that reported previously with oral cavity,

Table 3 Univariate analysis of potential risk factors for development of cGVHD

	Group A—with cGVHD (n = 40) n (%)	Group B—no cGVHD (n = 37) n (%)	P value
Patient related factors			
Median age at transplant	29	31	0.575
Males	28 (70)	24 (65)	0.631
Diagnosis			0.020
Acute lymphoid leukemia (ALL)	17 (43)	6 (16)	
Acute myeloid leukemia (AML)	23 (58)	29 (78)	
Biphenotypic leukemia	0 (0)	2 (5)	
Baseline risk			0.138
Good (or standard) risk	2	4	
Intermediate risk	7	14	
Poor risk	25	14	
Not known	6	5	
Relapse/refractory disease at transplant	5 (12)	10 (27)	0.231
Donor related factors			
Median donor age, years	32	33	0.860
ABO mismatch transplants	20 (50)	12 (32)	0.118
Gender mismatched transplants	25 (63)	15 (41)	0.054
Female to male transplants	17 (43)	6 (16)	0.012
Stem cell source			0.855
Bone marrow	2 (5)	3 (8)	
Umbilical cord	1 (3)	1 (3)	
Peripheral blood stem cells	37 (93)	33 (89)	
Degree of HLA matching			0.121
BM or PBSC			
Full matched (6/6 or 10/10)	34 (85)	27 (73)	
1 antigen mismatch	5 (13)	4 (11)	
Haplo-identical transplant	0 (0)	5 (14)	
Cord transplants	1 (3)	1 (3)	
Transplant related factors			
Type of transplant			0.321
Matched related transplant	35 (88)	30 (81)	
Matched unrelated transplant	5 (13)	5 (14)	
Haplo-identical transplant	0 (0)	2 (5)	
Type of conditioning regimen			0.093
Full intensity	18 (45)	9 (24)	
Reduced intensity	22 (55)	28 (76)	
Total body irradiation used	20 (50)	12 (32)	0.118
ATG used	5 (13)	7 (19)	0.438
Graft versus host disease prophylaxis			0.224
CsA+MTX	30 (75)	23 (62)	
CsA+MMF	10 (25)	14 (38)	
Cell doses infused			
TNC ($\times 10^8$)/kg	7.10	6.63	0.913
CD34 ($\times 10^6$)/kg	5.11	5.12	0.899
CD3 ($\times 10^6$)/kg	147.80	177.65	0.039
Prior acute GVHD, any grade, n (%)	21 (53)	13 (35)	0.630
Day100 median absolute lymphocyte count ($\times 10^9$ /L)	1.23 (n = 39)	1.41 (n = 28)	0.589
Day180 median absolute lymphocyte count ($\times 10^9$ /L)	1.61 (n = 39)	2.05 (n = 22)	0.131

Table 4 Multivariate analysis of potential risk factors for development of cGVHD

Risk factor	Hazard ratio	95 % CI	P value
Diagnosis of ALL	3.977	0.997–15.86	0.050
Female donor to male recipient	4.776	1.35–16.86	0.015
CD3 cell dose infused	0.995	0.989–1.001	0.082

Table 5 Comparison of transplant related outcomes in patients with and without cGVHD

	Group A—with cGVHD (n = 40)	Group B—no cGVHD (n = 37)	P value
Median days to platelet engraftment	13	12	0.719
Median days to myeloid engraftment	13	15	0.096
Incidence of relapse, n (%)	7 (18)	19 (51)	0.002
Slippage of chimerism, n (%)	6 (15)	16 (43)	0.006
Transplant related mortality, n (%)	5 (13)	6 (16)	0.63
Median relapse free survival, months	37.7	9.3	<0.001
4 year relapse free survival, %	46	29	<0.001
Median overall survival, months	Not reached	10.2	<0.001
4 year overall survival, %	62	29	<0.001

liver, skin and lung being the commonly involved sites [13]. Nearly half of patients with cGVHD had de novo cGVHD which is similar to the proportion reported previously in other studies [5, 13, 14]. Regarding transplant outcomes, it is relatively clear that cGVHD improves disease free survival [2, 5, 6] and our study also concurs with previous studies. The impact on overall survival is less consistent [2, 5]. Only few studies have shown a correlation between extensive stage cGVHD and overall survival [14]. In our study, we found a strong positive correlation between occurrence of cGVHD and improvement in OS. This improvement was noted in the entire cohort of patients with cGVHD irrespective of the severity of GVHD. The improvement in OS seen in patients who developed cGVHD is attributed to decrease in the incidence of leukemia relapse. This favorable impact of cGVHD on transplant outcome is related to increased graft versus

Fig. 2 Comparison of overall survival (OS) between patients with and without cGVHD **Fig. 3** Comparison of relapse free survival (RFS) between patients with and without cGVHD

leukemia effect in these patients. The improvement in OS might also be related to the fact that there was no increase in the incidence of infective complications in patients who developed cGVHD compared to those who did not. While previous studies have shown that infective complications are a major cause of mortality in patients with cGVHD (particularly fungal infections) [15], we found no increase in the incidence of infective complications in our cohort of patients with cGVHD.

Table 6 Comparison of infective complications in patients with and without cGVHD

	Group A—with cGVHD (n = 40)	Group B—no cGVHD (n = 37)	P value
CMV reactivation, n (%)	29 (73)	20 (54)	0.09
Median episodes of CMV	2	1.5	0.75
Adenoviral reactivation, n (%)	11 (28)	5 (14)	0.13
BK virus reactivation, n (%)	7 (18)	6 (16)	0.88
EBV reactivation, n (%)	2 (5)	3 (8)	0.58
Fungal infections	5 (13)	6 (16)	0.63

We conclude that cGVHD complicates nearly half of all acute leukemia transplants. Female donor to male recipient and diagnosis of ALL are important risk factors for the development of cGVHD. Occurrence of cGVHD is associated with decrease in leukemia relapse and an improvement in overall survival.

Conflict of interest The authors declare no conflict of interest.

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