transplantation procedures according to the time period are shown in Table 1. The overall proportions of AML, ALL and MDS were 53%, 34% and 13%, respectively. A total of 1354, 2292 and 2855 allo-HCTs were performed in 1997–2000, 2001–2004 and 2005–2008, respectively. The number and proportion of patients aged 50–70 years (1997–2000, $n=123$, 9%; 2001–2004, $n=617$, 27%; 2005–2008, $n=1054$, 37%), allo-HCT from an unrelated CB donor ($n=14$, 1%; $n=321$, 14%; $n=534$, 19%), and the use of a reduced-intensity conditioning regimen ($n=21$, 2%; $n=394$, 17%; $n=689$, 24%) increased over the three periods. Most of the myeloablative conditioning regimens (96%) consisted of high-dose CY with TBI or BU. Tacrolimus-based GVHD prophylaxis increased, especially in allo-HCT from an unrelated BM and CB donor (BM: $n=218$, 37%; $n=579$, 58%; $n=945$, 72%; CB: $n=3$, 21%; $n=99$, 31%; $n=229$, 43%).

Outcomes of allo-HCT over the three periods

The incidence of NRM of the entire 6501 patients was 23% at 3 years after allo-HCT (Figure 1a). Overall, 265 patients died of acute or chronic GVHD (median OS: 143 days, range: 18–3360), 497 died of infection (median OS: 116 days, range: 0–3184) and 500 died of organ failure (median OS: 145 days, range: 0–4013).

Older patients had a significantly higher incidence of NRM than younger patients (31% vs 20%, $P<0.001$, Figure 1b). The donor source significantly affected the incidence of NRM, and unrelated CB had the highest risk of NRM (related, 17%; unrelated BM, 25%; unrelated CB, 31%, $P<0.001$, Figure 1c). In a comparison of the outcome after allo-HCT among the three time periods in the overall 6501 patients (Figure 2), there were no linear improvements in NRM and OS over the three periods (NRM: 23%, 25% and 21%; OS: 61%, 57% and 60% at 3 years after allo-HCT). By the multivariate analysis that adjusted for disease type, patient age, patient gender, donor source and conditioning regimens, in younger patients (Table 2), the hazard ratios (HRs) for NRM in 2001–2004 and 2005–2008 compared with 1997–2000 were 0.78 (95% CI 0.65–0.93, $P=0.005$) and 0.64 (95% CI 0.54–0.78, $P<0.001$), respectively. The HR for overall mortality in 2005–2008 was significantly lower than that in 1997–2000 (HR 0.81, 95% CI 0.70–0.93, $P=0.004$). The HRs for relapse did not differ significantly among the periods. In older patients, the HRs for NRM and overall mortality in 2005–2008 compared with 2001–2004 were 0.56 (95% CI 0.46–0.68, $P<0.001$) and 0.66 (95% CI 0.47–0.93, $P=0.017$), respectively. However, the HR for relapse in 2005–2008 significantly increased (HR 1.53, 95% CI 1.20–1.97, $P=0.001$).

Allo-HCT from an HLA-matched or 1-Ag-mismatched related donor

In younger patients who received allo-HCT from a related donor (Figure 3a), the incidence of NRM remained rather low throughout the 12 years. Although NRM and OS slightly improved in 2005–2008, the differences were not statistically significant.

In older patients who received allo-HCT from a related donor, NRM was significantly reduced in 2005–2008 compared with 2001–2004 (Figure 3b, HR 0.62, 95% CI 0.44–0.88, $P=0.007$, Table 2). The incidences of death associated with organ failure and GVHD were significantly reduced in 2005–2008 (organ failure, 11 and 6%, $P=0.007$; GVHD, 6 and 3%, $P=0.015$, Figure 4a). In contrast, a significant increase in relapse was observed in 2005–2008 compared with 2001–2004 (21 and 36%, $P<0.001$, data not shown), and the same result was also shown by a multivariate analysis (HR 1.97, 95% CI 1.38–2.81, $P<0.001$, Table 2). This result remained the same when the analyses were restricted to HCT using reduced-intensity regimens or myeloablative regimens. Consequently, the improvement in OS in 2005–2008 was not statistically significant (Figure 3b and Table 2).

Allo-HCT from an unrelated BM donor

A significant reduction in NRM was seen over the three periods among younger patients who received allo-HCT from an unrelated BM donor (Figure 3c), with the HRs of 0.69 (95% CI 0.55–0.88, $P=0.003$) and 0.61 (95% CI 0.47–0.78, $P<0.001$) in 2001–2004 and 2005–2008, respectively (Table 2). The incidences of death associated with GVHD and organ failure were significantly reduced over the three periods (GVHD, 7, 4 and 4%, $P=0.011$; organ failure, 12, 10 and 8%, $P=0.002$, Figure 4b). OS significantly improved in 2005–2008 (Figure 3c and Table 2).

In older patients who received allo-HCT from an unrelated BM donor, NRM and OS significantly improved in 2005–2008 compared with 2001–2004 (Figure 3d). The HR for NRM in 2005–2008 was 0.58 (95% CI 0.41–0.82, $P=0.002$). The incidences of death associated with infection and organ failure were reduced in 2005–2008 (infection, 14 and 10%, $P=0.054$; organ failure,

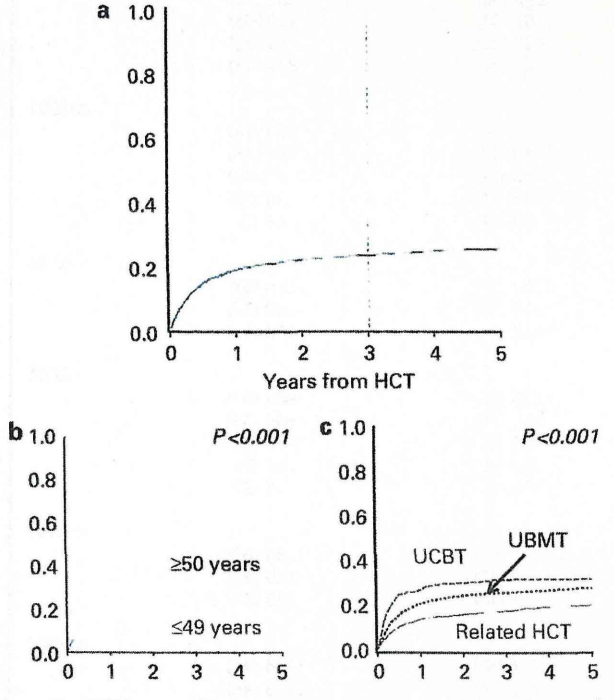

Figure 1. NRM over the past 12 years among 6501 patients who received allo-HCT in remission is shown in (**a**). NRM according to age (**b**) and donor source (**c**) are also shown.

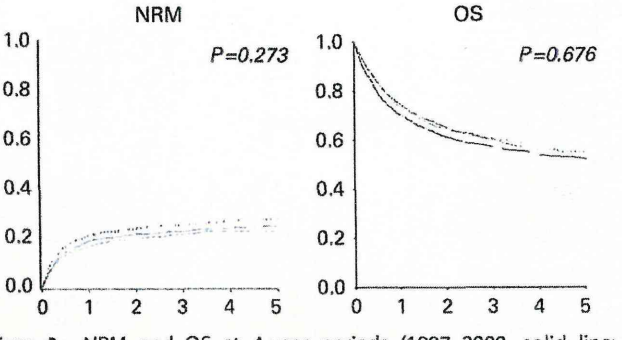

Figure 2. NRM and OS at 4-year periods (1997–2000, solid line; 2001–2004, dotted line; 2005–2008, dashed line) in the overall patients.

Table 2. Multivariate analyses for NRM, relapse and overall mortality after allo-HCT among the three periods

	All patients N = *6501*			*Related HCT* N = *2542*			*UBMT* N = *2898*			*UCBT* N = *869*		
	HR	*95% CI*	*P value*	*HR*	*95% CI*	*P value*	*HR*	*95% CI*	*P value*	*HR*	*95% CI*	*P value*
		N = 4707			*N* = 1846			*N* = 2202			*N* = 518	
Patient age at transplant, 16–49 years												
NRM												
1997–2000	1.00			1.00			1.00					
2001–2004	0.78	(0.65–0.93)	0.005	1.00	(0.75–1.33)	0.980	0.69	(0.55–0.88)	0.003	1.00		
2005–2008	0.64	(0.54–0.78)	<0.001	0.62	(0.44–0.88)	0.007	0.61	(0.47–0.78)	<0.001	1.04	(0.72–1.51)	0.830
Relapse												
1997–2000	1.00			1.00			1.00					
2001–2004	1.16	(0.98–1.37)	0.094	0.95	(0.74–1.21)	0.650	1.39	(1.39–1.06)	0.019	1.00		
2005–2008	1.12	(0.94–1.34)	0.220	1.20	(0.94–1.52)	0.150	1.20	(0.89–1.61)	0.240	0.66	(0.43–1.00)	0.049
Overall mortality												
1997–2000	1.00			1.00			1.00					
2001–2004	0.94	(0.82–1.06)	0.310	1.00	(0.82–1.22)	0.990	0.88	(0.73–1.06)	0.188	1.00		
2005–2008	0.81	(0.70–0.93)	0.004	0.89	(0.71–1.11)	0.285	0.77	(0.62–0.94)	0.010	0.84	(0.57–1.23)	0.373
		N = 1794			*N* = 696			*N* = 696			*N* = 351	
Patient age at transplant, 50–70 years												
NRM												
2001–2004	1.00			1.00			1.00			1.00		
2005–2008	0.56	(0.46–0.68)	<0.001	0.49	(0.33–0.71)	<0.001	0.58	(0.41–0.82)	0.002	0.57	(0.40–0.83)	0.003
Relapse												
2001–2004	1.00			1.00			1.00			1.00		
2005–2008	1.53	(1.20–1.97)	0.001	1.97	(1.38–2.81)	<0.001	1.46	(0.93–2.28)	0.100	0.96	(0.59–1.58)	0.880
Overall mortality												
2001–2004	1.00			1.00			1.00			1.00		
2005–2008	0.66	(0.47–0.93)	0.017	0.87	(0.67–1.15)	0.334	0.82	(0.61–1.09)	0.169	0.67	(0.49–0.91)	0.010

Abbreviations: CI = confidence interval; HCT = hematopoietic cell transplantation; HR = hazard ratio; NRM = non-relapse mortality; UBMT = unrelated BM transplantation; UCBT = unrelated cord blood transplantation. Year of allo-HCT (1997–2000 vs 2001–2004 or 2005–2008 among younger patients, 2001–2004 vs 2005–2008 among older patients or those who received UCBT), disease type (AML vs ALL or MDS), patient age (16–29 years vs 30–39 or 40–49 among younger patients, 50–59 vs 60–70 among older patients), patient gender (male vs female), donor source (HLA-matched sibling vs other family donors, HLA-matched unrelated BM, mismatched unrelated BM or unrelated CB), and conditioning regimens (myeloablative vs reduced-intensity) were considered as covariates. In the analysis for related HCT, donor source (HLA-matched sibling vs other family donors), year of allo-HCT, disease type, patient age, patient gender and conditioning regimens were considered as covariates. In the analysis for UBMT, donor source (HLA-matched BM vs mismatched unrelated BM), year of allo-HCT, disease type, patient age, patient gender and conditioning regimens were considered as covariates. In the analysis for UCBT, year of allo-HCT, disease type, patient age, patient gender and conditioning regimens were considered.

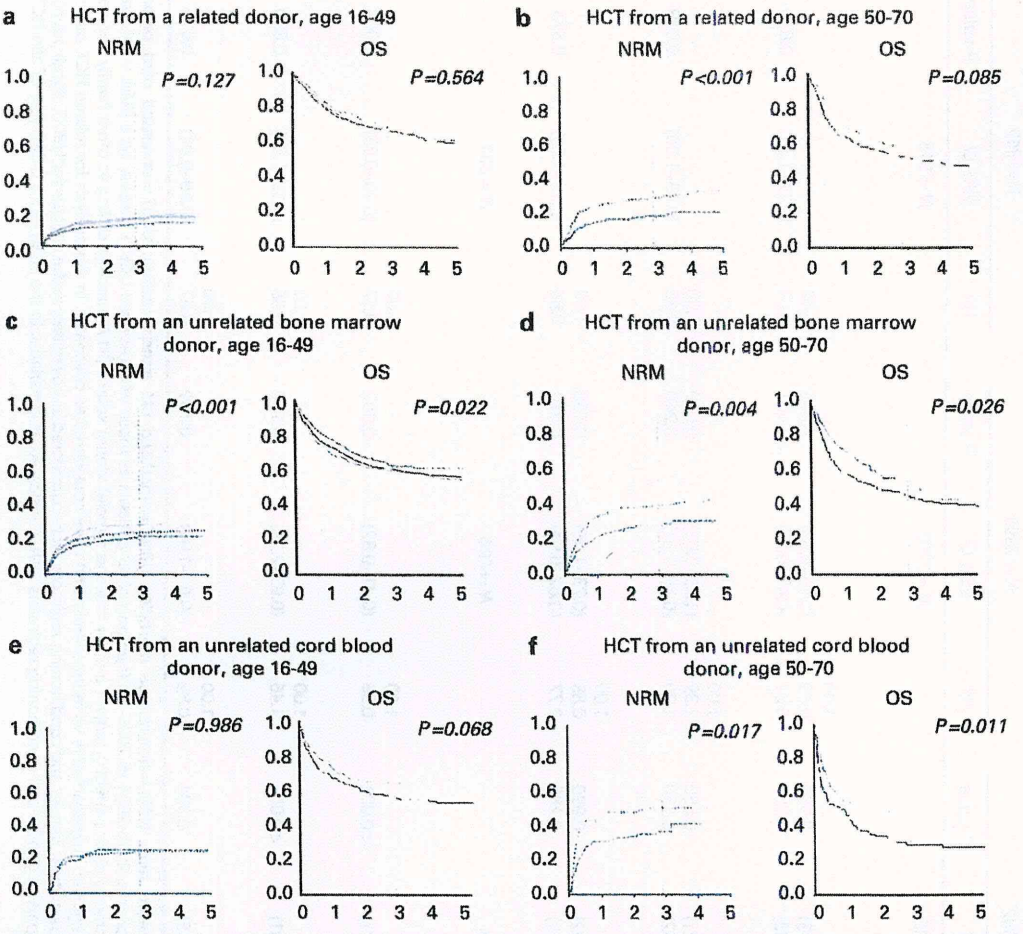

Figure 3. (**a**) NRM and OS at 3 years from HCT among younger patients (16–49 years) who received allo-HCT from a related donor were 15%, 16% and 12% ($P=0.127$), and 67%, 66% and 68% ($P=0.564$), respectively in the period of 1997–2000 ($n=587$, solid line), 2001–2004 ($n=620$, dotted line) and 2005-2008 ($n=639$, dashed line). (**b**) NRM and OS among older patients (50–70 years) who received related donor transplantation were 28% and 17% ($P<0.001$) and 52% and 57% ($P=0.085$), respectively in the period of 2001–2004 ($n=293$, dotted line) and 2005–2008 ($n=321$, dashed line). (**c**) NRM and OS among younger patients who received allo-HCT from an unrelated BM donor were 28%, 24% and 22% ($P<0.001$), and 60%, 60% and 63% ($P=0.022$), respectively in the period of 1997–2000 ($n=560$, solid line), 2001–2004 ($n=803$, dotted line) and 2005-2008 ($n=839$, dashed line). (**d**) NRM and OS among older patients who received allo-HCT from an unrelated BM donor were 39% and 27% ($P=0.004$) and 45% and 54% ($P=0.026$), respectively in the period of 2001–2004 ($n=195$, dotted line) and 2005–2008 ($n=473$, dashed line). (**e**) Non-relapse mortality and OS among younger patients who received allogeneic hematopoietic cell transplantation from an unrelated cord blood donor were 25% and 25% ($P=0.986$), and 55% and 65% ($P=0.068$), respectively in the period 2001–2004 ($n=214$, dotted line) and 2005–2008 ($n=292$, dashed line). (**f**) Non-relapse mortality and OS among older patients who received allogeneic hematopoietic cell transplantation from an unrelated cord blood donor were 51% and 37% ($P=0.017$), and 29% and 44% ($P=0.011$), respectively in the period of 2001–2004 ($n=107$, dotted line) and 2005–2008 ($n=242$, dashed line).

14 and 8%, $P=0.049$, Figure 4c). We found a significant reduction in mortality rates associated with bacterial and fungal infection.

Allo-HCT from an unrelated CB donor

In younger patients who received allo-HCT from an unrelated CB donor, there was no significant difference in the incidence of NRM between the two periods (Figure 3e). In this group, there was a marked reduction in the relapse rate (25 and 18%, $P=0.018$, data not shown; HR 0.66, 95% CI 0.43–1.00, $P=0.049$, Table 2). OS was better in 2005–2008; however, the difference was not statistically significant.

Significant improvements in NRM and OS were observed in 2005–2008 among older patients who received UCBT (Figure 3f). The HRs for NRM and overall mortality in 2005–2008 were 0.57 (95% CI 0.40–0.83, $P=0.003$) and 0.67 (95% CI 0.49–0.91, $P=0.010$), respectively. Reductions in the incidences of death associated with GVHD and infection seemed to contribute to the improvements in NRM (GVHD, 7 and 3%, $P=0.163$; infection, 23 and 13%, $P=0.136$). The mortality rate due to bacterial infection was significantly reduced.

Incidence of and mortality after severe acute GVHD

In subgroups that showed a significant reduction in the incidence of NRM, younger patients who received UBMT, older patients who received related HCT and older patients who received UCBT showed significant reductions in the incidence of GVHD-related mortality. In younger patients who received UBMT, the incidence of severe acute GVHD was significantly reduced over the three

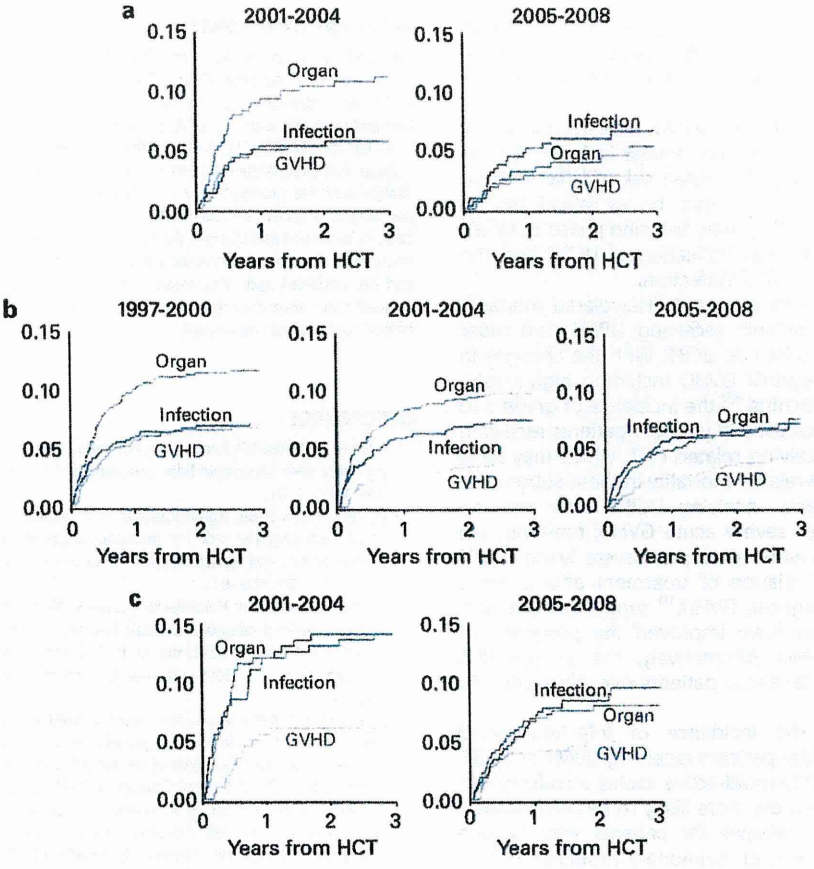

Figure 4. Change in the causes of NRM among different time periods is shown. Cumulative incidences of death due to GVHD, infection and organ failure are separately presented in each time period. **(a)** In older patients who received allo-HCT from a related donor, the incidences of death associated with organ failure and GVHD were significantly reduced in 2005–2008 (organ failure, 11 and 6%, $P=0.007$; GVHD, 6 and 3%, $P=0.015$). **(b)** In younger patients who received allo-HCT from an unrelated BM donor, the incidences of death associated with GVHD and organ failure were significantly reduced (GVHD, 7, 4 and 4%, $P=0.011$; organ failure, 12, 10 and 8%, $P=0.002$). **(c)** In older patients who received allo-HCT from an unrelated BM donor, the incidences of death associated with infection and organ failure were reduced in 2005–2008 (infection, 14 and 10%, $P=0.054$; organ failure, 14 and 8%, $P=0.049$).

periods (16, 15 and 12% at 100 days after allo-HCT, $P=0.021$). In older patients who received related HCT, the incidence of severe acute GVHD was reduced in 2005–2008 relative to 2001–2004, but this difference was not statistically significant (14 and 10%, $P=0.099$). In older patients who received UCBT, there was no remarkable reduction in the incidence of severe acute GVHD in the later period (18 and 16%, $P=0.542$). However, the mortality rate was significantly reduced among older patients who suffered severe acute GVHD after UCBT (92 and 67% at 3 years after allo-HCT, $P=0.022$).

DISCUSSION

In this study that used a large database of 6501 patients, we found that the incidence of NRM after allo-HCT for adult patients has significantly decreased over the past 12 years, which has led to an improvement of OS. As prior studies have primarily focused on the changes in NRM among younger patients who received allo-HCT with myeloablative conditioning,[2,4] this is the first study to show the changes in NRM in subgroups comprising older patients and UCBT.

We found that demographic, disease and transplantation characteristics have been changing, as previous studies reported.[1,2,4] The marked increase in the number of older patients, allo-HCT with reduced-intensity conditioning and UCBT might reflect an increase in allo-HCT for 'more vulnerable' patients. Gooley *et al.*[1] reported that the hematopoietic cell transplantation-specific comorbidity index (HCT-CI)[7] scores were higher in HCT recipients in more recent time periods. Unfortunately, we were not able to evaluate HCT-CI in the current study because of a lack of information.

Among patients who received related HCT, remarkable improvement in NRM was observed in older patients. Another distinguishing finding was an increase in relapse in overall older patients, especially among those who received related HCT in remission. There was no recent shift in the use of allo-HCT in a later remission state, and we obtained a similar result when the analyses were restricted to HCT using reduced-intensity regimens or myeloablative regimens. In addition, the proportional use of anti-thymocyte globulin has remained unchanged over the periods. Less use of PB donors and more aggressive selection of older patients as indicated for allo-HCT may have affected the result. Despite this increase in relapse, older patients who received HCT in remission showed, by multivariate analyses, a significant reduction in mortality with a remarkable reduction in HRs for NRM irrespective of donor sources.

In analyses based on the donor source, UBMT showed remarkable improvements in NRM and OS throughout the age subgroups. Along with high-resolution donor–recipient HLA

matching,[8,9] the lesser proportion of donor/patient pairs with allele mismatches may have reduced the incidence of GVHD-related mortality, and contributed to the improvement in outcomes after UBMT.

Among patients who received UCBT, we found a decreased risk of relapse in younger patients with no change in NRM. On the other hand, older patients had a decreased risk of NRM with no change in relapse. These outcomes may be explained by the changes in clinical practice in 2001–2004, 'learning phase' of UCBT, and that after 2005, including the indication of UCBT and the prophylaxis and treatment for GVHD/infection.

A recent reduction in the incidence of GVHD-related mortality was observed in younger patients receiving UBMT and older patients receiving related allo-HCT or UCBT. With the changes in prophylaxis and treatment against GVHD including high-resolution donor–recipient HLA matching,[8,9] the incidence of grade 3 to 4 severe acute GVHD has decreased in younger patients receiving UBMT and older patients receiving related HCT, which may have led to the reduction in GVHD-related mortality in these subgroups. Interestingly, in older patients receiving UCBT, there was no reduction in the incidence of severe acute GVHD; however, the mortality rate among those who developed severe acute GVHD was reduced. The prompt initiation of treatment after a more thorough examination to diagnose GVHD,[10] supportive care and nutritional management may have improved the prognosis of those who had severe GVHD. Alternatively, the unique HLA epidemiological genetics of Japanese patients may have affected the results.[11,12]

A recent reduction in the incidence of infection-related mortality was observed in older patients receiving UBMT or UCBT. New antifungal drugs, including mold-active azoles, micafungin or liposomal amphotericin B, are now more likely to be administered as empiric or preemptive strategies for patients who have a positive galactomannan Ag test or pulmonary nodules.[1,13,14] As GVHD and infection have been reported to be associated with each other's development and exacerbation,[13,15–18] an improved control of severe GVHD may have led to the reduction of the risk of infection-related mortality.[13,14]

We included all of the organ toxicities that were documented after allo-HCT as the cause of organ failure-related mortality, including conditioning regimen-related toxicity,[19,20] lung injury[15] and late effects on any organs.[21] We observed a reduction in the incidence of organ failure-related mortality in older patients receiving related HCT and those who received UBMT. In the future, more detailed analyses are warranted based on each specific organ toxicity.

As this analysis is based on a retrospectively collected multicenter database, our results may be susceptible to the disadvantages of any retrospective study, such as the heterogeneity in the treatment strategies chosen at the discretion of the physicians. Because of the nature of the multicenter registry, detailed data were not available regarding the incidences of infection and specific organ failure, and prophylactic treatment toward infection. Although we acknowledge this limitation, the results obtained from this large database that contains clinical data on over 6000 patients should provide valuable information. In addition, for the first time, we found reductions in NRM in subgroups consisting of older patients and those who received UCBT. We also showed the causes of death that contributed to the reduction of NRM in each donor/age subgroup. By further evaluating the risks of NRM and relapse in each demographic subgroup, we would be able to more clearly define the indications for allo-HCT, and tailor the strategy for individual patients.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

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Author contributions: SK designed the study, prepared the data file, performed the analysis, interpreted the data and wrote the manuscript; KY contributed to the study design, data file preparation, data analysis and interpretation of the data; TY was primarily responsible for the study design, data analysis and interpretation of the data; YA reviewed and cleaned the data, interpreted the data and helped to write the manuscript; TNI reviewed, cleaned and interpreted the data, HA, ST, KM, ST, TE, HO and MK obtained and interpreted the data; JT, KK, KK, RS, YM and HS reviewed, cleaned and interpreted the data; TF designed the study, interpreted the data and helped to write the manuscript.

REFERENCES

1 Gooley TA, Chien JW, Pergam SA, Hingorani S, Sorror ML, Boeckh M *et al.* Reduced mortality after allogeneic hematopoietic-cell transplantation. *N Engl J Med* 2010; **363**: 2091–2101.
2 Horan JT, Logan BR, Agovi-Johnson MA, Lazarus HM, Bacigalupo AA, Ballen KK *et al.* Reducing the risk for transplantation-related mortality after allogeneic hematopoietic cell transplantation: how much progress has been made? *J Clin Oncol* 2011; **29**: 805–813.
3 Giebel S, Labopin M, Holowiecki J, Labar B, Komarnicki M, Koza V *et al.* Outcome of HLA-matched related allogeneic hematopoietic stem cell transplantation for patients with acute leukemia in first complete remission treated in Eastern European centers. Better results in recent years. *Ann Hematol* 2009; **88**: 1005–1013.
4 Gratwohl A, Brand R, Frassoni F, Rocha V, Niederwieser D, Reusser P *et al.* Cause of death after allogeneic haematopoietic stem cell transplantation (HSCT) in early leukaemias: an EBMT analysis of lethal infectious complications and changes over calendar time. *Bone Marrow Transplant* 2005; **36**: 757–769.
5 Atsuta Y, Suzuki R, Yoshimi A, Gondo H, Tanaka J, Hiraoka A *et al.* Unification of hematopoietic stem cell transplantation registries in Japan and establishment of the TRUMP System. *Int J Hematol* 2007; **86**: 269–274.
6 Giralt S, Ballen K, Rizzo D, Bacigalupo A, Horowitz M, Pasquini M *et al.* Reduced-intensity conditioning regimen workshop: defining the dose spectrum. Report of a workshop convened by the center for international blood and marrow transplant research. *Biol Blood Marrow Transplant* 2009; **15**: 367–369.
7 Sorror ML, Maris MB, Storb R, Baron F, Sandmaier BM, Maloney DG *et al.* Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. *Blood* 2005; **106**: 2912–2919.
8 Flomenberg N, Baxter-Lowe LA, Confer D, Fernandez-Vina M, Filipovich A, Horowitz M *et al.* Impact of HLA class I and class II high-resolution matching on outcomes of unrelated donor bone marrow transplantation: HLA-C mismatching is associated with a strong adverse effect on transplantation outcome. *Blood* 2004; **104**: 1923–1930.
9 Lee SJ, Klein J, Haagenson M, Baxter-Lowe LA, Confer DL, Eapen M *et al.* High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation. *Blood* 2007; **110**: 4576–4583.
10 Martin PJ, McDonald GB, Sanders JE, Anasetti C, Appelbaum FR, Deeg HJ *et al.* Increasingly frequent diagnosis of acute gastrointestinal graft-versus-host disease after allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant* 2004; **10**: 320–327.
11 Oh H, Loberiza Jr FR, Zhang MJ, Ringden O, Akiyama H, Asai T *et al.* Comparison of graft-versus-host-disease and survival after HLA-identical sibling bone marrow transplantation in ethnic populations. *Blood* 2005; **105**: 1408–1416.
12 Hahn T, McCarthy Jr PL, Zhang MJ, Wang D, Arora M, Frangoul H *et al.* Risk factors for acute graft-versus-host disease after human leukocyte antigen-identical sibling transplants for adults with leukemia. *J Clin Oncol* 2008; **26**: 5728–5734.
13 Upton A, Kirby KA, Carpenter P, Boeckh M, Marr KA. Invasive aspergillosis following hematopoietic cell transplantation: outcomes and prognostic factors associated with mortality. *Clin Infect Dis* 2007; **44**: 531–540.
14 Yokoe D, Casper C, Dubberke E, Lee G, Munoz P, Palmore T *et al.* Infection prevention and control in health-care facilities in which hematopoietic cell transplant recipients are treated. *Bone Marrow Transplant* 2009; **44**: 495–507.
15 Fukuda T, Boeckh M, Carter RA, Sandmaier BM, Maris MB, Maloney DG *et al.* Risks and outcomes of invasive fungal infections in recipients of allogeneic hematopoietic stem cell transplants after nonmyeloablative conditioning. *Blood* 2003; **102**: 827–833.
16 Marr KA, Seidel K, Slavin MA, Bowden RA, Schoch HG, Flowers ME *et al.* Prolonged fluconazole prophylaxis is associated with persistent protection against

candidiasis-related death in allogeneic marrow transplant recipients: long-term follow-up of a randomized, placebo-controlled trial. *Blood* 2000; **96**: 2055–2061.
17 Paulin T, Ringden O, Nilsson B. Immunological recovery after bone marrow transplantation: role of age, graft-versus-host disease, prednisolone treatment and infections. *Bone Marrow Transplant* 1987; **1**: 317–328.
18 Sayer HG, Longton G, Bowden R, Pepe M, Storb R. Increased risk of infection in marrow transplant patients receiving methylprednisolone for graft-versus-host disease prevention. *Blood* 1994; **84**: 1328–1332.
19 Barrett AJ. Conditioning regimens for allogeneic stem cell transplants. *Curr Opin Hematol* 2000; **7**: 339–342.
20 Feinstein L, Storb R. Reducing transplant toxicity. *Curr Opin Hematol* 2001; **8**: 342–348.
21 Abou-Mourad YR, Lau BC, Barnett MJ, Forrest DL, Hogge DE, Nantel SH *et al*. Long-term outcome after allo-SCT: close follow-up on a large cohort treated with myeloablative regimens. *Bone Marrow Transplant* 2010; **45**: 295–302.

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ORIGINAL ARTICLE

Unrelated cord blood transplantation vs related transplantation with HLA 1-antigen mismatch in the graft-versus-host direction

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Little information is available regarding whether an unrelated cord blood (UCB) unit or a related donor with a 1-antigen mismatch at the HLA-A, HLA-B or HLA-DR locus in the graft-versus-host direction (RD/1AG-MM-GVH) should be selected as an alternative donor for patients without an HLA-matched related/unrelated donor. Therefore, we conducted a retrospective study using national registry data on patients with leukemia or myelodysplastic syndrome who received transplantation using a single UCB ($n = 2288$) unit or an RD/1AG-MM-GVH ($n = 525$). We found that the survival rate in the UCB group was comparable to that in the RD/1AG-MM-GVH group, although the RD/1AG-MM-GVH group with an HLA-B mismatch showed significantly higher overall and non-relapse mortality. Neutrophil and platelet engraftment were significantly faster, whereas the incidence of acute or chronic graft-versus-host disease (GVHD) was significantly higher in the RD/1AG-MM-GVH group. The incidence of acute or chronic GVHD in the RD/1AG-MM-GVH group with *in vivo* T-cell depletion was comparable to that in the UCB group, which translated into a trend toward better overall survival, regardless of the presence of an HLA-B mismatch. In conclusion, UCB and RD/1AG-MM-GVH are comparable for use as an alternative donor, except for RD/1AG-MM-GVH involving an HLA-B mismatch.

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Keywords: cord blood transplantation; related transplantation; HLA mismatch; alternative donor

INTRODUCTION

For patients who lack an HLA-identical sibling, an HLA-matched unrelated donor (MUD) is considered to be the preferred alternative donor in allogeneic hematopoietic cell transplantation (HCT).[1–5] However, it is difficult to find an MUD for patients with rare HLA haplotypes. Furthermore, it takes at least a few months from the start of an unrelated donor search to actually receive a graft. Therefore, there is a large demand for an alternative source to an HLA-identical sibling or MUD, particularly for patients who have a rare haplotype or who need immediate transplantation.

Unrelated cord blood (UCB) has emerged as a promising alternative source for pediatric and adult patients.[6–17] In UCB transplantation, up to two antigen/allele mismatches between a recipient and cord blood unit are acceptable without an increased risk of acute graft-versus-host disease (GVHD). The clinical outcome in UCB transplantation is improving, and is almost comparable to that in HLA 8/8 allele MUD transplantation, although a high risk of graft failure and early treatment-related complications are still major issues.[15–17]

Another alternative source is an HLA-mismatched related donor, particularly when a related donor with a 1-antigen mismatch at the HLA-A, HLA-B, or HLA-DR locus in the graft-versus-host (GVH) direction (RD/1AG-MM-GVH) is available. HCT from an RD/1AG-MM-GVH results in a higher but acceptable incidence of acute GVHD.[18–20] In previous studies, HLA mismatches in the host-versus-graft (HVG) direction were associated with a higher incidence of graft failure and lower overall survival (OS).[18,19,21] However, the risk of graft failure might have been improved by the use of conditioning regimens that strongly suppress the recipient's immune system.[22] Therefore, in current clinical practice in Japan, stem cell transplantation from an RD/1AG-MM-GVH is being performed while accepting multiple antigen mismatches in the HVG direction without specific *ex vivo* stem cell manipulation.[18,19,23] We have recently reported that OS in transplantation from an RD/1AG-MM-GVH involving an HLA-B antigen mismatch was inferior, whereas that from an RD/1AG-MM-GVH involving an HLA-A or -DR antigen mismatch was comparable to that from an 8/8-MUD in standard-risk diseases.[23]

Unlike transplantation from an MUD, transplantation using a UCB unit or an RD/1AG-MM-GVH can be performed immediately when necessary. However, little information is available regarding the priority in selecting these alternative donors. Therefore, we conducted a retrospective study using national registry data on 2813 patients with leukemia or myelodysplastic syndrome (MDS)

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who received transplantation using a single UCB or an RD/1AG-MM-GVH.

MATERIALS AND METHODS

Data collection

Data for patients (age: ⩾16 years) with acute myeloid leukemia, acute lymphoblastic leukemia, MDS and chronic myelogenous leukemia who received a first HCT using a single HLA 0–2 antigen-mismatched UCB unit or an RD/1AG-MM-GVH between 1 January 1998 and 31 December 2009 were obtained from the Transplant Registry Unified Management Program (TRUMP),[24] which includes data from the Japan Cord Blood Bank Network (JCBBN) and the Japan Society for Hematopoietic Cell Transplantation (JSHCT). Our analysis included 2306 patients who received a single UCB graft (UCB group) and 541 patients who received a graft from an RD/1AG-MM-GVH (RD/1AG-MM-GVH group). As of January 2012, double UCB grafts for HCT are not available in Japan. The following patients were excluded: 26 patients who lacked data on survival status, survival date, sex of recipient, or GVHD prophylaxis and 8 patients who received stem cells that had been manipulated by *ex vivo* T-cell depletion or CD34 selection. Overall, 2288 patients who received a UCB unit and 525 who received a graft from an RD/1AG-MM-GVH fulfilled the criteria. The study was approved by the data management committees of TRUMP and by the institutional review boards of Japanese Red Cross Nagoya First Hospital and Saitama Medical Center, Jichi Medical University, where this study was organized.

Histocompatibility

Histocompatibility data for the HLA-A, HLA-B and HLA-DR loci were obtained from reports from the institution where the transplantation was performed or from cord blood banks. To reflect current practice in Japan, HLA matching in UCB or RD/1AG-MM-GVH transplantation was assessed by serological data for HLA-A, HLA-B, and HLA-DR loci. An HLA mismatch in the GVH direction was defined as when the recipient's antigens or alleles were not shared by the donor, whereas a mismatch in the HVG direction was defined as when the donor's antigens or alleles were not shared by the recipient.

End points

The primary end point of the study was to compare OS rates between the UCB and RD/1AG-MM-GVH groups. Other end points were the cumulative incidences of neutrophil and platelet engraftment, acute and chronic GVHD, relapse, and non-relapse mortality (NRM). Neutrophil recovery was considered to have occurred when the absolute neutrophil count exceeded 0.5×10^9/l for 3 consecutive days following transplantation. Platelet recovery was considered to have occurred when the absolute platelet count exceeded 50×10^9/l without platelet transfusion. The physicians who performed transplantation at each center diagnosed and graded acute and chronic GVHD according to the traditional criteria.[25,26] The incidence of chronic GVHD was evaluated in patients who survived for at least 100 days.

Statistical analysis

Descriptive statistics were used to summarize variables related to the patient characteristics. Comparisons between groups were performed with the χ^2-test or extended Fisher's exact test as appropriate for categorical variables and the Mann–Whitney *U*-test for continuous variables. The probability of OS was estimated according to the Kaplan–Meier method, and the groups were compared with the log-rank test. The adjusted probability of OS was estimated according to the Cox proportional-hazards model, with other significant variables considered in the final multivariate model. The probabilities of neutrophil and platelet engraftment, acute and chronic GVHD, NRM, and relapse were estimated on the basis of cumulative incidence methods, and the groups were compared with the Gray test;[27,28] competing events were death without engraftment for neutrophil and platelet engraftment, death or relapse without GVHD for acute and chronic GVHD, death without relapse for relapse, and relapse for NRM. The Cox proportional-hazards model was used to evaluate variables that may affect OS, whereas the Fine and Gray proportional-hazards model was used to evaluate variables that may affect engraftment, GVHD, NRM and relapse.[29] We classified the conditioning regimen as myeloablative if either total body irradiation >8 Gy, oral busulfan ⩾9 mg/kg, intravenous busulfan ⩾7.2 mg/kg, or melphalan >140 mg/m^2 was used in the conditioning regimen, and otherwise classified it as reduced intensity, based on the report by the Center for International Blood and Marrow Transplant Research.[30] For patients for whom the doses of agents used in the conditioning regimen were not available, we used the information on conditioning intensity (myeloablative or reduced intensity) reported by the treating clinicians. Acute leukemia in the first or second remission, chronic myelogenous leukemia in the first or second chronic phase or accelerated phase, and MDS with refractory anemia or refractory anemia with ringed sideroblasts were defined as standard-risk diseases, and other conditions were defined as high-risk diseases. The following variables were considered when comparing the UCB and RD/1AG-MM-GVH groups: the recipient's age group (⩽50 years or >50 years at transplantation), sex of recipient, disease (acute myeloid leukemia, acute lymphoblastic leukemia, chronic myelogenous leukemia or MDS), disease status before transplantation (standard- or high-risk), type of conditioning regimen (myeloablative or reduced intensity), type of GVHD prophylaxis (calcineurin inhibitor and methotrexate, calcineurin inhibitor only, or other), year of transplantation (1998–2004, 2005–2009), and the time from diagnosis to transplantation (<6 months or ⩾6 months). In the analysis within the RD/1AG-MM-GVH group, the use of *in vivo* T cell depletion (no vs yes), stem cell source (peripheral blood (PB) stem cells vs bone marrow (BM)), and the number of HLA mismatches in the HVG direction (0–1 vs 2–3) were also considered. Factors without a variable of main interest were selected in a stepwise manner from the model with a variable retention criterion of $P<0.05$. We then added a variable of main interest to the final model. All tests were two-sided, and $P<0.05$ was considered to indicate statistical significance. All statistical analyses were performed with Stata version 12 (Stata Corp., College Station, TX, USA) and EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan).[31] EZR is a graphical user interface for R (The R Foundation for Statistical Computing, version 2.13.0, Vienna, Austria). More precisely, it is a modified version of R commander (version 1.6-3) that was designed to add statistical functions that are frequently used in biostatistics.

RESULTS

Characteristics of patients and transplants

Table 1 shows the patient and transplant characteristics. Recipients of an RD/1AG-MM-GVH were younger than recipients of a UCB unit. Approximately half of the recipients in the RD/1AG-MM-GVH group received PB. The number of HLA mismatches in the GVH direction between a UCB unit and recipient was 0 in 10%, 1 in 33% and 2 in 57%. In the RD/1AG-MM-GVH group, the number of antigen mismatches in the HVG direction was 0 in 12%, 1 in 68%, 2 in 18% and 3 in 3%. Most of the recipients of an RD/1AG-MM-GVH received a calcineurin inhibitor with methotrexate for GVHD prophylaxis, whereas 25% of UCB recipients received only calcineurin inhibitor. *In vivo* T-cell depletion including antithymocyte globulin (ATG) or alemtuzumab was used in 10% of the RD/1AG-MM-GVH group, but in only 1% of the UCB group. Alemtuzumab was used in only one patient, who received transplantation from an RD/1AG-MM-GVH. Information regarding the dose and type of ATG was missing in two-third of the patients who received ATG. Available data showed that the median dose of thymoglobulin was 2.5 (range 2.5–9.0, $n=9$) and 2.5 (range 1.25–5.0, $n=10$) mg/kg and the median dose of ATG-Fresenius was 8.0 (range 5.0–10.0, $n=3$) and 8.0 (range 5.0–10.0, $n=7$) mg/kg, in the UCB and RD/1AG-MM-GVH groups, respectively. Two-third of UCB transplantations were performed between 2005 and 2009. The median duration of follow-up for survivors was 2 and 4 years in the UCB and RD/1AG-MM-GVH groups, respectively.

Neutrophil and platelet engraftment

The incidence of neutrophil engraftment at day 50 in the RD/1AG-MM-GVH group was higher than that in the UCB group (UCB group, 73%, 95% confidence interval (CI), 71–75%; RD/1AG-MM-GVH group, 93%, 95% CI, 91–95%; Gray test, $P<0.001$; Figure 1a). The incidence of platelet engraftment at day 150 in the

Table 1. Patient characteristics

Variable	*UCB (n = 2288)*	*RD/1AG-MM-GVH (n = 525)*	P
Age at transplant, median (range)	49 (16–82)	43 (16–74)	<0.001
Recipient sex			
Female	1004 (44%)	239 (46%)	0.494
Male	1284 (56%)	286 (54%)	
Disease			
Acute myelogenous leukemia	1365 (60%)	269 (51%)	0.003
Acute lymphoblastic leukemia	498 (22%)	137 (26%)	
Chronic myelogenous leukemia	124 (5%)	42 (8%)	
Myelodysplastic syndrome	301 (13%)	77 (15%)	
Duration from diagnosis to transplant			
Median time (range), months	7.9 (0.2–768.5)	7.6 (0–251.7)	0.233
Disease risk			
Standard	959 (42%)	249 (47%)	0.050
High	1217 (53%)	257 (49%)	
Unknown	112 (5%)	19 (4%)	
Source of stem cells			
Bone marrow	—	251 (48%)	—
Peripheral blood	—	274 (52%)	
Cord blood	2288 (100%)	—	
HLA compatibility in the graft-versus-host direction			
Matched	225 (10%)	—	<0.001
One-antigen mismatch	753 (33%)	525 (100%)	
Two-antigen mismatch	1310 (57%)	—	
HLA compatibility in the host-versus-graft direction			
Matched	233 (10%)	62 (12%)	<0.001
One-antigen mismatch	716 (31%)	355 (68%)	
Two-antigen mismatch	1339 (59%)	94 (18%)	
Three-antigen mismatch	—	14 (3%)	
Conditioning regimen			
Myeloablative	1390 (61%)	253 (48%)	<0.001
CY + TBI ±	1062	164	
Other TBI regimen	130	20	
BU + CY ±	88	45	
Other non-TBI regimen	110	24	
Reduced intensity	894 (39%)	162 (31%)	
FLU ± TBI ±	840	138	
Other regimen	54	24	
Unclassifiable	4 (0.2%)	110 (21%)	
GVHD prophylaxis			
CSA/TAC + MTX	1410 (62%)	448 (85%)	<0.001
CSA/TAC + MMF	246 (11%)	12 (2%)	
CSA/TAC + Steroid	28 (1%)	13 (2%)	
CSA/TAC only	571 (25%)	45 (9%)	
Unknown	33 (1%)	7 (1%)	
Use of in vivo *T-cell depletion*			
No	2258 (99%)	472 (90%)	<0.001
Yes	30 (1%)	53 (10%)	
Year at transplant			
1998–2004	760 (33%)	260 (50%)	<0.001
2005–2009	1528 (67%)	265 (50%)	
Follow-up of survivors			
Median time (range), years	2.1 (0.0–10.0)	4.0 (0.1–12.2)	<0.001

Abbreviations: BU, busulfan; CSA, cyclosporine; CY, cyclophosphamide; FLU, fludarabine; MMF, mycophenolate mofetil; MTX, methotrexate; TAC, tacrolimus; TBI, total body irradiation; UCB, unrelated cord blood.

RD/1AG-MM-GVH group was also higher than that in the UCB group (UCB group, 53%, 95% CI, 51–55%; RD/1AG-MM-GVH group, 70%, 95% CI, 66–74%; Gray test, $P<0.001$; Figure 1b). The use of RD/1AG-MM-GVH was significantly associated with a higher incidence of neutrophil and platelet engraftment in the multivariate analysis (neutrophil engraftment, hazard ratio (HR), 3.46,

95% CI, 3.00–3.98, $P<0.001$; platelet engraftment, HR 2.20, 95% CI, 1.89–2.57, $P<0.001$; Supplementary Table 1). As our previous study revealed that an HLA-B mismatch had an adverse effect on OS in transplantation from an RD/1AG-MM-GVH, patients in the RD/1AG-MM-GVH group with an HLA-A, -B, or -DR mismatch were separately compared with the UCB group. We consistently observed superior neutrophil and platelet engraftment in each RD/1AG-MM-GVH group as compared with the UCB group (Supplementary Table 1).

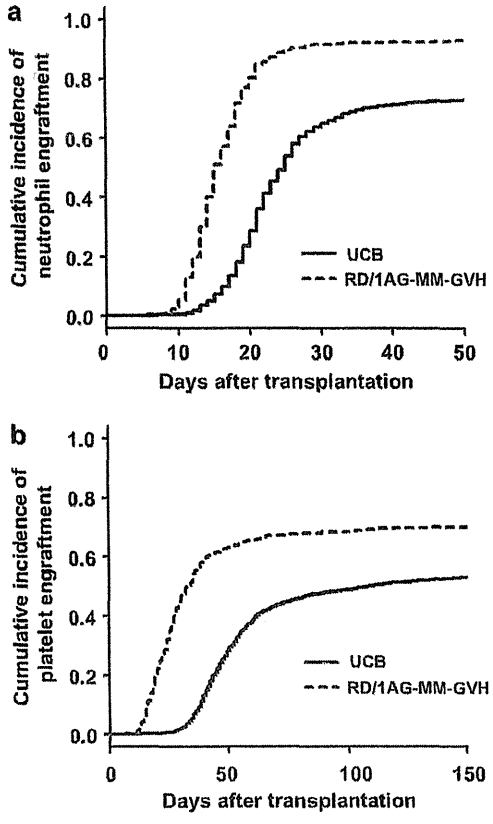

Figure 1. Neutrophil (**a**) and platelet engraftment (**b**).

Acute and chronic GVHD

The incidence of grade II–IV or grade III–IV acute GVHD in the RD/1AG-MM-GVH group was significantly higher than that in the UCB group (grade II–IV acute GVHD at day 100: UCB group, 34%, 95% CI, 32–36%; RD/1AG-MM-GVH group, 50%, 95% CI, 45–54%; Gray test, $P<0.001$; grade III–IV acute GVHD at day 100: UCB group, 11%, 95% CI, 10–13%; RD/1AG-MM-GVH group, 21%, 95% CI, 17–24%; Gray test, $P<0.001$; Figures 2a and b). The incidence of chronic GVHD or extensive type of chronic GVHD in the RD/1AG-MM-GVH group was also significantly higher than that in the UCB group (chronic GVHD at 3 years: UCB group, 25%, 95% CI, 23–27%; RD/1AG-MM-GVH group, 42%, 95% CI, 38–47%; Gray test, $P<0.001$; extensive chronic GVHD at 3 years: UCB group, 11%, 95% CI, 10–13%; RD/1AG-MM-GVH group, 29%, 95% CI, 25–34%; Gray test, $P<0.001$; Figures 2c and d). A multivariate analysis confirmed a higher risk of grade II–IV or grade III–IV acute GHVD, chronic or extensive chronic GVHD in the RD/1AG-MM-GVH group than in the UCB group (grade II–IV acute GVHD; HR 1.64, 95% CI, 1.43–1.90, grade III–IV acute GVHD; HR 2.28, 95% CI, 1.80–2.88, chronic GVHD; HR 1.47, 95% CI, 1.24–1.73, extensive chronic GVHD; HR 2.35, 95% CI, 1.90–2.91, Supplementary Table 2).

OS

The 3-year unadjusted OS rates in the UCB and RD/1AG-MM-GVH groups were 38% (36–41%) and 39% (34–43%), respectively ($P=0.115$). The use of either UCB or RD/1AG-MM-GVH was not associated with OS rates in the multivariate analysis (UCB vs RD/1AG-MM-GVH, HR, 0.99, 95% CI, 0.87–1.12, $P=0.833$) in all-risk patients, or either standard-risk ($P=0.588$) or high-risk patients ($P=0.639$; Table 2), after adjusting for the following significant risk factors: age >50 years, male recipient, acute myeloid leukemia vs MDS, high-risk disease, GVHD prophylaxis using only calcineurin inhibitor vs calcineurin inhibitor + methotrexate, and earlier year

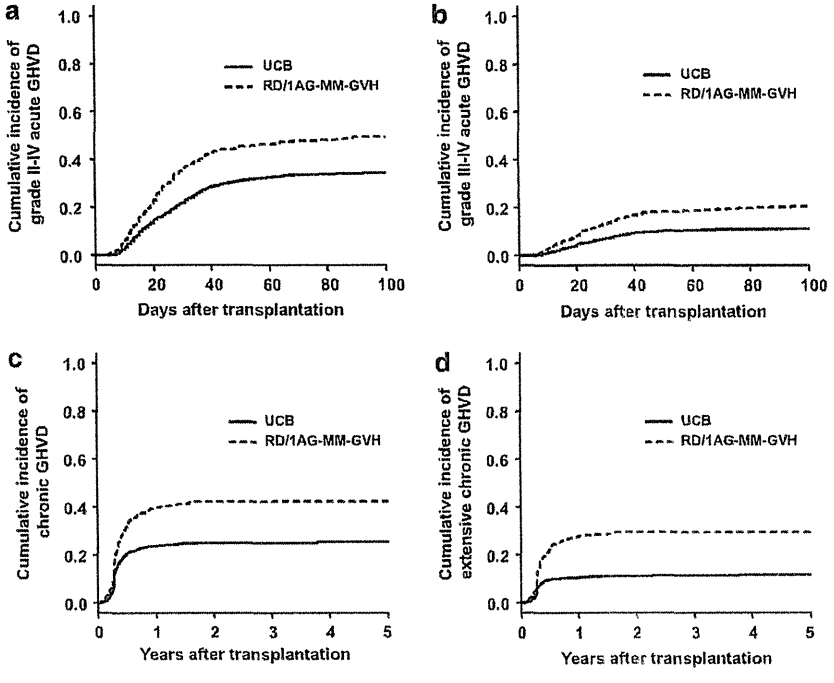

Figure 2. Acute and chronic GVHD. Cumulative incidences of grade II–IV (**a**) and grade III–IV acute GVHD (**b**) and chronic (**c**) and extensive chronic GVHD (**d**) are shown.