Table 3. Causes of death for adult Ph(−) ALL patients undergoing myeloablative allo-SCT in CR1 ($N = 917$)

	Related		Unrelated BM		Cord blood		
No. of patients	130	(%)	148	(%)	33	(%)	*P*
Relapse	59	45	37	25	8	24	0.001
Infection	17	13	26	18	8	24	0.26
Organ failure	17	13	23	16	1	3	0.15
GVHD	11	8	19	13	2	6	0.34
Interstitial pneumonia	8	6	15	10	4	12	0.38
Hemorrhage	3	2	7	5	5	15	0.009
TMA	7	5	3	2	2	6	0.27
ARDS	1	1	5	3	0	0	0.20
Graft failure	2	2	2	1	2	6	0.18
SOS	2	2	2	1	0	0	0.78
Secondary malignancy	1	1	0	0	0	0	0.50
Other	2	2	9	6	1	3	0.14

Ph(−), ALL indicates Philadelphia chromosome-negative acute lymphoblastic leukemia; allo-SCT, allogeneic stem cell transplantation; CR, complete remission; BM, bone marrow; GVHD, graft-versus-host disease; TMA, thrombotic microangiopathy; ARDS, acute respiratory distress syndrome; SOS, sinusoidal obstruction syndrome.

recipients, time to neutrophil engraftment was associated with a CD34-positive cell number ($<1 \times 10^5$/kg: day 22 versus $\geq 1 \times 10^5$/kg: day 19, $P = 0.02$).

The cumulative incidence of grade III–IV acute GVHD was significantly higher in patients who underwent URD allo-SCT (8% in RD, 18% in URD, and 11% in CB at day 100; $P = 0.008$).

Among assessable patients who survived at least 100 days after allo-SCT, no significant difference was observed between RD, URD, and CB allo-SCTs in the incidence of chronic GVHD (34% in RD, 38% in URD, and 31% in CB at 3 years; $P = 0.52$)

allo-SCT in subsequent CR

Although it was concluded from the results of a study by MRC/ECOG that RD allo-SCT in CR1 could achieve the best result [10], there is still plenty of room to discuss allo-SCT beyond CR1 for patients who could not find a suitable donor or maintain CR1. Among 300 patients transplanted in subsequent CR, there were no significant differences in OS between RD, URD, and CB allo-SCTs (47% in RD, 39% in URD, and 48% in CB at 4 years; $P = 0.33$). The results of multivariate analysis showed that JALSG intermediate- or high-risk and cytogenetic abnormalities [hypodiploid, $t(4;11)$ or $t(8;14)$] were significant risk factors for OS (Table 4). The donor source was not a significant risk factor [URD: HR 1.28 (95% CI 0.90–1.82), $P = 0.17$; CB: HR 1.01 (95% CI 0.61–1.67), $P = 0.97$ (versus RD)].

The cumulative incidence of relapse was not statistically different among patients who underwent RD, URD, and CB allo-SCTs (31% in RD, 26% in URD, and 29% in CB at 3 years; $P = 0.48$). The results of multivariate analysis showed

Table 4. Outcomes for adult Ph(−) ALL patients undergoing myeloablative allo-SCT in subsequent CR: multivariate analyses ($N = 300$)

Covariates	Relative risk (95%CI)	*P*
Overall survival		
JALSG risk		
Low	1.00	
Intermediate	1.45 (1.05–2.02)	0.03
High	1.92 (1.08–3.41)	0.03
Cytogenetics		
Normal	1.00	
Hypodiploid, $t(4;11)$ or $t(8;14)$	2.48 (1.11–5.52)	0.03
Others [no $t(9;22)$]	1.24 (0.89–1.71)	0.20
Relapse		
Cytogenetics		
Normal	1.00	
Hypodiploid, $t(4;11)$ or $t(8;14)$	4.13 (1.54–11.1)	0.005
Others [no t(9;22)]	1.29 (0.82–2.02)	0.27
Non-relapse mortality		
Age at allo-SCT, year		
16≤, <45	1.00	
≥45	1.82 (1.08–3.07)	0.02
JALSG risk		
Low	1.00	
Intermediate	1.52 (0.94–2.47)	0.09
High	2.39 (1.12–5.10)	0.02

Ph(−) ALL indicates Philadelphia chromosome-negative acute lymphoblastic leukemia; allo-SCT, allogeneic stem cell transplantation; CR, complete remission.

that cytogenetic abnormalities [hypodiploid, $t(4;11)$, or $t(8;14)$] were significant risk factors for relapse (Table 4).

Similarly, the cumulative incidence of NRM was not statistically different among patients who underwent RD, URD, and CB allo-SCTs (21% in RD, 36% in URD, and 27% in CB at 3 years; $P = 0.46$). The results of multivariate analysis showed that ≥45 years of age at allo-SCT and JALSG intermediate or high risk were significant risk factors for NRM (Table 4).

allo-SCT in non-CR

Among 509 patients transplanted in non-CR, there were no significant differences in OS among patients who underwent RD, URD, and CB allo-SCTs (15% in RD, 21% in URD, and 18% in CB at 4 years; $P = 0.20$). The results of multivariate analysis showed that ≥45 years of age at allo-SCT, cytogenetic abnormalities, HLA partially matched or mismatched, and non-TBI preparative regimens were significant risk factors for OS (Table 5). The donor source was not a significant risk factor [URD: HR 0.99 (95% CI 0.79–1.24), $P = 0.96$; CB: HR 1.09 (95% CI 0.78–1.53), $P = 0.61$ (versus RD)].

The cumulative incidence of relapse was not statistically different among patients who underwent RD, URD, and CB allo-SCTs (59% in RD, 42% in URD, and 58% in CB at 3 years; $P = 0.35$). However, the results of multivariate analysis showed that the donor source as well as cytogenetic abnormalities [hypodiploid, $t(4;11)$, or $t(8;14)$] and non-TBI

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Table 5. Outcomes for adult Ph(−) ALL patients undergoing myeloablative allo-SCT in non-CR: multivariate analyses ($N = 509$)

Covariates	Relative risk (95% CI)	P
Overall survival		
Age at allo-SCT, year		
16≤, <45	1.00	
≥45	1.65 (1.30–2.11)	<0.0001
Cytogenetics		
Normal	1.00	
Hypodiploid, $t(4;11)$ or $t(8;14)$	2.04 (1.38–3.03)	<0.0001
Others [no $t(9;22)$]	1.26 (1.02–1.55)	0.03
HLA		
Well matched	1.00	
Partially matched	1.44 (1.15–1.81)	0.002
Mismatched	1.37 (1.04–1.81)	0.02
Conditioning		
TBI regimens	1.00	
Non-TBI regimens	1.83 (1.28–2.62)	0.001
Relapse		
Cytogenetics		
Normal	1.00	
Hypodiploid, $t(4;11)$ or $t(8;14)$	2.45 (1.52–3.97)	<0.0001
Others [no $t(9;22)$]	1.26 (0.97–1.62)	0.08
Conditioning		
TBI regimens	1.00	
Non-TBI regimens	1.84 (1.19–2.84)	0.006
Source		
Related	1.00	
Unrelated BM	0.74 (0.56–0.97)	0.03
Cord blood (CB)	1.54 (1.09–2.17)	0.02
Non-relapse mortality		
Age at allo-SCT, year		
16≤, <45	1.00	
≥45	2.00 (1.47–2.73)	<0.0001
Cytogenetics		
Normal	1.00	
Hypodiploid, $t(4;11)$ or $t(8;14)$	1.98 (1.18–3.34)	0.01
Others [no $t(9;22)$]	1.14 (0.86–1.51)	0.37
HLA		
Well matched	1.00	
Partially matched	1.59 (1.16–2.17)	0.004
Mismatched	1.67 (1.16–2.41)	0.006
Conditioning		
TBI regimens	1.00	
Non-TBI regimens	1.99 (1.26–3.14)	0.003

Ph(−), ALL indicates Philadelphia chromosome-negative acute lymphoblastic leukemia; allo-SCT, allogeneic stem cell transplantation; TBI, total body irradiation.

preparative regimens were significant risk factors for relapse (Table 5).

The cumulative incidence of NRM was not statistically different among patients who underwent RD, URD, and CB allo-SCTs (39% in RD, 42% in URD, and 45% in CB at 3 years; $P = 0.17$). The results of multivariate analysis showed that ≥45 years of age at allo-SCT, cytogenetic abnormalities, HLA partially matched or mismatched, and non-TBI preparative regimens were significant risk factors for NRM (Table 5). Post-transplant lymphoproliferative disorder was observed in one patient, and there was no association with the use of ATG.

discussion

This report presents the results for the largest series of adult Ph(−) ALL patients who underwent allo-SCT. There were no significant differences between RD, URD, and CB allo-SCTs in any disease stage, suggesting that CB allo-SCT could be a treatment of choice for all disease stages of patients without a suitable RD or URD. There were no significant survival differences between BM and PBSC recipients in any disease stage (data not shown), which was consistent with other studies [27, 28]. Interestingly, OS after CB allo-SCT was significantly better than that after HLA-mismatched URD allo-SCT for CR1 patients younger than 45 years of age. These results might indicate advantages of CB allo-SCT when carried out for patients without an HLA-matched donor at an appropriate timing.

The major finding in this study is that OS was compatible between RD, URD, and CB allo-SCTs for Ph(−) ALL in CR1, even though NRM rates were higher in URD and CB allo-SCTs than RD allo-SCT. This is because of higher relapse rates in RD allo-SCT compared with URD and CB allo-SCTs. The low NRM rates due to the lower incidence of acute GVHD in our population might result from the differences in ethnic background [29, 30]. Although the NRM rates after allo-SCT in CR1 were not significantly different from those of URD allo-SCT in CR1, the causes of NRM would be different between URD and CB allo-SCTs. Hemorrhage due to insufficient platelet recovery and infection due to graft failure or delayed neutrophil recovery would be the major causes of NRM after CB allo-SCT in CR1. Since delayed engraftment is one of the most common limitations of CB allo-SCT [31–33], several attempts such as double cord units [34–36], intra-BM injection [37–39], and *ex vivo* expansion [40, 41] have been made to ensure engraftment. Although CD34-positive cell dose was not a significant risk factor for OS in this study, engraftment was delayed among patients who received fewer CD34-positive cells as previously reported [42]. Although all patients who underwent CB allo-SCT administered single CB intravenously, the technical progression of CB all-SCT could also improve the outcome of Ph(−) ALL as well as other hematological malignancies [21, 43–49] by reducing NRM.

Our results also indicated that CB allo-SCT beyond CR1 could achieve OS similar to that of RD or URD allo-SCT. It is noteworthy that some, but not all, patients with refractory disease could be rescued by CB allo-SCT as well as RD or URD allo-SCT [12]. Among patients transplanted in non-CR, survival of patients transplanted at ≥10 months from diagnosis was significantly superior to that of those transplanted <10 months from diagnosis (data not shown), suggesting that patients who could await a suitable donor or those with late relapse could obtain the advantages of allo-SCT. These patients could not have survived long with chemotherapy alone, and therefore, CB could be a hope of survival for patients with refractory disease who do not have a suitable RD or URD.

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To our knowledge, this is the first and largest analysis of CB allo-SCT for Ph(−) ALL alone ($N = 233$). Recently, the results of a large retrospective analysis of a donor source that included data of 1525 patients (including 165 patients who underwent CB allo-SCT) were reported by the Center for International Blood and Marrow Transplant Research, the National Cord Blood Program, the European Group for Blood and Marrow Transplantation, and the Eurocord-Netcord registry [50]. The number of ALL patients who underwent CB allo-SCT was limited to 89 including both Ph(+) and Ph(−) ALL patients. The results of disease-specific analyses were also reported from Japan [CB; Ph(+): $N = 43$, Ph(−): $N = 71$] [51] and Minnesota (CB: $N = 69$) [52], with data of Ph(+) and Ph(−) ALL patients being analyzed together. Statistical techniques to adjust heterogeneities of the study population were used in those studies. Although we agree with the methodology of the above-described studies and the conclusions that support the use of CB for ALL patients without a suitable RD or URD, Ph(+) and Ph(−) ALL should be analyzed separately in an era of TKIs to obtain data which would be useful in clinical situations [9]. Our study clearly confirmed the usefulness of CB for Ph(−) ALL in any disease status.

In this type of retrospective study, selection biases from different backgrounds of patients who underwent RD, URD, and CB allo-SCTs could not be eliminated [12]. Considering that CB allo-SCT has not yet been recognized as a standard treatment of Ph(−) ALL, the background of CB recipients might be worse than that of other sources, that is, CB allo-SCT might be carried out for patients whose prognosis is considered to be poor without allo-SCT. In addition, since the median interval from diagnosis to allo-SCT in CR1 was similar between RD and CB allo-SCTs, the time-censoring effect, a major bias described elsewhere [12, 53, 54], did not affect our results. Although we could not make a comparison between chemotherapy and allo-SCT, our study could suggest promising data to broaden the choices of donor source.

In conclusion, the outcomes were comparable between RD, URD, and CB allo-SCTs in any disease status, and these may be considered equivalent options for patients with Ph(−) ALL. In the absence of a suitable RD or URD, CB allo-SCT should be planned promptly for Ph(−) ALL patients so as not to miss the appropriate timing.

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disclosure

The authors have declared no conflicts of interest.

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ORIGINAL ARTICLE

Allogeneic hematopoietic stem cell transplantation for intermediate cytogenetic risk AML in first CR

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Allogeneic hematopoietic SCT (allo-HCT) from matched sibling donor (MSD) is recommended for younger patients with intermediate cytogenetic risk AML in first CR (CR1), whereas the role of alternative donor transplants in these patients is unknown. We retrospectively analyzed 605 patients with intermediate-risk AML, who received myeloablative allo-HCT in CR1. The 4-year OS for MSD ($n = 290$) and matched unrelated donor (MUD; $n = 141$) was 65% and 68% ($P = 0.50$), respectively. In multivariate analysis, MUD had a similar risk of overall mortality as MSD (hazard ratio = 0.90; 95% confidence interval, 0.62–1.30; $P = 0.58$), whereas older age, female donor/male recipient (FDMR) combination, and requiring more than one course of induction chemotherapy to achieve CR1 were poor prognostic factors for OS. Thus, OS after MUD HCT with sex combinations other than FDMR was significantly higher than that after MSD HCT from female donors to male recipients (4-year OS 72% versus 55%, $P = 0.04$). These results suggest that HCT, not only from MSD, but also from MUD, should be considered in younger patients with intermediate-risk AML in CR1, and that the donor–recipient sex combination is more important than the donor type in donor selection.

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Keywords: AML; first CR; allogeneic hematopoietic SCT

INTRODUCTION

The current standard treatment strategy for young patients with AML consists of induction chemotherapy and subsequent post-remission therapy. The post-remission therapy includes intensive consolidation chemotherapy and allogeneic hematopoietic SCT (allo-HCT). Although the toxicity of consolidation chemotherapy is relatively low, a substantial proportion of patients relapse, and the risk of relapse depends on cytogenetic risk.[1,2] On the other hand, allo-HCT as a post-remission therapy is associated with the lowest relapse rates. However, this benefit is limited by the high nonrelapse mortality (NRM) and the donor type has a significant impact on NRM.[3] The risk of NRM associated with allo-HCT needs to be balanced with the risk of relapse, and hence, the indication for allo-HCT among patients with AML in the first CR (CR1) depends on the cytogenetic risk and available donor type.[4]

Regarding those patients with favorable cytogenetic risk AML, who achieved CR1, the long-term disease-free survival after intensive consolidation chemotherapy of approximately 60% is reported, and they did not benefit from allo-HCT in CR1.[5–7] Thus, these patients are not considered candidates for allo-HCT in CR1.[8]

As for patients with unfavorable cytogenetic risk AML in CR1, previous prospective studies that assigned allo-HCT versus alternative post-remission therapies, on an intent-to-treat donor versus no-donor basis showed significant disease-free survival and OS benefit with allo-HCT, not only from a matched sibling donor (MSD), but also from a matched unrelated donor (MUD).[5–7,9] Accordingly, allo-HCT in CR1 from MSD or MUD is recommended for unfavorable risk AML.[8]

The indication for allo-HCT in CR1 depends on the available donor type in patients with intermediate cytogenetic risk AML. As meta-analyses of prospective studies showed that allo-HCT in CR1 from MSD offered significant disease-free survival and OS benefit,[5,6] allo-HCT in CR1 from MSD is recommended. In contrast, the indication for allo-HCT from alternative donors among these patients is unknown, because higher NRM may offset therapeutic benefits.[3] Although several studies reported comparable outcome after MUD or MSD transplantation,[10–13] these studies included only a small number of patients with intermediate-risk AML in CR1, and information regarding the outcome of allo-HCT from alternative donors in this group of patients is limited. Collectively, further investigation of the outcome of allo-HCT from alternative donors in patients with intermediate-risk AML in CR1 is warranted. In the present study, we retrospectively analyzed the impact of donor type on

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transplant outcomes among patients with intermediate-risk AML in CR1.

MATERIALS AND METHODS

Collection of data and data source

The recipients' clinical data were provided by the Japan Society for Hematopoietic Cell Transplantation (JSHCT) and the Japan Marrow Donor Program (JMDP). The registry data is managed using the 'Transplant Registry Unified Management Program' system.[14] Both JSHCT and JMDP collect recipients' clinical data at 100 days after allo-HCT. The patient's data on survival, disease status and long-term complications, including chronic GVHD and second malignancies, are renewed annually by follow-up forms. This study was approved by the data management committees of JSHCT. Informed consent was provided according to the Declaration of Helsinki.

Patients

Between January 1996 and December 2008, a total of 682 adult patients aged 16 to 70 years, with intermediate cytogenetic risk AML in CR1, received first BM or PBSC transplantation with myeloablative conditioning regimens. Excluding 66 patients without complete HLA data and 11 patients whose follow-up data were not available, we analyzed 605 patients. Only BM grafts were used in unrelated HCT, because the PBSC donation from unrelated donors was not permitted in Japan. HLA compatibility was determined by serological typing for HLA-A, -B and -DR in related donor (RD) HCT, and by high-resolution typing for HLA-A, -B, -C and -DRB1 in unrelated donor HCT. A MSD was defined as a serologically MSD, whereas other RDs were defined as RDs other than MSD. A MUD was defined as an eight/eight identical unrelated donor, whereas a mismatched unrelated donor (MMUD) was defined as an unrelated donor who had at least one locus mismatch.

Definitions

Neutrophil recovery was defined by an ANC of at least 500 cells per mm^3 for three consecutive points. Acute and chronic GVHD were diagnosed and graded according to defined criteria.[15,16] Relapse was defined as a recurrence of underlying hematological malignant diseases. NRM was defined as death during continuous remission. For OS, failure was death due to any cause, and surviving patients were censored at the last follow-up. The date of transplantation was the starting time point for calculating all outcomes. Cytogenetic risk-group assignment was done according to the Southwest Oncology Group/Eastern Cooperative Oncology Group classification.[2]

Statistical analysis

The two-sided χ^2-test was used for categorical variables, and the two-sided Wilcoxon rank sum test was used for continuous variables. OS was calculated using the Kaplan–Meier method. The log-rank test was used for group comparisons. Cumulative incidence curves were used in a competing-risks setting to calculate the probability of acute and chronic GVHD, relapse and NRM.[17] For GVHD, death without GVHD and relapse were the competing events; for relapse, death without relapse was the competing event; and for NRM, relapse was the competing event. Gray's test was used for group comparison of cumulative incidence.[18] The Cox proportional hazards regression model was used to test the statistical significance of several potential prognostic factors for relapse, NRM and OS. Variables with a significance level less than 0.1 in univariate analysis were entered into multivariable models and sequentially eliminated in a stepwise backward fashion. Each step of model building contained the main effect of donor type. Factors with a significance level less than 0.05 were kept in the final model. The median value was used as a cut-off point for year of transplant. For WBC counts at diagnosis, 50×10^9/L was used as a cut-off point according to the previous report.[10] All *P*-values were two-sided, and *P*-values of less than 0.05 were considered statistically significant.

RESULTS

Patient characteristics

Characteristics of the patients are summarized in Table 1. Among the 605 patients analyzed, 290 had MSD HCT, 53 had other RD HCT, 141 had MUD HCT and 121 had MMUD HCT. Of 53 patients with other RD, HLA was matched in 14 and mismatched in 39 patients. Of 121 patients with MMUD, 69 were one locus mismatched and 52 were two or more loci mismatched. The median age of patients was 37 (range, 16–59) years, and median time from diagnosis to HCT was 7.43 (range, 0.43–54.3) months. The median follow-up period of survivors was 4.2 (range, 0.1–13) years. The proportions of male patients, normal karyotype, conditioning regimens, including TBI, and BMT were significantly higher, whereas those of M1/M2/M3/M4/M5 FAB classification and CYA-based GVHD prophylaxis were significantly lower in the unrelated HCT than in the related HCT. The time from diagnosis to HCT was longer in the unrelated HCT compared with related HCT. Other characteristics were not significantly different between related and unrelated HCT.

Acute and chronic GVHD

The unadjusted cumulative incidences of grade II–IV acute GVHD for the MSD and MUD HCT were 26% and 25% at 100 days ($P = 0.89$), respectively, and those of grade III–IV acute GVHD were 10% and 7% at 100 days ($P = 0.46$), respectively (Table 2). The unadjusted cumulative incidences of chronic GVHD for the MSD and MUD HCT were 45% and 44% at 2 years ($P = 0.98$), respectively, and those of extensive chronic GVHD were 28% and 23% at 2 years ($P = 0.37$), respectively (Table 2).

Survival

OS rates for the MSD and MUD HCT were 65% and 68% at 4 years, respectively ($P = 0.50$; Table 2, Figure 1a). Univariate analysis of risk factors for overall mortality showed that the following factors were significant at the 0.1 level: patient age $\geqslant 40$ years, female donor/male recipient (FDMR) combination, and requiring more than one course of induction chemotherapy to achieve CR1 (Table 3). In multivariate analysis, MUD was not a significant factor for overall mortality (hazard ratio (HR) = 0.90; 95% confidence interval (CI), 0.62–1.30; $P = 0.58$). Significant factors for overall mortality were patient age $\geqslant 40$ years (HR = 1.55; 95% CI, 1.17–2.06; $P < 0.01$), FDMR combination (HR = 1.42; 95% CI, 1.03–1.95; $P = 0.03$) and requiring more than one course of induction chemotherapy to achieve CR1 (HR = 1.81; 95% CI, 1.36–2.41; $P < 0.01$) (Table 4). As the donor–recipient sex combination, but not donor type, was a significant factor for overall mortality, OS after MUD HCT with sex combinations other than FDMR was significantly higher than that after MSD HCT from female donors to male recipients (4-year OS 72% versus 55%, $P = 0.04$) (Figure 1b).

Nonrelapse mortality

The cumulative incidences of NRM for the MSD and MUD HCT were 17% and 19% at 4 years, respectively ($P = 0.52$) (Table 2, Figure 2a). Univariate analysis of risk factors for NRM showed that the following factors were significant at the 0.1 level: patient age $\geqslant 40$ years, FDMR combination and MMUD (Table 3). In multivariate analysis, MUD HCT was not a significant factor for NRM compared with MSD HCT (HR = 1.26; 95% CI, 0.77–2.06; $P = 0.35$; Table 4). Significant factors for higher NRM were patient age $\geqslant 40$ years (HR = 1.71; 95% CI, 1.17–2.50; $P < 0.01$), FDMR combination (HR = 1.68; 95% CI, 1.12–2.52; $P = 0.01$) and MMUD (HR = 1.83; 95% CI, 1.16–2.86; $P < 0.01$).

Relapse

The cumulative incidences of relapse for the MSD and MUD HCT were 24% and 19% at 4 years, respectively ($P = 0.25$; Table 2, Figure 2b). Univariate analysis of risk factors for relapse showed that the following factors were significant at the 0.1 level: longer interval between diagnosis and transplantation, peripheral blood

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Table 1. Patient characteristics

Characteristics	*MSD*	*Other RD*	*MUD*	*MMUD*	P-*values*[a]
No. of patients	290	53	141	121	
Median patient age at HCT, years	39	36	35	37	0.09
Range	16–58	17–58	16–59	16–59	
Patient sex, n *(%)*					0.02
Male	155 (53)	24 (45)	86 (61)	75 (62)	
Female	135 (47)	29 (55)	55 (39)	46 (38)	
Sex matching, n *(%)*					0.61
Others	202 (77)	45 (87)	112 (79)	98 (81)	
Female to male	61 (23)	7 (13)	29 (21)	23 (19)	
Not available	27	1	0	0	
FAB classification, n *(%)*					<0.01
M1–M5	227 (82)	39 (80)	90 (70)	83 (74)	
M0, M6, M7	51 (18)	10 (20)	39 (30)	29 (26)	
Others, not available	12	4	12	9	
Prior myelodysplastic syndrome, n *(%)*					0.52
No	279 (97)	49 (92)	134 (98)	116 (96)	
Yes	10 (3)	4 (8)	3 (2)	5 (4)	
Not available	1	0	4	0	
Cytogenetics, n *(%)*					0.03
Normal	272 (94)	49 (92)	138 (98)	117 (97)	
+8, +6, -Y, del(12p)	18 (6)	4 (8)	3 (2)	4 (3)	
Conditioning regimen					<0.01[b]
CY + TBI	94 (32)	25 (47)	65 (46)	64 (53)	
CY + CA + TBI	40 (14)	3 (6)	18 (13)	10 (8)	
CY + BU + TBI	12 (4)	1 (2)	13 (9)	5 (4)	
Other TBI regimen	36 (12)	8 (15)	12 (9)	16 (13)	
BU + CY	102 (35)	12 (23)	31 (22)	17 (14)	
Other non-TBI regimen	6 (2)	4 (8)	2 (1)	9(7)	
GVHD prophylaxis, n *(%)*					<0.01[c]
CsA-based	268 (94)	29 (55)	55 (39)	40 (34)	
FK-based	9 (3)	21 (40)	79 (56)	69 (59)	
Others[d]	9 (3)	3 (6)	7 (5)	8 (9)	
Not available	4	0	0	4	
Time from diagnosis to HCT[e]					
Median	5.79	7.60	8.62	10.2	<0.01
Range	0.43–47.6	2.83–27.6	2.50–54.3	3.49–27.7	
<6 months	153 (54)	17 (33)	20 (14)	10 (8)	<0.01
6 to < 9 months	97 (34)	21 (41)	53 (38)	35 (29)	
9 months or longer	34 (12)	13 (25)	68 (48)	75 (63)	
Not available	6	2	0	1	
Year of transplant, n *(%)*					0.76
1996–2003	156 (54)	23 (43)	74 (52)	66 (55)	
2004–2008	134 (46)	30 (57)	67 (48)	55 (45)	
Stem cell source, n *(%)*					<0.01
BM	175 (60)	33 (62)	141 (100)	121 (100)	
Peripheral blood	115 (40)	20 (38)	0 (0)	0 (0)	
WBC counts at diagnosis, $\times 10^9$*/L*					
<50	196 (71)	36 (75)	108 (79)	82 (75)	0.14
⩾50	79 (29)	12 (25)	29 (21)	27 (25)	
Not available	15	5	4	12	
No. of induction courses to achieve CR, n *(%)*					0.43
1	187 (68)	31 (62)	88 (67)	68 (60)	
⩾2	88 (32)	19 (38)	43 (33)	45 (40)	
Not available	15	3	10	8	

Abbreviations: CA = cytarabine; FK = tacrolimus; HCT = hematopoietic SCT; MMUD = mismatched unrelated donor; MSD = matched sibling donor; MUD = matched unrelated donor; RD = related donor. [a]*P*-value between related and unrelated donors. [b]*P*-value between TBI regimen and non-TBI regimen. [c]*P*-value between CsA-based prophylaxis and FK-based prophylaxis. [d]Others include T-cell depletion. [e]The median time from diagnosis to transplant was 7.43 months for the whole group.

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Table 2. Clinical outcomes

	MSD	*Other RD*		*MUD*		*MMUD*	
	% (95% CI)	*% (95% CI)*	*P-values*[a]	*% (95% CI)*	*P-values*[a]	*% (95% CI)*	*P-values*[a]
Acute GVHD, grades II–IV at 100 days	26 (21–31)	38 (25–51)	0.04	25 (18–32)	0.89	51 (42–59)	<0.01
Acute GVHD, grades III–IV at 100 days	10 (6–13)	15 (7–26)	0.19	7 (4–12)	0.46	14 (9–21)	0.16
Chronic GVHD at 2 years	45 (39–51)	48 (33–62)	0.75	44 (35–53)	0.98	41 (32–51)	0.55
Extensive chronic GVHD at 2 years	28 (23–34)	31 (18–44)	0.73	23 (16–31)	0.37	23 (15–31)	0.25
OS at 4 years	65 (59–71)	53 (37–68)	0.26	68 (59–76)	0.50	61 (51–70)	0.25
Nonrelapse mortality at 4 years	17 (12–22)	18 (9–30)	0.73	19 (13–27)	0.52	25 (18–34)	<0.01
Relapse at 4 years	24 (19–29)	29 (17–42)	0.45	19 (13–27)	0.25	12 (7–19)	0.02

Abbreviations: CI = confidence interval; MSD = matched sibling donor; RD = related donor; MUD = matched unrelated donor; MMUD = mismatched unrelated donor. [a]*P*-values for comparison with MSD.

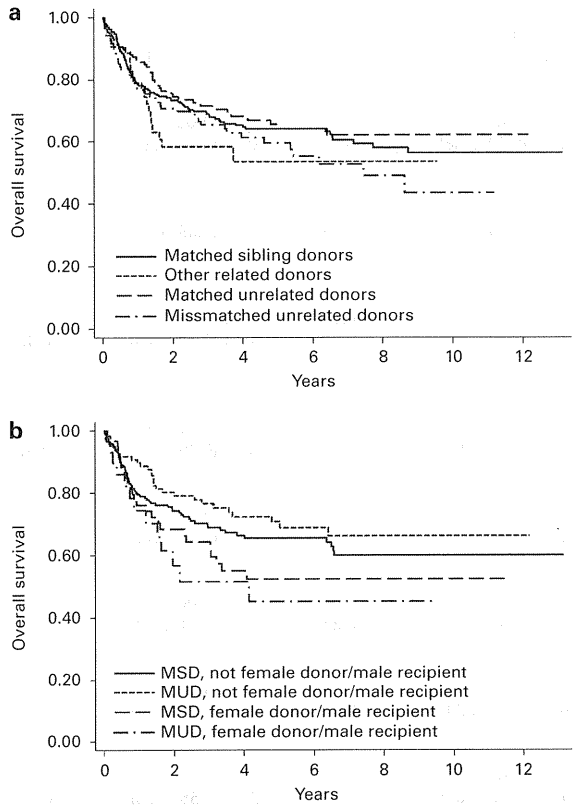

Figure 1. OS. (**a**) Comparison of MSD, other RD, MUD and MMUD transplantation. (**b**) Comparison according to the donor–recipient sex combination and donor type among patients with MSD and MUD.

as stem cell source, WBC counts at diagnosis $\geqslant 50 \times 10^9$/L, requiring more than one course of induction chemotherapy to achieve CR1, and MMUD (Table 3). In multivariate analysis, MUD HCT was not a significant factor for relapse compared with MSD HCT (HR = 0.98; 95% CI, 0.58–1.64; $P = 0.93$; Table 4). Significant factors for relapse were WBC counts at diagnosis $\geqslant 50 \times 10^9$/L (HR = 1.77; 95% CI, 1.20–2.63; $P < 0.01$) and requiring more than one course of induction chemotherapy to achieve CR1 (HR = 2.24; 95% CI, 1.54–3.27; $P < 0.01$), and 9 months or longer interval between diagnosis and transplantation (HR = 0.56; 95% CI, 0.32–0.98; $P = 0.04$).

DISCUSSION

We retrospectively analyzed the impact of donor type on transplant outcomes among patients with intermediate-risk AML in CR1. We observed comparable survival after MSD or MUD HCT, but the donor–recipient sex combination had a significant impact on transplant outcomes. The prognosis of older patients was poorer than that of younger patients because of higher NRM. These findings have important implications for the treatment of intermediate-risk AML in CR1.

The prognosis of younger patients with intermediate-risk AML could be improved by performing allo-HCT in CR1 when MSD is available.[5,6] On the other hand, it is unknown whether these patients without MSD may benefit from alternative donor transplantation, because higher NRM associated with alternative donor transplantation may offset therapeutic benefits.[3] In our study, NRM for a MUD HCT was 19% at 4 years, which was similar to that for a MSD HCT and appeared acceptable. The comparable outcomes after a MSD or a MUD HCT observed in our study suggest that HCT, not only from MSD, but also from MUD, should be considered in younger patients with intermediate-risk AML in CR1.

The FDMR combination had a crucial negative impact on transplant outcome in the present study, whereas it had no or a modest effect on transplant outcome in other studies.[19–21] We suggest two possible explanations for this discrepancy. First, it has been reported that the negative effect of the FDMR combination on survival was more pronounced in the standard-risk disease group than in the high-risk disease group, because the negative impact of the FDMR combination on NRM was stronger in the former than in the latter group, whereas the GVL effect associated with the FDMR combination becomes less important in the standard-risk disease group.[21,22] In the current study, subjects were restricted to patients with intermediate-risk AML in CR1. This may have resulted in a pronounced impact of the FDMR combination on transplant outcome in the current study. Second, as the impact of the FDMR combination on NRM is reported to be at least partially independent from that of GVHD on NRM,[21] and Japanese patients have lower incidence of GVHD,[23] the impact of sex combination on transplant outcome may be more evident in the Japanese than in the western populations.[22] The results of the present study suggest that the donor–recipient sex combination is a more important factor than the donor type in donor selection, in a certain subgroup of patients. As this may alter the current strategies in donor selection, verification in future studies is warranted.

Regarding older patients with intermediate-risk AML, a recent retrospective study showed that patients who underwent allo-HCT in CR1 had better survival than those who were treated with conventional chemotherapy alone, because the latter patients were associated with high relapse rates.[24] On the other hand, previous prospective studies, including patients with AML of all

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Table 3. Univariate analysis of OS, nonrelapse mortality and relapse

Variables	N	*OS*		*NRM*		*Relapse*	
		HR (95% CI)	*P-values*	*HR (95% CI)*	*P-values*	*HR (95% CI)*	*P-values*
Patient age							
20–39	290	1.00		1.00		1.00	
<20	45	0.83 (0.47–1.46)	0.52	0.67 (0.29–1.57)	0.36	1.05 (0.53–2.06)	0.89
⩾40	270	1.47 (1.11–1.95)	<0.01	1.65 (1.14–2.41)	<0.01	1.13 (0.78–1.65)	0.52
Sex matching							
Others	457	1.00		1.00		1.00	
Female to male	120	1.39 (1.01–1.91)	0.04	1.68 (1.12–2.53)	0.01	0.80 (0.49–1.31)	0.38
FAB classification							
M1–M5	439	1.00		1.00		1.00	
M0, M6, M7	129	0.89 (0.63–1.25)	0.51	1.01 (0.65–1.56)	0.97	0.87 (0.56–1.37)	0.55
Prior MDS							
No	578	1.00		1.00		1.00	
Yes	22	0.67 (0.28–1.64)	0.39	0.46 (0.11–1.86)	0.28	0.70 (0.22–2.19)	0.54
Cytogenetics							
Normal	576	1.00		1.00		1.00	
+8, +6, -Y, del(12p)	29	0.72 (0.35–1.46)	0.36	1.11 (0.52–2.38)	0.80	0.31 (0.08–1.25)	0.10
TBI							
Yes	422	1.00		1.00		1.00	
No	183	1.06 (0.80–1.42)	0.68	1.01 (0.69–1.50)	0.94	1.01 (0.68–1.49)	0.97
GVHD prophylaxis							
CsA-based	392	1.00		1.00		1.00	
FK-based	178	1.13 (0.84–1.53)	0.42	1.14 (0.77–1.71)	0.51	1.10 (0.73–1.64)	0.65
Others	27	1.19 (0.63–2.27)	0.59	1.06 (0.43–2.63)	0.89	1.48 (0.68–3.20)	0.32
Time from diagnosis to HCT							
< 6 months	200	1.00		1.00		1.00	
6 to <9 months	206	0.86 (0.62–1.20)	0.37	0.92 (0.58–1.48)	0.74	0.77 (0.51–1.17)	0.23
9 months or longer	190	0.88 (0.63–1.22)	0.45	1.26 (0.81–1.96)	0.31	0.48 (0.29–0.77)	<0.01
Year of transplant							
2004–2008	286	1.00		1.00		1.00	
1996–2003	319	0.91 (0.69–1.21)	0.53	1.08 (0.73–1.59)	0.69	0.83 (0.57–1.19)	0.31
Stem cell source							
BM	470	1.00		1.00		1.00	
Peripheral blood	135	1.08 (0.78–1.49)	0.64	0.76 (0.47–1.23)	0.27	1.64 (1.11–2.42)	0.01
WBC counts at diagnosis							
$<50 \times 10^9$/L	422	1.00		1.00		1.00	
$\geqslant 50 \times 10^9$/L	147	1.15 (0.84–1.57)	0.38	0.77 (0.49–1.24)	0.28	1.86 (1.27–2.74)	<0.01
No. of induction courses							
1	374	1.00		1.00		1.00	
⩾2	195	1.76 (1.32–2.33)	<0.01	1.36 (0.92–2.01)	0.12	2.25 (1.55–3.26)	<0.01
Donor							
MSD	290	1.00		1.00		1.00	
Other RD	53	1.34 (0.84–2.15)	0.23	1.17 (0.58–2.39)	0.66	1.31 (0.73–2.33)	0.36
MUD	141	0.88 (0.61–1.26)	0.49	1.12 (0.69–1.79)	0.65	0.77 (0.48–1.23)	0.28
MMUD	121	1.21 (0.86–1.71)	0.27	1.73 (1.11–2.67)	0.02	0.56 (0.32–0.99)	0.046

Abbreviations: CI = confidence interval; FK = tacrolimus; HCT = hematopoietic SCT; HR = hazard ratio; MDS = myelodysplastic syndrome; MSD = matched sibling donor; MMUD = mismatched unrelated donor; MUD = matched unrelated donor; NRM = nonrelapse mortality; RD = related donor.

cytogenetic risk groups, showed that the beneficial effect of allo-HCT in CR1 on OS was absent in patients older than 35–40 years, because the benefits of the reduced relapse rate were offset by a higher NRM.[6,25] In accordance with these prospective studies, older patients had higher NRM and overall mortality than younger patients in the current study. Our study revealed that a substantial number of older patients received allo-HCT in CR1, but the results of our study and others indicate that prospective studies to evaluate the efficacy of allo-HCT in CR1 for older patients with intermediate-risk AML are necessary before it becomes a general practice.

The proportion of patients who received TBI regimens tended to be lower in the older patients than in the younger patients in the current study (data not shown), perhaps in an attempt to

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Table 4. Significant factors in multivariate analysis for OS, nonrelapse mortality and relapse

Variables	N	*OS*		*NRM*		*Relapse*	
		HR (95% CI)	*P-values*	*HR (95% CI)*	*P-values*	*HR (95% CI)*	*P-values*
Patient age							
20–39	290	1.00		1.00		—	—
<40	45	0.85 (0.48–1.50)	0.58	0.67 (0.28–1.57)	0.35	—	—
⩾40	270	1.55 (1.17–2.06)	<0.01	1.71 (1.17–2.50)	<0.01	—	—
Sex matching							
Others	457	1.00		1.00		—	—
Female to male	120	1.42 (1.03–1.95)	0.03	1.68 (1.12–2.52)	0.01	—	—
WBC counts at diagnosis							
$<50 \times 10^9$/L	422	—	—	—	—	1.00	
$\geqslant 50 \times 10^9$/L	147	—	—	—	—	1.77 (1.20–2.63)	<0.01
No. of induction courses							
1	374	1.00	—	—	—	1.00	
⩾2	195	1.81 (1.36–2.41)	<0.01	—	—	2.24 (1.54–3.27)	<0.01
Time from diagnosis to HCT							
<6 months	200	—	—	—	—	1.00	
6 to <9 months	206	—	—	—	—	0.85 (0.55–1.31)	0.45
9 months or longer	190	—	—	—	—	0.56 (0.32–0.98)	0.04
Donor							
MSD	290	1.00		1.00		1.00	
Other RD	53	1.35 (0.84–2.18)	0.21	1.31 (0.64–2.68)	0.47	1.44 (0.80–2.61)	0.22
MUD	141	0.90 (0.62–1.30)	0.58	1.26 (0.77–2.06)	0.35	0.98 (0.58–1.64)	0.93
MMUD	121	1.17 (0.83–1.67)	0.37	1.83 (1.16–2.86)	<0.01	0.71 (0.38–1.32)	0.28

Abbreviations: CI = confidence interval; HCT = hematopoietic SCT; HR = hazard ratio; MMUD = mismatched unrelated donor; MSD = matched sibling donor; MUD = matched unrelated donor; NRM = nonrelapse mortality; RD = related donor.

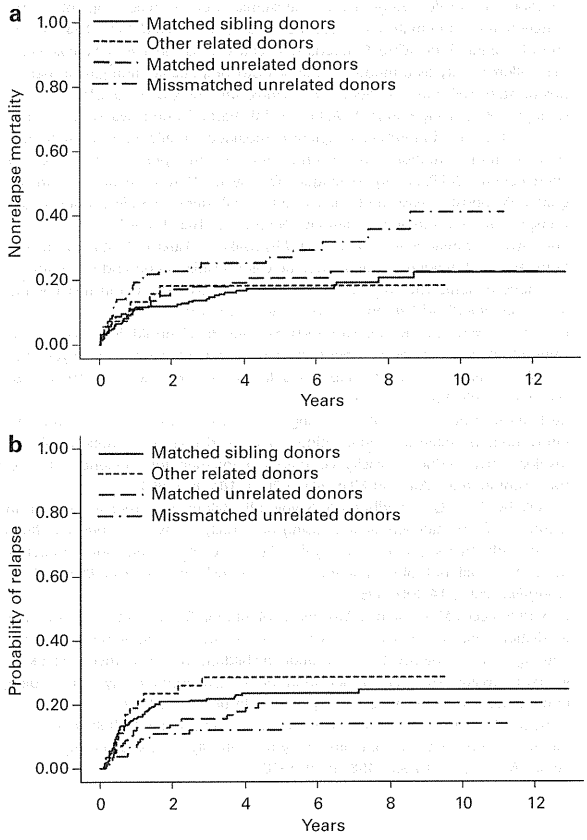

Figure 2. Comparison of MSD, other RD, MUD, and MMUD transplantation. (**a**) Cumulative incidence of NRM. (**b**) Cumulative incidence of relapse.

reduce toxicity. However, there was no significant difference in NRM between TBI and non-TBI regimens among older patients (data not shown). Recently, reduced toxicity myeloablative regimens, such as the combination of fludarabine with myeloablative doses of BU, were developed with an aim to decrease toxicity without compromising antileukemic effects.[26] These regimens might be beneficial for older patients, especially for those with standard-risk disease.[27] The optimal conditioning regimens for older patients need to be determined in the future studies.

OS after other RD and MMUD HCT did not differ significantly from that after MSD HCT in the current study, but these results need to be interpreted with caution. First, the small number of patients with other RD limited the power to detect significant differences in survival between MSD and other RD HCT. Second, other RD and MMUD included donors with various degrees of HLA incompatibilities. Thus, it is difficult to draw firm conclusions regarding the role of other RD and MMUD HCT from this study. Nonetheless, considering that other RD and MMUD HCT yielded a 4-year OS of 53% and 61%, respectively, allo-HCT from these donors might be an option for patients with unfavorable features. For example, as patients who required more than one course of induction therapy to achieve CR1 have poor outcomes with conventional chemotherapy,[8] they might benefit from allo-HCT from other RD or MMUD, when MSD and MUD are not available.

Our study has several limitations. First, this is a non-randomized, retrospective observational study using registry data, which would allow for the introduction of bias. To minimize bias, we conducted multivariate analyses to adjust for baseline differences. However, some factors which might have influenced transplant outcomes (such as performance score and extramedullary disease) could not be included in the Cox proportional hazards regression model due to a high frequency of missing values. Second, a time-censoring effect might have influenced the results.[28] Patients who undergo transplantation late after achievement of CR may be at a lower risk of relapse, by virtue of having remained in remission a time long enough for a transplantation to be performed.[28] This effect might have favorably affected the outcome of unrelated donor HCT. However, there was no significant difference in OS between MSD

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and MUD HCT, even when the time from diagnosis to transplantation was included in the final model of multivariate analyses (data not shown). Third, although the role of allo-HCT according to genetic mutations, such as *FLT3-ITD*, *NPM1* and *CEBPA*, is now being explored,[29] the information about these mutations was not available and this was beyond the scope of the present study. However, the results of our study do support the inclusion of not only MSD HCT, but also MUD HCT, in the prospective studies, which evaluate the role of allo-HCT according to these genetic mutations.

In conclusion, the results of our study suggest that HCT, not only from MSD, but also from MUD ,should be considered in younger patients with intermediate-risk AML in CR1, and that the donor–recipient sex combination is more important than the donor type in donor selection. Prospective studies to evaluate the role of allo-HCT in CR1 for older patients are warranted.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

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ORIGINAL ARTICLE

Hyperferritinemia after adult allogeneic hematopoietic cell transplantation: quantification of iron burden by determining non-transferrin-bound iron

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Abstract Iron overload is a common complication in allogeneic hematopoietic cell transplantation (HCT). We studied the prevalence of iron overload using serum ferritin from 122 allogeneic HCT survivors who had survived a median of 1259 (range 134–4261) days. We also quantified iron overload by determining non-transferrin-bound iron (NTBI), which reflects iron overload more directly than ferritin, and compared the results with those of the ferritin assay. Fifty-two patients (43 %) showed hyperferritinemia (HF) (serum ferritin >1000 ng/mL), and there was a moderate correlation between serum ferritin and the number of transfused red blood cell units ($\rho = 0.71$). In multivariate analyses, HF was a significant risk factor for liver dysfunction ($P = 0.0001$) and diabetes ($P = 0.02$), and was related to a lesser extent with performance status ($P = 0.08$). There was a significant correlation between serum ferritin and NTBI ($\rho = 0.59$); however, the association of NTBI with these outcomes was weaker than that of serum ferritin. In conclusion, serum ferritin is a good surrogate marker of iron overload after allogeneic HCT, and reflects organ damage more accurately than NTBI.

Keywords Iron overload · Hyperferritinemia · Hematopoietic cell transplantation · Ferritin · Non-transferrin-bound iron

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Introduction

Allogeneic hematopoietic cell transplantation (HCT) is a curative treatment for many patients with malignant and nonmalignant hematological disorders. Allogeneic HCT recipients are at risk of developing iron overload because they have large red blood cell (RBC) transfusions both during the initial treatment of their disease and during the period of transplantation. Iron overload is considered to be a common complication of HCT, and published consensus guidelines recommend screening for it in HCT survivors [1]. Iron overload has been reported to relate to liver dysfunction and to increase the risk of infections late after allogeneic HCT [2–6]. However, the iron overload on survivors after allogeneic HCT, and its clinical impact, remains unclear. The estimation of iron overload is currently based on serum ferritin levels, but in HCT recipients, many confounding factors such as inflammation, ineffective erythropoiesis, and liver disease can be related to ferritin overestimation [7–9]. Non-transferrin-bound iron (NTBI), which is increased during iron overload, is considered to be a marker of iron toxicity, and recently, a variety of analytical approaches for measuring NTBI have been reported [10–18]. NTBI is a low-molecular weight

form of iron that is detected during iron overload, when transferrin becomes fully saturated and unable to bind excess iron. NTBI is thought to catalyze the formation of reactive radicals [19, 20]. Several studies have demonstrated that NTBI is a good index of iron overload in patients with thalassemia [21–23]. We studied the prevalence of iron overload in adult allogeneic HCT survivors using serum ferritin and quantified iron overload by determining NTBI. Further analyses include the impact of iron overload on hepatic function, diabetes mellitus, and performance status.

Materials and methods

Patients

Allogeneic HCT survivors followed at the Japanese Red Cross Nagoya First Hospital were eligible for this study if they met all of the following criteria: (1) underwent their first allogeneic HCT in our institute; (2) survived ≥100 days from HCT in continuous remission; (3) independent of RBC transfusion during recent 3 months; (4) did not have active infection; (5) did not receive iron chelating therapy or phlebotomy; and (6) returned for follow-up at our institute between August 2009 and August 2010. The study protocol was approved by the Japanese Red Cross Nagoya First Hospital's Institutional Review Board, and all patients provided informed consent in accordance with the Declaration of Helsinki.

Laboratory studies and assessment of complications

Iron overload was initially assessed by measuring serum iron, transferring saturation, and serum ferritin using standard commercial assays. Hyperferritinemia (HF) was defined as a serum ferritin level >1000 ng/mL, because iron-related liver function test abnormalities have been reported to increase in patients with this ferritin level [24, 25]. Patients' plasma was frozen and sent to Asahikawa Medical University for measurements of NTBI. Liver function tests (LFTs) were performed at the same time as the iron status assessment, including aspartate aminotransferase (AST; reference range, 8–30 IU/L), alanine aminotransferase (ALT; reference range, 5–35 IU/L), gammaglutamyl transpeptidase (γ-GTP; reference range, 8–35 IU/L), and alkaline phosphatase (ALP; reference range, 42–141 IU/L). Liver dysfunction was defined as having at least one abnormal LFT value. Data on the unit of RBC transfusion since the initial diagnosis of underlying hematological disorders were obtained from the blood bank of the Japanese Red Cross Nagoya First Hospital and from the institutions where the patients were reported to have received transfusions. Patients, who had current symptoms or signs of chronic graft-versus-host disease (GVHD) and were under treatment of immunosuppressant therapy, were classified as active chronic GVHD. Patients who were under the treatment for diabetes mellitus, except for only diet therapy, at the same time of iron status assessment, were defined as patients with diabetes. Performance status at the time of iron status assessment was evaluated by Karnofsky score (KS). Poor performance status was defined as a KS less than 100.

Quantification of NTBI

Serum NTBI concentrations were measured as described previously with some modifications [13]. Serum samples had been kept frozen at −20 °C until the time of measurement. At first, 50 μL of 5 mM triscarbonatecobalt (III) ($Na_3[Co(CO_3)_3]\cdot 3H_2O$) was added to 450 μL of serum, and incubated at 37 °C for 60 min. The resulting mixed solution should be 500 μL. Two-hundred twenty-five μL of solution was then transferred to a new tube and 25 μL of 80 mM nitrilotriacetic acid (NTA) was added to chelate all NTBI in a serum, which should be non-specifically and weakly bound to serum proteins such as albumin and citrate. We prepared another tube in which 225 μL of mixed solution was also transferred, and 25 μL of sorbent without NTA was added. Triscarbonatecobalt (III) was added to saturate apo-transferrin (apo-Tf) first before adding NTA, because displacement of iron from the NTA–NTBI complex to apo-Tf had to be prevented. Both tubes were incubated at room temperature for 30 min, and then ultrafiltrated by Amicon Ultra-0.5 mL 30K (Ultracel-30K) (Millipore/Merck) under 14,000 g for 30 min at 20 °C. Twenty μL of each ultrafiltrate was directly injected into the metal-free high-performance liquid-chromatography (HPLC) system. This system utilized nonmetallic polyether-ethyl ketone tubing throughout a 2796 BioSeparation Module with Degasser and Sample Heater-Cooler, and a 2998 Photodiode Array Detector (Waters). Analytical columns were OmniSpher 5 μm C18, G100 × 3 mm, and a glass column with ChromSep guard column SS 10 × 2 mm (Varian/Agilent Technologies). Chromatographic conditions were flow rate of 0.8 mL/min; mobile phase isocratic containing 20 % acetonitrile and 3 mM 3-hydroxy-2-methyl-1-propyl-pyridine-4-one (CP22) in a 5 mM morpholinopropanesulfonic acid (MOPS) buffer, pH 7.0; visible detection wavelength of 450 nm. Finally, the measured NTBI value of the tube without NTA was subtracted from the one containing NTA. By this subtraction, contamination of iron in the sorbent used for NTA solution and the influence of the remainder of cobalt that was not used for occupation of unsaturated Tf binding sites would be offset. NTBI was detected by our method even in healthy volunteers, although the concentrations were extremely low;

the average NTBI was 0.206 ± 0.091 μmol/L (males, $n = 20$) and 0.212 ± 0.095 μmol/L: (females, $n = 16$). There was no negative NTBI value even in healthy volunteers. We therefore believe this subtraction method raised the sensitivity of quantification, because negative values of NTBI had often been observed in previous reports, presumably due to iron contamination in the reagents [10]. The between-day imprecision was estimated by analysis of one individual serum sample on 4 different days with duplicate. The calculated mean value, standard deviation, and between-day imprecision coefficient of variation were 0.45 μmol/L, 0.05 μmol/L, and 11.1 %, respectively.

Statistical analyses

The main aims of this study were to estimate the prevalence of iron overload using serum ferritin in ≥100-day survivors of allogeneic HCT and to compare serum ferritin with NTBI. The other aims were to find correlations between serum ferritin and NTBI levels and the amount of RBC transfusions, and the impact iron overload has on liver dysfunction, diabetes mellitus, and performance status. Differences between groups were assessed using the Fisher's exact, Chi-square, and Mann–Whitney test, as deemed appropriate. Univariate and multivariate logistic regression models were used to analyze the effect of some relevant variables on liver dysfunction, diabetes, and performance status. The correlation between serum ferritin and the days since HCT, NTBI level, the value of LFTs, and the amount of red blood cell were measured was assessed by Spearman's rank correlation. A significance level of $P < 0.05$ was used for all analyses, which were based on all data available as of November 30, 2010.

Results

Patient characteristics

Among 141 patients who returned for follow-up during this study period, 122 were enrolled. However, 19 were not eligible for enrollment because they had disease relapse, 6; ongoing RBC transfusion, 7; active infection, 2; second malignancy, 2; second allogeneic transplantation, 1; or were under the treatment of deferasirox, 1. The median age at allogeneic HCT was 37 years (range 17–65 years); 63 patients were males and 59 were females. Primary diagnoses included acute myeloid leukemia (AML; $n = 31$), myelodysplastic syndromes (MDS; $n = 27$), acute lymphoblastic leukemia (ALL; $n = 24$), chronic myeloid leukemia (CML; $n = 16$), aplastic anemia (AA; $n = 13$), non-Hodgkin lymphoma (NHL; $n = 5$), paroxysmal nocturnal hemoglobinuria (PNH; $n = 3$), chronic active Epstein-Barr virus infection (CAEBV; $n = 2$), and multiple myeloma (MM; $n = 1$). Conditioning regimens were myeloablative ($n = 85$) and reduced intensity ($n = 37$). Donors were HLA-matched ($n = 103$) and mismatched ($n = 19$); related ($n = 48$) and unrelated ($n = 74$). Graft sources were bone marrow ($n = 93$), peripheral blood ($n = 17$), and cord blood ($n = 12$). Graft-versus-host disease (GVHD) prophylaxis consisted of a combination of short-term methotrexate and tacrolimus ($n = 72$) or cyclosporine ($n = 50$).

Iron overload

The median serum ferritin level was 854 ng/mL (range 14–10500 ng/mL), and 112 patients (92 %) had an above-normal ferritin value (reference range, male: 18.6–261; female: 4.0–64.2 ng/mL). Overall, 52 patients (43 %) had HF (serum ferritin >1000 ng/mL). The characteristics of patients with and without HF are shown in Table 1. The median time from allogeneic HCT to serum ferritin assessment was 1259 days (range 134–4261 days) and was similar between patients with and without HF (1245 and 1277 days, respectively, $P = 0.55$). There was no correlation between serum ferritin and days since HCT (Fig. 1, $P = 0.13$). Also, there were no significant differences related to age at HCT, sex, primary diagnosis, disease risk, conditioning regimen, graft source, donor type, or HLA disparity and GVHD prophylaxis between patients with and without HF.

Grade II–IV acute GVHD occurred in 27 patients (22 %). Fourteen (27 %) of the patients with HF had a history of grade II–IV acute GVHD, and there was no significant difference compared with 13 (19 %) of the patients without HF ($P = 0.28$). Twenty-five patients (20 %) had active chronic GVHD at the time of ferritin assessment. No significant correlation was found between HF and the presence of active chronic GVHD ($P = 0.17$). Two patients with HF were HCV-positive ($P = 0.18$).

Compared with patients without HF, those with HF had received more RBC transfusion (median 55 vs. 24 U, $P < 0.0001$) (Table 2). There was a statistically significant correlation between serum ferritin and the number of packed RBC units transfused from the diagnosis of underlying disease ($\rho = 0.71$; $P < 0.0001$) (Fig. 2).

Association of serum ferritin with outcomes

At the time of ferritin assessment, 54 of the 122 (44 %) patients had liver dysfunction. Thirty-three (63 %) of the patients with HF had liver dysfunction, whereas only 21 (30 %) of the patients were without it ($P = 0.0004$). The

Table 1 Patient and transplantation characteristics

Characteristic	Patients without HF ($n = 70$)	Patients with HF ($n = 52$)	P	Patients without high NTBI ($n = 56$)	Patients with high NTBI ($n = 55$)	P
Median age at HCT, years (range)	40 (17–65)	32 (18–63)	0.46	40 (17–61)	36 (19–65)	0.79
Median time since HCT, days (range)	1277 (275–4261)	1245 (134–4213)	0.55	1228 (280–4261)	1475 (134–4213)	0.53
Females	38 (54 %)	21 (40 %)	0.15	25 (45 %)	26 (47 %)	0.85
Diagnosis			0.25			0.62
Acute leukemia/MDS	45 (64 %)	37 (71 %)		36 (64 %)	37 (67 %)	
Aplastic anemia	6 (9 %)	7 (13 %)		5 (9 %)	7 (13 %)	
Other[a]	19 (27 %)	8 (15 %)		15 (27 %)	11 (20 %)	
Disease risk			0.57			0.56
High	25 (36 %)	22 (42 %)		20 (36 %)	23 (42 %)	
Low	45 (64 %)	30 (58 %)		36 (64 %)	32 (58 %)	
Conditioning regimen			0.16			1.0
Myeloablative	45 (64 %)	40 (77 %)		38 (68 %)	37 (67 %)	
Reduced intensity	25 (36 %)	12 (23 %)		18 (32 %)	18 (33 %)	
Donor			0.71			0.56
Related	29 (41 %)	19 (37 %)		25 (45 %)	21 (38 %)	
Unrelated	41 (59 %)	33 (63 %)		31 (55 %)	34 (62 %)	
Graft source			0.52			1.0
BM	56 (80 %)	37 (71 %)		43 (77 %)	42 (76 %)	
PB	8 (11 %)	9 (17 %)		8 (14 %)	8 (15 %)	
CB	6 (9 %)	6 (12 %)		5 (9 %)	5 (9 %)	
HLA disparity, match			0.45			0.44
Match	61 (87 %)	42 (81 %)		49 (88 %)	45 (82 %)	
Mismatch	9 (13 %)	10 (19 %)		7 (13 %)	10 (18 %)	
GVHD prophylaxis			1.0			0.85
CsA base	29 (41 %)	21 (40 %)		23 (41 %)	24 (44 %)	
FK base	41 (59 %)	31 (60 %)		33 (59 %)	31 (56 %)	
Acute GVHD			0.28			0.26
Grade 0–I	57 (81 %)	38 (73 %)		46 (82 %)	40 (73 %)	
Grade II–IV	13 (19 %)	14 (27 %)		10 (18 %)	15 (27 %)	
Chronic GVHD			0.17			0.25
None or not active	59 (84 %)	38 (73 %)		47 (84 %)	41 (75 %)	
Active	11 (16 %)	14 (27 %)		9 (16 %)	14 (25 %)	
Hepatitis	0 (0 %)	2 (4 %)	0.18	0 (0 %)	2 (4 %)	0.24

HF indicates hyperferritinemia defined as serum ferritin level higher than 1000 ng/mL; high NTBI, NTBI level higher than 0.38 μmol/L; HCT, hematopoietic cell transplantation; disease risk low, AML in first or second remission; Philadelphia chromosome-negative ALL in first remission; CML in chronic phase; MDS, refractory anemia or nonmalignant hematological disease; disease risk high, all other diagnoses; HLA match, identical HLA-A, -B, and –DRB1 loci; HLA mismatch, at least one disparity at one of these loci

BM bone marrow, *PB* peripheral blood, *CB* cord blood, *CsA* cyclosporine, *FK* tacrolimus

[a] Twenty-seven patients with "other" diagnoses included: CML, 16; non-Hodgkin lymphoma, 5; PNH, 3; chronic active Epstein-Bar virus infection, 2; multiple myeloma, 1

rate of patients with above the upper limit of normal AST values (19 vs. 4 %; $P = 0.01$) and ALT (48 vs. 11 %; $P < 0.0001$), were significantly higher in patients with HF compared with those without it. Median AST, ALT, and γ-GTP values were significantly higher in the patients with HF (Table 2). Among these LFTs, serum ferritin correlated most closely to ALT values ($\rho = 0.49$; $P < 0.0001$) compared with AST ($\rho = 0.36$; $P < 0.0001$) and γ-GTP ($\rho = 0.36$; $P = 0.0006$). In multivariate analysis, HF was a significant risk factor for liver dysfunction (odds ratio 4.92; 95 % CI, 2.19–11.1; $P = 0.0001$) (Table 3). Among 25 patients who had active chronic GVHD, 6 (24 %) patients

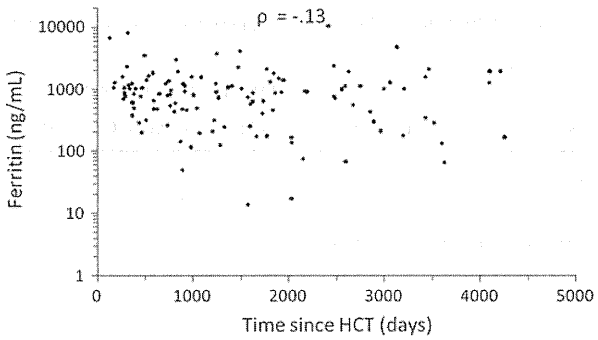

Fig. 1 Relation between ferritin and days since HCT. There was no statistically significant correlation between serum ferritin level and days since allogeneic HCT ($\rho = -13$; $P = 0.13$)

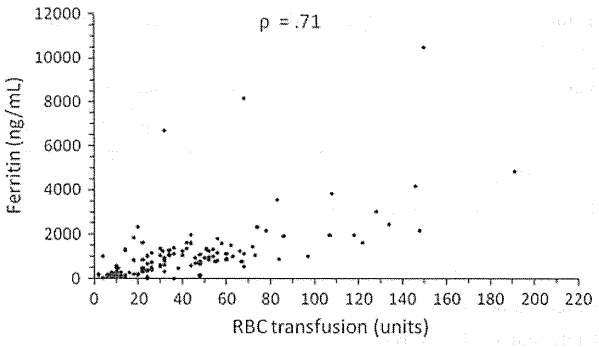

Fig. 2 Relation between ferritin and red blood cell transfusion. There was a statistically significant correlation between serum ferritin level and the number of packed red blood cell units transfused from the diagnosis of underlying disease ($\rho = 0.71$; $P < 0.0001$)

had liver dysfunction diagnosed as being associated with GVHD. Even if patients with GVHD-associated liver dysfunction were excluded, HF was a risk factor for liver dysfunction (odds ratio 4.67; 95 % CI, 1.99–10.9; $P = 0.0004$). The rate of diabetes tended to be higher in patients with HF compared with those without it (27 vs. 11 %; $P = 0.03$) (Table 2). In multivariate analyses, steroid therapy (odds ratio 15.5; 95 % CI, 3.86–61.9; $P = 0.0001$), age at HCT (odds ratio 1.07; 95 % CI, 1.01–1.12; $P = 0.01$), being a male patient (odds ratio 5.06; 95 % CI, 1.39–18.5; $P = 0.01$), and HF (odds ratio 4.20; 95 % CI, 1.24–14.3; $P = 0.02$) were significant risk factors for diabetes (Table 3). The rate of patients who had a poor performance status, which was defined as a KS less

Table 2 Iron burden measurements, liver dysfunction, diabetes, and Karnofsky score at the time of ferritin assessment

Characteristic	Patients without HF	Patients with HF	*P*	Patients without high NTBI	Patients with high NTBI	*P*
Ferritin, ng/mL (range)	466 (13.6–969)	1420 (1010–10500)	<0.0001	491 (13.6–2330)	1300 (49.4–10500)	<0.0001
Hyperferritinemia, no. (%)	0 (0)	52 (100)	<0.0001	9 (16 %)	39 (71 %)	<0.0001
NTBI, μmol/L (range)	0.28 (0.03–1.62)	0.68 (0.12–2.09)	<0.0001	0.24 (0.03–0.38)	0.68 (0.39–2.09)	<0.0001
High NTBI, no. (%)	16 (23)	39 (75)	<0.0001	0 (0)	55 (100)	<0.0001
Pre-HCT ferritin, ng/mL (range)	531 (6.3–1770)	1320 (185–5180)	0.0001	483 (6.3–5180)	1300 (185–4990)	0.01
Serum iron, ìg/dL (range)	98 (33–172)	135 (48–308)	<0.0001	105 (33–190)	125 (48–308)	0.003
Transferrin saturation, % (range)	39 (12–78)	55 (22–119)	<0.0001	39 (12–93)	50 (22–118)	<0.0001
Median RBC transfusion, units (range)	24 (2–84)	55 (4–191)	<0.0001	24 (2–86)	51 (4–191)	<0.0001
LFTs above UNL, no. (%)						
AST	3 (4 %)	10 (19 %)	0.01	4 (7 %)	9 (16 %)	0.15
ALT	8 (11 %)	25 (48 %)	<0.0001	10 (18 %)	22 (40 %)	0.01
ALP	12 (17 %)	10 (19 %)	0.82	8 (14 %)	13 (24 %)	0.23
γ-GTP	14 (20 %)	19 (37 %)	0.06	15 (27 %)	15 (27 %)	0.99
LFTs, median, IU/L (range)						
AST	23 (13–54)	28 (13–248)	0.003	24 (15–54)	26 (13–248)	0.21
ALT	22 (9–50)	33 (7–428)	<0.0001	25 (9–62)	28 (7–428)	0.07
ALP	229 (86–585)	256 (103–791)	0.05	229 (108–585)	258 (86–791)	0.08
γ-GTP	29 (7–204)	50 (21–1012)	0.0004	33 (7–392)	39 (11–1012)	0.13
Liver dysfunction, no. (%)	21 (30 %)	33 (63 %)	0.0004	19 (34 %)	31 (56 %)	0.02
Diabetes, no. (%)	8 (11 %)	14 (27 %)	0.03	10 (18 %)	12 (22 %)	0.64
Poor performance status, no. (%)	20 (29 %)	27 (52 %)	0.01	17 (30 %)	26 (47 %)	0.08

HF indicates hyperferritinemia defined as serum ferritin level higher than 1000 ng/mL; high NTBI indicates NTBI level higher than 0.38 μmol/L

HCT hematopoietic cell transplantation, *RBC* red blood cell, *LFTs* liver function tests, *UNL* upper normal limit, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *Y-GTP* gammaglutamyl transpeptidase, *ALP* alkaline phosphatase, Poor performance status, Karnofsky score less than 100

Table 3 Multivariate analyses of risk factors of liver dysfunction, diabetes, and poor performance status (using hyperferritinemia as a factor of iron overload)

Variable	Liver dysfunction		Diabetes		Poor performance status	
	Odds ratio (95 % CI)	*P*	Odds ratio (95 % CI)	*P*	Odds ratio (95 % CI)	*P*
Age at HCT (Continuous)	–	–	1.07 (1.01–1.12)	0.01	–	–
Time since HCT (Continuous)	–	–	–	–	–	–
Male	–	–	5.06 (1.39–18.5)	0.01	–	–
High disease risk	–	–	–	–	–	–
Unrelated donor	–	–	–	–	–	–
Graft source CB (vs. BM)	–	–	–	–	–	–
Graft source PB (vs. BM)	–	–	–	–	–	–
HLA mismatch	0.19 (0.06–0.68)	0.01	–	–	–	–
Myeloablative conditioning regimen	–	–	–	–	–	–
CsA base GVHD prophylaxis	–	–	–	–	–	–
Grade II–IV acute GVHD	–	–	–	–	4.93 (1.73–14.1)	0.003
Active chronic GVHD	–	–	NA	NA	9.75 (2.84–33.5)	0.0003
Steroid therapy	NA	NA	15.5 (3.86–61.9)	0.0001	NA	NA
Diabetes	NA	NA	NA	NA	3.63 (1.10–12.0)	0.03
Hyperferritinemia	4.92 (2.19–11.1)	0.0001	4.20 (1.24–14.3)	0.02	2.28 (0.91–5.70)	0.08

Poor performance status indicates Karnofsky score less than 100

CI confidence interval, *HCT* hematopoietic cell transplantation, *BM* bone marrow, *PB* peripheral blood, *CB* cord blood, *CsA* cyclosporine; hyperferritinemia, serum ferritin level higher than 1000 ng/mL

than 100, was significantly higher in patients with HF (52 vs. 29 %, $P = 0.01$) (Table 2). In multivariate analysis, the presence of an active chronic GVHD (odds ratio 9.75; 95 % CI, 2.84–33.5; $P = 0.0003$), a history of grade II–IV acute GVHD (odds ratio 4.93; 95 % CI, 1.73–14.1; $P = 0.003$), and diabetes (odds ratio 3.63; 95 % CI, 1.10–12.0; $P = 0.03$) were significant risk factors for poor performance status (Table 3). Only in univariate analysis, HF was significant risk factor for poor performance status (odds ratio 2.70; 95 % CI, 1.27–5.73; $P = 0.01$). No patients had distinctive clinical heart failure at the time of ferritin assessment (data not shown).

Association of NTBI with outcomes

We measured serum NTBI from 111 of the 122 patients. The median NTBI value was 0.38 μmol/L (range 0.03–2.09 μmol/L). Forty-five of the 48 patients (94 %) with HF showed more than the normal range of NTBI (males: 0.206 ± 0.091; females: 0.212 ± 0.095 μmol/L) in this assay, compared with 44 of the 63 patients (70 %) without HF ($P = 0.002$). The median NTBI value was significantly higher in patients with HF (median, 0.68 μmol/L; range, 0.12–2.09 μmol/L) compared with those without it (median, 0.28 μmol/L; range, 0.03–1.62 μmol/L) ($P < 0.0001$) (Table 2). In addition, there was a statistically significant correlation between serum ferritin and the NTBI level ($\rho = 0.59$; $P < 0.0001$) (Fig. 3a). NTBI values also correlated with the number of packed RBC units transfused from the diagnosis of underlying disease ($\rho = 0.50$; $P < 0.0001$) (Fig. 3b) and transferrin saturation ($\rho = 0.45$; $P < 0.0001$), and to a lesser extent with the RBC units transfused before HCT ($\rho = 0.33$; $P = 0.0006$), after HCT ($\rho = 0.31$; $P = 0.001$) and serum iron ($\rho = 0.37$; $P = 0.0001$). Between patients with and without high NTBI, which was defined as above the median NTBI level (0.38 μmol/L), there were no significant differences related to characteristics of patients (Table 1). The rate of patients with liver dysfunction was significantly higher in patients with high NTBI compared with those without it (56 vs. 34 %; $P = 0.02$). There were no significant differences related to diabetes (22 vs. 18 %; $P = 0.64$) and poor performance status (47 vs. 30 %; $P = 0.08$) (Table 2). In multivariate analyses, high NTBI was a significant risk factor for only liver dysfunction (odds ratio 3.01; 95 % CI, 1.32–6.85; $P = 0.009$), but not diabetes (odds ratio 1.32; 95 % CI, 0.43–4.05; $P = 0.63$) and poor performance status (odds ratio 1.83; 95 % CI, 0.69–4.85; $P = 0.22$) (Table 4).

LIC estimated by the R2 MRI technique

Five patients with HF (median serum ferritin 4170 ng/mL, range 2160–10500 ng/mL) underwent magnetic resonance imaging (MRI) of the liver to estimate the liver iron concentration (LIC, normal range: 0.17–1.8 mg/g dry tissue)

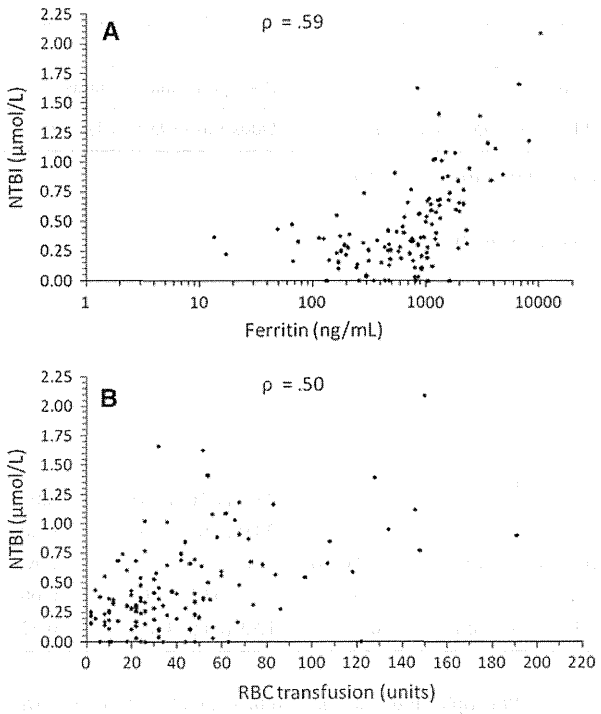

Fig. 3 Relation between NTBI and serum ferritin level (**a**) and red blood cell transfusion (**b**). There was a statistically significant correlation between serum ferritin and NTBI level ($\rho = 0.59$; $P < 0.0001$). NTBI values also correlated to the number of packed red blood cell units ($\rho = 0.50$; $P < 0.0001$)

by the R2 MRI technique (Ferriscan®, Perth, Australia) and all had high LIC (median 12.8 mg/g dry tissue, range 9.6–36.4 mg/g dry tissue). Four of these 5 patients had liver dysfunction. The median AST, ALT, ALP, and γ-GTP values of these 5 patients were 32 IU/L (range 27–108 IU/L), 47 IU/L (range 26–130 IU/L), 217 IU/L (range 192–421 IU/L), and 51 IU/L (range 28–69 IU/L), respectively. All five patients also had high NTBI (median 1.12 μmol/L, range 0.77–2.09 μmol/L).

Discussion

We observed a relatively high prevalence of iron overload in survivors of allogeneic HCT. Overall, 43 % of our patients exhibited evidence of iron overload based on their serum ferritin levels, a relatively high prevalence in agreement with the 32–58 % previously reported [2, 3, 26]. These results emphasize the need for routine screening for iron overload in survivors of allogeneic HCT.

Serum ferritin is commonly used to make indirect estimations of body iron stores, but is not specific for iron overload because it can be elevated by other factors, such as inflammation, ineffective erythropoiesis, and liver disease [27]. Serum ferritin also has had a poor correlation with LIC in multiple studies of patients with thalassemia and sickle cell disease [27–29]. In previous reports which systematically evaluated iron overload in allogeneic HCT recipients, LIC was estimated by noninvasive imaging techniques such as T2* or R2 MRI, or by the superconducting quantum interference device (SQUID) [2, 3, 26]. Majhail et al. [3] reported only a modest correlation ($\rho = 0.47$) between serum ferritin and LIC that was measured by MRI in allogeneic HCT survivors. In the present study, we assessed the iron burden by determining NTBI, which has been shown to be significantly correlated with LIC in patients with thalassemia [23]. In the HCT setting, some reports showed that the conditioning regimen itself can increase NTBI levels in the peritransplantation period, due to inhibition of erythropoiesis or tissue injury which results in the release of stored iron from the liver [30, 31]. In the current study, serum ferritin was well correlated with the number of packed RBC units and NTBI in patients without active infection, relapse, or second malignancy. These findings confirmed that serum ferritin was a good marker of iron overload in survivors after allogeneic HCT.

Pullarkat et al. [32] reported that elevated pretransplant ferritin increased acute GVHD. Tissue injury caused by iron overload in the patients undergoing allogeneic HCT may be the initiation of the pathogenesis of GVHD. In the current study, there were no significant differences in acute GVHD and active chronic GVHD between patients with and without HF assessed at post-transplantation. We could not exactly evaluate the correlation between iron overload and GVHD, because we assessed only patients who had survived with or without GVHD. But, it may be suggested that the statement of active chronic GVHD did not elevate the serum ferritin level. It remains to be determined whether iron overload initiates or aggravates acute and chronic GVHD.

Iron overload is known to contribute to the etiology of liver dysfunction, but it also can mimic exacerbation of hepatic GVHD, thus resulting in unnecessary continuation or intensification of immunosuppressive therapy [4, 33]. In a study assessing the role of liver biopsy for evaluating the cause of liver dysfunction late after HCT, iron overload was found in 75 % and sole histopathologic abnormality in 33 % [33]. The current study demonstrated that HF was an independent risk factor for liver dysfunction in survivors after allogeneic HCT, and this was in agreement with the previous report [2]. ALT was the LFT value most closely correlated with serum ferritin levels in the current study. Busca et al. [2] reported that ALT and γ-GTP were the LFT values that most frequently exceeded the upper limit of normal in patients with hyperferritinemia, after HCT. Elevation of ALT is not specific to, but might be suggestive of, hepatic iron overload. The current study also showed that not only steroid therapy but also HF was a risk factor

Table 4 Multivariate analyses of risk factors of liver dysfunction, diabetes, and poor performance status (using high NTBI as a factor of iron overload)

Variable	Liver dysfunction		Diabetes		Poor performance status	
	Odds ratio (95 % CI)	*P*	Odds ratio (95 % CI)	*P*	Odds ratio (95 % CI)	*P*
Age at HCT (Continuous)	–	–	1.05 (1.00–1.10)	0.04	–	–
Time since HCT (Continuous)	–	–	–	–	–	–
Male	2.61 (1.15–5.91)	0.02	5.38 (1.43–20.2)	0.01	–	–
High disease risk	–	–	–	–	–	–
Unrelated donor	–	–	–	–	–	–
Graft source CB (vs. BM)	–	–	–	–	–	–
Graft source PB (vs. BM)	–	–	–	–	–	–
HLA mismatch	0.28 (0.08–0.97)	0.04	–	–	–	–
Myeloablative conditioning regimen	–	–	–	–	–	–
CsA base GVHD prophylaxis	–	–	–	–	–	–
Grade II–IV acute GVHD	–	–	–	–	3.63 (1.16–11.4)	0.03
Active chronic GVHD	–	–	NA	NA	20.0 (4.09–97.5)	0.0002
Steroid therapy	NA	NA	16.9 (4.22–68.1)	<0.0001	NA	NA
Diabetes	NA	NA	NA	NA	3.41 (0.97–12.0)	0.06
High NTBI	3.01 (1.32–6.85)	0.009	1.32 (0.43–4.05)	0.63	1.83 (0.69–4.85)	0.22

Poor performasnce status indicates Karnofsky score less than 100

CI confidence interval, *HCT* hematopoietic cell transplantation, *BM* bone marrow, *PB* peripheral blood, *CB* cord blood, *CsA* cyclosporine, *High NTBI* NTBI level higher than 0.38 μmol/L

for diabetes in survivors after allogeneic HCT. Iron overload can be a cause of diabetes due to insulin resistance as well as islet cell insufficiency [34]. An improvement of liver dysfunction has been demonstrated with phlebotomy or iron chelating therapy [2]. The management of iron overload might reverse pancreatic function. Since humans do not have any physiological mechanisms to excrete excess iron [35], iron chelating therapy or phlebotomy might improve the general conditions of patients with iron overload after allogeneic HCT. In this study, assessment of cardiac complications, such as ejection fraction and cardiac iron loading, was not enough done, but none of the patients had distinctive heart failure. Our study did not address the changes in serum ferritin or NTBI between pre- and post-HCT. Further studies are warranted to address this topic.

There are a variety of factors which can lead us to overestimate the amount of ferritin in HCT recipients. We therefore examined NTBI in expecting its stronger association with outcomes than serum ferritin. Our results, however, showed a weaker association of NTBI with outcomes than serum ferritin, although there was a statistically significant correlation between serum ferritin and NTBI levels. The weaker association could be explained by the following: serum ferritin reflects total body iron which is mainly stored in the liver or reticuloendothelial organ, but NTBI refers only to the iron in plasma that binds to ligands other than transferrin. Thus, it is reasonable to consider that the number of transfusions and organ damage at the time of ferritin assessment may be more closely related with serum ferritin than NTBI. Moreover, patients may already have organ damage when we find an elevation in the serum ferritin level. On the other hand, NTBI is considered to be a marker of iron toxicity even at the early stage of organ damage, and determination of NTBI is crucial for evaluating and monitoring the risk of iron toxicity [10]. In this study, NTBI was assessed within a wide range of days after HCT, and systematic and sequential assessment of NTBI is lacking. It remains to be determined whether NTBI at the fixed time points (for example, at 1-year follow-up after HCT) predicts future iron overload-related complications better than ferritin by sequential assessment of NTBI and organ damages. Further investigations with LIC, serum ferritin and with labile plasma iron (LPI), which is pathologically relevant component of NTBI [20, 22], are needed to establish the assay and clinical significance of NTBI.

In conclusion, our study demonstrates that iron overload is a common complication in survivors after allogeneic HCT. Serum ferritin was well correlated with NTBI and was demonstrated to be a good surrogate marker of iron overload even after allogeneic HCT. Iron overload was shown to be a significant risk factor for liver dysfunction and diabetes, and was related to a lesser extent with performance status. Further evaluations are warranted to better understand the impact of iron overload on late morbidity and mortality and the benefit of iron chelating therapy or phlebotomy for patients who suffer from iron overload.

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