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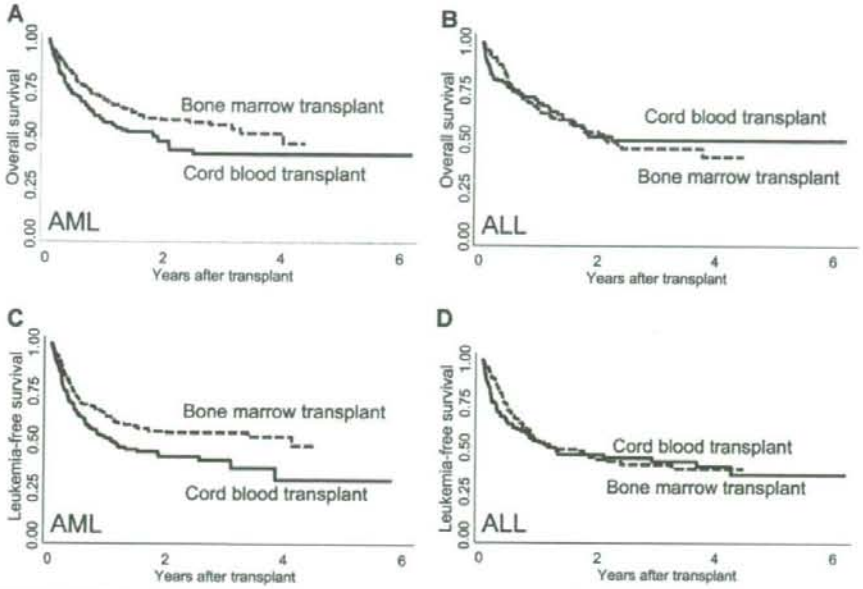

Figure 1. Adjusted OS and LFS of recipients with AML or ALL of CB or BM from unrelated donors. For patients with AML, adjusted probabilities of (A) OS (CB vs BM = 48% vs 59% at 2 years, $P = .010$) and (C) LFS (CB vs BM = 42% vs 54% at 2 years, $P = .004$) were both lower in CB recipients. For patients with ALL, the adjusted probabilities of (B) OS (CB vs BM = 52% vs 53% at 2 years, $P = .99$) and (D) LFS (CB vs BM = 46% vs 44% at 2 years, $P = .41$) were similar between CB recipients and BM recipients.

short-term methotrexate (CB vs BM = 80% vs 96% in AML patients, and CB vs BM = 80% vs 93% in ALL patients) were used preferentially in BM recipients. The median follow-up period for survivors was 1.9 years (range, 0.1-6.2 years) for CB recipients and 1.4 years (range, 0.3-4.5 years) for BM recipients.

Outcome

OS. For patients with AML, the unadjusted probabilities of OS were lower for CB recipients at 1 year (51% vs 69%) and 2 years (43% vs 60%) compared with BM recipients ($P < .001$). For patients with ALL, there were no significant differences between the 2 groups (CB vs BM = 66% vs 66% at 1 year, 49% vs 57% at 2 years, $P = .40$).

Among patients with AML, the use of CB remained a significant risk factor for overall mortality after adjustment for other factors (HR = 1.5; 95% confidence interval [CI], 1.0-2.0; $P = .028$; Table 2). However, in patients with ALL, the use of CB was not a significant factor for overall mortality on multivariate analysis (HR = 1.1; 95% CI, 0.7-1.6; $P = .78$). The adjusted probability of OS was significantly lower for CB recipients (57% vs 69% at 1 year, and 48% vs 59% at 2 years, $P = .010$; Figure 1A) compared with BM recipients for patients with AML, whereas the adjusted probability of OS was similar (69% vs 64% at 1 year, and 52% vs 53% at 2 years, $P = .99$; Figure 1B) between the groups for patients with ALL.

Results of the subgroup analyses showed that the difference in survival among AML patients was prominent in patients demonstrating 1CR at transplantation (RR = 2.9, 95% CI = 1.4-6.2, $P = .005$; Table 3).

LFS. For patients with AML, the unadjusted probabilities of LFS were significantly lower for CB recipients at 1 year (43% vs 62%) and 2 years (36% vs 54%) compared with BM recipients ($P < .001$). For patients with ALL, the unadjusted probabilities of LFS were lower with marginal significance for CB recipients at 1 year (52% vs 58%) and 2 years (45% vs 51%) compared with BM recipients ($P = .06$).

Among patients with AML, the use of CB remained as a significant risk factor for treatment failure (ie, relapse or death) after adjustment for other factors (HR = 1.5; 95% CI, 1.1-2.0; $P = .012$; Table 2). However, in patients with ALL, the use of CB was not a significant factor for treatment failure by multivariate analysis (HR = 1.2; 95% CI, 0.9-1.8; $P = .28$). The adjusted probability of LFS was significantly lower for CB recipients (51% vs 62% at 1 year, and 42% vs 54% at 2 years, $P = .004$; Figure 1C) compared with BM recipients for patients with AML, whereas the adjusted probability of LFS was similar (53% vs 53% at 1 year, and 46% vs 44% at 2 years, $P = .41$; Figure 1D) between the groups for patients with ALL.

Relapse

On univariate analyses, the cumulative incidence of relapse was higher for CB recipients with marginal significance in both AML (27% vs 20% at 1 year, and 31% vs 24% at 2 years) and ALL (27% vs 19% at 1 year, and 31% vs 24% at 2 years) ($P = .067$, and .085, respectively; Figure 2A,B).

On multivariate analyses adjusted by other factors, there was no significantly higher risk of relapse for CB recipients with either AML (RR = 1.2, 95% CI = 0.8-1.9, $P = .38$) or ALL (RR = 1.4, 95% CI = 0.8-2.4, $P = .19$; Table 2).

TRM

For patients with AML, the unadjusted cumulative incidence of TRM was significantly higher for CB recipients at 1 year (30% vs 19%) and 2 years (33% vs 22%) compared with those for BM recipients ($P = .004$; Figure 2C). For patients with ALL, the

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Table 2. Results of multivariate analysis of outcomes in 173 recipients of cord blood and 311 recipients of bone marrow with acute myeloid leukemia, and 114 recipients of cord blood and 222 recipients of bone marrow with acute lymphoblastic leukemia

Outcome	Acute myeloid leukemia		Acute lymphoblastic leukemia	
	RR (95% CI)	P	RR (95% CI)	P
Overall survival*				
BM	1.00		1.00	
CB	1.45 (1.04-2.01)	.028	1.06 (0.71-1.57)	.78
Leukemia-free survival†				
BM	1.00		1.00	
CB	1.48 (1.09-2.01)	.012	1.22 (0.85-1.76)	.28
Relapse‡				
BM	1.00		1.00	
CB	1.21 (0.79-1.87)	.38	1.42 (0.84-2.41)	.19
TRM§				
BM	1.00		1.00	
CB	1.47 (0.95-2.28)	.085	1.01 (0.59-1.73)	.98
Neutrophil recovery‖				
BM	1.00		1.00	
CB	0.41 (0.33-0.51)	< .001	0.37 (0.29-0.48)	< .001
Platelet recovery¶				
BM	1.00		1.00	
CB	0.34 (0.27-0.44)	< .001	0.43 (0.33-0.56)	< .001
Acute GVHD#				
BM	1.00		1.00	
CB	0.80 (0.56-1.15)	.23	0.61 (0.39-0.95)	.028
Chronic GVHD**				
BM	1.00		1.00	
CB	0.94 (0.63-1.42)	.79	1.08 (0.66-1.77)	.77
Chronic GVHD, extensive type††				
BM	1.00		1.00	
CB	0.36 (0.18-0.72)	.004	0.58 (0.28-1.20)	.14

RR indicates relative risk; CI, confidence interval; BM, bone marrow; CB, cord blood; and GVHD, graft-versus-host disease.

*For overall survival, other significant variables for AML were patient age more than 45 years at transplantation, more advanced disease status at conditioning, M5/M6/M7 French-American-British classification, and female donor to male recipient donor-recipient sex mismatch; other significant variables for ALL were second or after complete remission disease status, more advanced disease status, and Philadelphia chromosome abnormality.

†For leukemia-free survival, other significant variables for AML were patient age more than 45 years at transplantation, more advanced disease status at conditioning, M5/M6/M7 French-American-British classification, and female donor to male recipient donor-recipient sex mismatch; other significant variables for ALL were second or after complete remission disease status, more advanced disease status, and Philadelphia chromosome abnormality.

‡For relapse, other significant variables for AML were more advanced disease status at conditioning, donor-recipient ABO major mismatch, chromosome abnormality other than favorable abnormalities, and cyclophosphamide and total body irradiation or busulfan and cyclophosphamide conditioning regimen; other significant variables for ALL were second or after complete remission disease status, more advanced disease status, and cyclophosphamide and total body irradiation conditioning.

§For TRM, other significant variables for AML were patient age more than 45 years at transplantation, second or after complete remission disease status, more advanced disease status, and chromosome abnormality other than favorable abnormalities; other significant variables for ALL were patient age more than 45 years at transplantation, more advanced disease status at conditioning, and conditioning other than cyclophosphamide and total body irradiation.

‖For neutrophil recovery, other significant variables for AML were second or after complete remission disease status and more advanced disease status; other significant variables for ALL were more advanced disease status at conditioning and cyclosporine-based GVHD prophylaxis.

¶For platelet recovery; other significant variables for AML were second or after complete remission disease status, more advanced disease status, female donor to male recipient donor-recipient sex mismatch, and tacrolimus-based GVHD prophylaxis; other significant variables for ALL were more advanced disease status at conditioning and conditioning other than cyclophosphamide and total body irradiation.

#For acute GVHD, no other significant variables were identified for both AML and ALL.

**For chronic GVHD, other significant variables for AML were more advanced disease status and conditioning other than cyclophosphamide and total body irradiation or busulfan and cyclophosphamide; there were no other significant variables identified for ALL.

††For extensive chronic GVHD, there were no other significant variables identified for AML; another significant variable for ALL was patient male sex.

cumulative incidence of TRM was similar between the 2 groups (CB vs BM = 21% vs 23% at 1 year, 24% vs 25% at 2 years, $P = .83$; Figure 2D).

On multivariate analyses adjusted by other factors, the risk for TRM was higher for CB recipients compared with that for BM recipients among patients with AML (RR = 1.5, 95% CI = 1.0-2.3, $P = .085$; Table 2) with marginal significance. For patients with ALL, the risk for TRM was similar between CB and BM recipients (RR = 1.0, 95% CI = 0.6-1.7, $P = .98$).

Cause of death

Recurrence of the primary disease was the leading cause of death in each group (CB vs BM = 37% vs 33% in patients with AML and 36% vs 41% in patients with ALL). The following causes were infection and organ failure in all groups (Table 4).

Other outcomes of transplantation

Neutrophil and platelet recovery. The unadjusted cumulative incidence of neutrophil recovery or platelet recovery at day 100 was significantly lower in CB recipients for both AML (77% vs 94%) and ALL (80% vs 97%) compared with that among BM recipients ($P < .001$ for both). On multivariate analyses, neutrophil recovery was significantly lower among CB recipients for both AML (RR = 0.4, 95% CI = 0.3-0.5, $P < .001$) and ALL (RR = 0.4, 95% CI = 0.3-0.5, $P < .001$; Table 2).

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Table 3. Results of multivariate analysis of overall survival according to disease status at transplantation

	First complete remission			Second or after complete remission			More advanced		
Overall survival	n	RR (95% CI)	P	n	RR (95% CI)	P	n	RR (95% CI)	P
AML									
UBMT	130	1.00		82	1.00		95	1.00	
UCBT	50	2.92 (1.38-6.18)	.005	39	1.24 (0.51-3.04)	.63	81	1.29 (0.84-1.98)	.25
ALL									
UBMT	130	1.00		48	1.00		42	1.00	
UCBT	63	1.60 (0.84-3.05)	.16	21	0.62 (0.22-1.74)	.36	30	0.80 (0.38-1.69)	.57

RR indicates relative risk; CI, confidence interval; UBMT, unrelated bone marrow transplantation; and UCBT, unrelated cord blood transplantation.

The unadjusted cumulative incidence of platelet recovery greater than 50 000/μL at 4 months was significantly lower among CB recipients for both AML (59% vs 85%) and ALL (61% vs 83%) compared with that of BM recipients ($P < .001$ for both). The difference was also significant on multivariate analyses for both AML (RR = 0.3, 95% CI = 0.3-0.4, $P < .001$) and ALL (RR = 0.4, 95% CI = 0.3-0.6, $P < .001$; Table 2).

Acute GVHD. The unadjusted cumulative incidence of grade 2 to 4 acute GVHD was lower among CB recipients compared with that among BM recipients (32% vs 35% in AML, 28% vs 42% in ALL); the difference was significant in patients with ALL ($P = .39$ in AML, $P = .008$ in ALL). The difference was also significant on multivariate analyses in ALL (RR = 0.6, 95% CI = 0.4-1.0, $P = .028$). There was no significant difference in patients with AML (RR = 0.8, 95% CI = 0.6-1.2, $P = .23$; Table 2).

Chronic GVHD. The unadjusted cumulative incidence of chronic GVHD at 1 year after transplantation did not significantly differ between CB recipients and BM recipients in both AML (28% vs 32%, $P = .46$) and ALL (27% vs 30%, $P = .50$). The cumulative incidence of extensive-type chronic GVHD was significantly lower among CB recipients compared with that among BM recipients in both AML (8% vs 20%, $P < .001$) and ALL (10% vs 17%, $P = .034$). On multivariate analyses, the risk of developing chronic GVHD was similar in CB recipients and BM recipients in both AML (RR = 0.9, 95% CI = 0.6-1.4, $P = .79$) and ALL (RR = 1.1, 95% CI = 0.7-1.8, $P = .77$). The risk of developing extensive chronic GVHD was lower in CB recipients compared with BM recipients (RR = 0.4, 95% CI = 0.2-0.7, $P = .004$ in AML, and RR = 0.6, 95% CI, 0.3-1.2, $P = .14$ in ALL) and was significantly different in patients with AML (Table 2).

Discussion

The objective of our study was to investigate the outcomes of HLA-A, -B, low-resolution, and -DRB1 high-resolution 0 to 2 mismatched single-unit unrelated CBT in adult patients with acute leukemia compared with those of HLA-A, -B, -C, and -DRB1 (8 of 8) allele-matched unrelated BMT. Although AML and ALL are different diseases, previous comparisons of unrelated BMT and

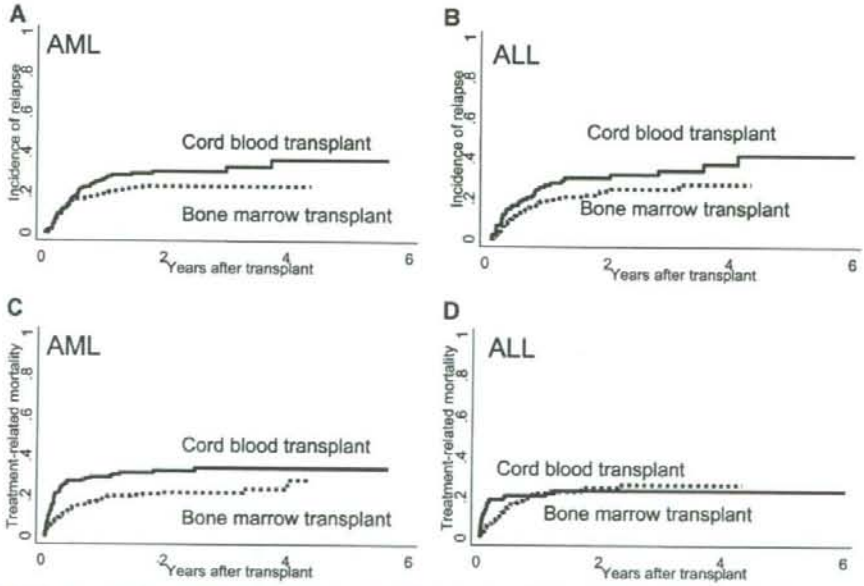

Figure 2. Cumulative incidence of relapse or TRM of recipients of CB or BM among patients with AML or ALL. For patients with AML, the cumulative incidence of (A) relapse (CB vs BM = 31% vs 24% at 2 years, $P = .068$) and (C) TRM (CB vs BM = 33% vs 22% at 2 years, $P = .004$) was higher in CB recipients. For patients with ALL, the cumulative incidence of relapse (B) was higher in CB recipients with marginal significance (CB vs BM = 31% vs 24% at 2 years, $P = .085$), but the incidence of TRM (D) was similar in CB and BM recipients (CB vs BM = 24% vs 25% at 2 years, $P = .83$).

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Table 4. Causes of death after transplantation of unrelated cord blood or unrelated bone marrow among patients with acute myeloid leukemia or acute lymphoblastic leukemia

	Acute myeloid leukemia		Acute lymphoblastic leukemia	
Cause of death	UCBT	UBMT	UCBT	UBMT
Recurrence of disease	35 (37)	34 (33)	18 (36)	34 (41)
Graft failure/rejection	3 (3)	4 (4)	0 (0)	3 (4)
Graft-versus-host disease	6 (6)	7 (7)	3 (6)	5 (6)
Infection	22 (23)	19 (18)	13 (26)	11 (13)
Idiopathic pneumonia	4 (4)	4 (4)	2 (4)	6 (7)
Organ failure	17 (18)	17 (16)	8 (16)	10 (12)
Secondary cancer	0 (0)	1 (1)	0 (0)	0 (0)
Other causes	5 (5)	5 (5)	2 (4)	4 (5)
Unknown/data missing	2 (2)	13 (13)	4 (8)	10 (12)
Total	94 (100)	104 (100)	50 (100)	83 (100)

Data are presented as n (%).

UCBT indicates unrelated cord blood transplantation; and UBMT, unrelated bone marrow transplantation.

unrelated CBT did not separate these 2 diseases. Our report is the first to show the result of disease-specific analyses with a sufficient number of patients.

For AML patients, the recipients of CB were more likely to have advanced leukemia at the time of transplantation, as reported previously, suggesting that CB was used as an alternative stem cell source in the later phase of unrelated donor searches, especially in adults.[11,12,14] A larger proportion of CB recipients with ALL had the Philadelphia chromosome abnormality, which correlates with highly aggressive ALL and usually requires urgent transplantation, in which CB has an advantage over BM.[21]

Different outcomes of mortality were found between AML and ALL in a controlled comparison using multivariate analyses. Whereas significantly lower OS and LFS rates were observed in CB recipients with AML, rates of overall mortality and treatment failure were similar between CB and BM recipients with ALL. The relapse rate was not different between CBT and BMT in patients with both AML and ALL, which was consistent with previous reports.[11-13] In adult patients with ALL, a previous report showed no difference in the outcome of related compared with unrelated BM or peripheral blood transplantation in 1CR.[22] Favorable disease status at transplantation could be a more important factor affecting outcome rather than the type of stem cell source or donor type in patients with ALL. It is notable that TRM in HLA allele-matched unrelated BM recipients with AML was quite low in our study. This is probably associated with the low incidence of acute and chronic GVHD in the Japanese population, which is thought to be the result of genetic homogeneity.[23-26] Among patients with AML, although the difference was not statistically significant, a higher trend of TRM observed in CB recipients might be associated with higher overall and TRM rates in CB recipients. Reasons for higher TRM could include the graft source and delayed neutrophil recovery. Better supportive care is required after CBT for patients going through a prolonged neutropenic period. Development of better graft engineering or better conditioning regimens would help to decrease the TRM rate in CB recipients. Because relapse was the major cause of death in all groups, any attempt to decrease TRM should preserve the antileukemia effect to improve OS and LFS. Another reason for the higher TRM could be a higher risk patient population, higher risk for both disease status and comorbid conditions, requiring rapid transplantation. Searching for unrelated donors earlier and providing transplantation earlier in the disease course could help to decrease TRM in CB recipients.

Neutrophil and platelet recovery was slower in CB recipients with either AML or ALL, consistent with the results of previous reports.[11,12,27] Multiple studies have reported lower incidence of acute GVHD in CB recipients.[8-10,12,13] In our study, particularly in patients with ALL, the risk of developing grade 2 to 4 acute GVHD in CB recipients was lower compared with BM recipients, which was reported to be lower compared with the incidence reported from Western countries.[23-25] The risk of developing chronic GVHD was similar between CB and BM recipient with either disease, but the risk of developing extensive-type chronic GVHD was lower in CB recipients; the difference was significant in patients with AML. It is notable that there was no increase in the incidence of acute or chronic GVHD in CB recipients among patients with either AML or ALL, despite HLA disparity.

For differences in outcomes between AML and ALL, one possibility is a difference of treatment before conditioning therapy. Most AML patients received a more intense treatment for induction and consolidation therapy compared with that for ALL. There was no adjustment made for previous treatment, and this could be the reason for higher mortality in CBT, which requires a longer time for neutrophil recovery. Another possible cause of the difference in outcomes is the difference in conditioning regimens. Preparative regimens were similar between CB and BM recipients among ALL patients. However, in patients with AML, the proportion of standard regimens, such as cyclophosphamide and TBI or busulfan and cyclophosphamide, was smaller among CB recipients. These differences in the distribution of preparative regimens were also seen in a previous report.[11] Although the final model was adjusted for conditioning regimens, we cannot rule out the possibility of an effect that larger CB recipients received additional or different chemotherapeutic agents compared with BM recipients among patients with AML. Although the difference was small, the median age of CB recipients with AML was 4 years older than CB recipients with ALL (median age, 38 vs 34 years, $P = .021$), which might have affected the higher mortality rate among CB recipients with AML. It is also possible that some unknown biologic aspects have contributed to these differences, and this would require further evaluation in future studies.

Further subgroup analyses indicated that the superiority of HLA allele-matched BM versus CB for OS was mostly found in patients with AML showing 1CR at conditioning. However, because of the limited numbers of patients in these subgroup analyses and the possibility of an unidentified bias in stem cell source selection, our findings should be verified by further analysis in a larger population.

In conclusion, we found different outcomes between patients with AML and ALL, indicating the importance of disease-specific analyses in alternative donor studies. HLA-A, -B low-resolution, and -DRB1 high-resolution 0 to 2 mismatched single-unit CB is a favorable alternative stem cell source for patients without a suitable related or 8 of 8 matched unrelated BM donor. In the absence of a suitable donor, unrelated CBT should be planned promptly to transplant the patient while in a better disease status and better clinical condition. For patients with AML, decreasing mortality, especially in the early phase of transplantation, is required to improve the outcome for CB recipients.

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Authorship

Contribution: Y.A. and R.S. designed the study and wrote the paper; Y.A. analyzed results and made the figures; S. Kato and Y.M. designed the research; T.-N.I., H.A., and M. Takanashi reviewed and cleaned the Japan Cord Blood Bank Network data and reviewed the results; S. Taniguchi, S. Takahashi, S. Kai, H.S., Y. Kouzai, M.K., and T.F. submitted and cleaned the data; and S.O., M. Tsuchida, K.K., Y.M., and Y. Kodera reviewed and cleaned the Japan Marrow Donor Program data and reviewed the results.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

A complete list of members from the Japan Marrow Donor Program and the Japan Cord Blood Bank Network can be found in the Supplemental Appendix (available on the *Blood* website; see the Supplemental Materials link at the top of the online article).

Correspondence: Yoshiko Atsuta, Department of Hematopoietic Stem Cell Transplantation Data Management, Nagoya University School of Medicine, 1-1-20 Daiko-Minami, Higashi-ku Nagoya, 461-0047 Japan; e-mail: y-atsuta@med.nagoya-u.ac.jp.

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LETTER TO THE EDITOR

Fatal giant cell myocarditis after allogeneic bone marrow transplantation

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Giant cell myocarditis (GCM) is a rare and often fatal disease that frequently affects young, predominantly healthy adults and patients with autoimmune disease.[1] We describe the first case of GCM after allogeneic haematopoietic SCT (HSCT).

A 9-year-old boy with ALL was referred to our institution after failing to achieve a second remission of his disease. Immunohistochemically, leukaemic cells were positive for CD10, CD19, CD20, CD22 and CD34. Prior to HSCT, the cumulative dose of anthracyclines was 330 mg/m^2, which was calculated as the equivalent dose of native doxorubicin.[2] Findings on ultrasound cardiography and electrocardiography were normal at transplant. The patient underwent HSCT from an HLA-DR mismatched unrelated donor while in a state of ALL relapse. The conditioning regimen consisted of TBI (12 Gy), etoposide 60 mg/kg once daily i.v. and CY 60 mg/kg once daily i.v. for 2 days (total dose 120 mg/kg), with MTX and tacrolimus as prophylaxis for GVHD. BM showed rapid engraftment with full donor chimerism and no evidence of relapse. Stage 3 gut, stage 3 skin and stage 1 liver (overall grade III acute GVHD) were noted on day 8, and progressive onset, extensive chronic GVHD developed. Following combined immunosuppressive therapy using tacrolimus, prednisolone, MTX and thalidomide, the patient's chronic GVHD improved, and he was discharged 7 months after HSCT with no evidence of cardiomyopathy on ultrasound cardiography and electrocardiography. Monitoring of haematopoietic chimerism was repeated monthly, and he had 100% donor cell engraftment in unseparated nucleated BM and peripheral blood cells.

Two months after discharge, the patient was readmitted following fever (38.9 °C), vomiting and abdominal pain. His creatine kinase level was slightly elevated at admission (559 U/l). His temperature returned to normal on the next day after administering antibiotics, but he suddenly complained of severe dyspnea. Electrocardiogram showed ventricular fibrillation. Attempts at resuscitation were unsuccessful, and he died 6 h after the first complaint of dyspnea. An autopsy was performed with the permission of his parents, which showed diffuse cardiac myocyte damage with infiltration of lymphocytes, histiocytes, eosinophils and giant multinucleated cells (Figure 1a). Immunohistochemistry showed that lymphocytes were mostly CD8$^+$ T cells (Figure 1b). There was no evidence of haemorrhagic myocardial necrosis with interstitial oedema, fibrin deposition and vascular endothelial damage, which are sometimes observed in patients as a complication of high-dose CY with or without irradiation.[3] Myocardial damage because of anthracycline-containing chemotherapy leads to either early- or late-onset cardiotoxicity. But neither myofibril loss nor vascular degeneration were observed in this case, in contrast to the characteristic findings in patients with doxorubicin-induced cardiotoxicity.[4] Electron microscopy showed the absence of viral inclusions. No viral infectious agents were found in the myocardial tissue, and all cultures obtained during hospitalization and at autopsy remained negative. Clustering of CD19$^+$ and CD10$^+$ leukaemic cells were detected in BM and spleen, but there were no signs of microscopic relapse in the myocardium.

To our knowledge, there is only one case report of GCM in association with high-dose chemotherapy and autologous HSCT with IL-2 immunomodulation.[5] The authors suggested that the cytokine imbalance produced by IL-2 might have initiated a preferential activation of T helper cells, resulting in an autoimmune process that manifested as GCM. Their immunohistochemical studies demonstrated that the majority of infiltrating lymphocytes were CD4$^+$ helper lymphocytes, and CD4$^+$ cells have been implicated as primary mediators in some autoimmune diseases with GCM.[1] On the other hand, Platzbecker *et al.*[6] reported a case of allogeneic unrelated peripheral blood SCT (PBSCT) in a patient with AML, who had irreversible heart damage caused by massive infiltration of CD8$^+$ lymphocytes. In the case reported by Platzbecker *et al.*,[6] the patient again presented with relapsing disease on day 120 after the first PBSCT, and a second transplant was performed using unmanipulated PBSC from the same donor with no GVHD prophylaxis. On day 7 after the second PBSCT, he developed fever along with acute GVHD grade III of the skin, and treatment with CYA and prednisone was started with good response. On day 15, the patient suffered acute ventricular fibrillation and died. Autopsy revealed severe damage with cytolysis and massive infiltration by donor-derived CD8$^+$ T cells. This finding suggested that CD8$^+$ T cells might have induced a cytotoxic effect against cardiomyocytes, raising the possibility of acute GVHD of the heart.

Furthermore, therapy with CYA and anti-T-lymphocyte antibodies has been shown to prevent GCM in laboratory experiments.[7] Some patients with GCM have had a dramatic clinical response to anti-T-cell immunosuppression. Although our patient experienced chronic GVHD, resembling autoimmune disease, his chronic GVHD was responding well to treatment with immunosuppressive agents such as tacrolimus, prednisolone and thalidomide. One month before he developed heart failure, analysis of a sub-population of his peripheral lymphocytes showed 67% of CD8$^+$ CD11$^-$ T cells, which is thought to include the precursor and effectors of cytotoxic T cells. The increase

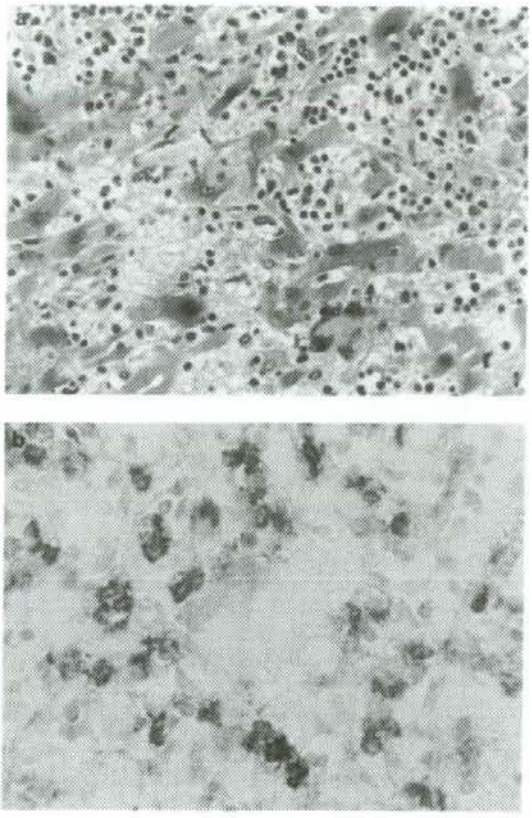

Figure 1 (a) High-power magnification of GCM displaying giant cells, lymphocytes, histiocytes, eosinophils and damaged myocytes (HE, × 300). (b) Most of the infiltrating cells are $CD8^+$ cytotoxic T cells (Immunostaining, × 300). GCM = giant cell myocarditis; HE = haematoxylin and eosin.

of $CD8^+$ $CD11^-$ T cells closely correlates with chronic GVHD,[8] but no active GVHD findings in skin, liver and gut were noted during autopsy. Although it is unknown whether chronic GVHD against the cardiomyocytes correlated with GCM in our case, it is important that the heart might be a target of alloreactive T cells after HSCT. GCM is distinct from lymphocytic myocarditis, because GCM has a more fulminant clinical course. GCM may respond to treatment with a combination of immunosuppressive drugs. For patients receiving immunosuppressive therapy after BMT, it is very difficult to diagnose GCM and rescue them from sudden cardiac failure. Further systemic investigation of epidemiology, causes, natural history and effects of treatment, including cardiac transplantation is needed.

M Yabe[1], H Ishiguro[2], Y Yasuda[2], I Takakura[2], S Matsuda[2], K Shimamura[3], S Kato[1] and H Yabe[1]
[1]*Department of Cell Transplantation, Tokai University School of Medicine, Isehara, Japan;*
[2]*Specialized Clinical Science, Pediatrics, Tokai University School of Medicine, Isehara, Japan and*
[3]*Department of Pathology, Tokai University School of Medicine, Isehara, Japan*
E-mail: miharu@is.icc.u-tokai.ac.jp

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Patient Report

Progressive multifocal leukoencephalopathy after allogeneic bone marrow transplantation for Wiskott–Aldrich syndrome

Yukiharu Yasuda,[1] Hiromasa Yabe,[1] Hiroyasu Inoue,[1] Takashi Shimizu,[1] Miharu Yabe,[1] Yoshiaki Yogo[2] and Shunichi Kato[1]

[1]*Department of Pediatrics, Tokai University School of Medicine, Bohseidai, Isehara, Kanagawa and* [2]*Department of Viral Infection, Institute of Medical Science, University of Tokyo, Tokyo Japan*

Key words bone marrow transplantation, JC virus, progressive multifocal leukoencephalopathy, Wiskott–Aldrich syndrome.

Progressive multifocal leukoencephalopathy (PML) is a demyelinating disease of the central nervous system caused by JC virus (JCV), neurotropic polyomavirus. PML is well known as an opportunistic infection because it has been recognized with increasing frequency in HIV-infected patients.[1] Wiskott–Aldrich syndrome (WAS) is an X-linked recessive combined immunodeficiency that manifests the triad of thrombocytopenia, eczema, and recurrent infection. Hematopoietic stem cell transplantation (HSCT) has been used as curative treatment for WAS, but an immunosuppressive state persists for months or years after HSCT. JCV infections occur in <10% of marrow transplant patients.[2] PML is very rare as a complication of WAS or HSCT.[3] We report a case of WAS with PML following bone marrow transplantation (BMT), focusing on nested polymerase chain reaction (PCR) of JCV, which amplified target for the regulated region, as a very efficacious method to confirm the diagnosis of PML.

Case report

A 2-month-old boy was diagnosed with WAS including eczema, melena, thrombocytopenia and infections in 1984. He had frequent viral and bacterial infections. As treatment of acyclovir-resistant HSV-type 1 infection became very difficult, he had been treated with many antiviral drugs and with activated autologous T-lymphocyte. In February 1998 he was referred to Tokai University Hospital for allogeneic BMT. BMT was done from an HLA allele-matched unrelated donor on 15 April 1998. He received a conditioning regimen of thoraco-abdominal irradiation (TAI, 10 Gy, five fractions), cyclophosphamide 50 mg/kg per day for 4 days, and anti-thymocyte globulin (ATG) 2.5 mg/kg per day for 4 days. Cyclosporin (CsA, 3 mg/kg per day, from day −1), short-term methotrexate (15 mg on day +1, 10 mg on days +3,+6,+11) and prednisolone (1 mg/kg per day, day+7→+35) were administered as graft-versus-host disease (GVHD) prophylaxis. A total of 4.44×10^8/kg of nucleated cells were infused. The recovery of myelopoiesis was comparatively slow; for example, peripheral white blood cells exceeded 1000/μL on day +14. But this recovery was mixed chimeric, with the recipient type having an advantage over the donor type. On day +50, Epstein–Barr virus-associated lymphoproliferative disorder occurred, and he received donor lymphocyte infusion (DLI) twice on day +60 and day +69. Chimerism analysis on DNA fingerprinting (variable number tandem repeat) showed a rapid change from recipient dominant chimera to the complete donor chimera after DLI. Grade II of acute GVHD, which was not present before DLI, appeared on the skin (3+) following DLI and progressed into chronic GVHD. His post-BMT course was complicated by recurrent and severe infections, mucocutaneous infection of acyclovir-resistant Herpes simplex type 1, mycotic pneumonia, which made a fungus ball, and methicillin-resistant *Staphylcoccus aureus* (MRSA) sepsis.

In November 1999, 6 months after BMT, he complained of nausea and headache at first, and approximately 2 weeks later he developed progressive impairmenrt of ocular movement, and anarthria. Magnetic resonance imaging (MRI) of the brain showed T1 low signal intensity in sequence and T2-high signal intensity in the right occipital lobe, cerebellum and pons (Fig. 1). There were similar legions in the right corona radiate, left thalamus and basal ganglia (Fig. 2). Cerebrospinal fluid (CSF) cytology, sugar and protein were normal, and bacterial culture was negative. His course was progressive with aphasia and clouding of consciousness, and nested PCR for JCV of his CSF was reported to be positive. The diagnosis of PML was confirmed by the presence of JCV, in addition to clinical symptoms and brain MRI findings. His consciousness and pain response became lowered and respiration was weakened. Because of these symptoms he was intubated approximately 1 month after onset. Several attempts to treat PML, using acyclovir, rivabirin, interferon-α (IFN-α) and infusion of activated autologous lymphocytes, were not effective. He died approximately 2 months after onset of PML (8 months after BMT). In this patient, autopsy of the brain, restricted to the affected areas, showed demyelinated white matter and oligodendrocytes and there were some inclusion bodies

Correspondence: Yukiharu Yasuda, MD, Department of Pediatrics, Tokai University School of Medicine, Bohseidai, Isehara, Kanagawa, 259-1193, Japan. Email: yyasuda@cameo.plala.or.jp

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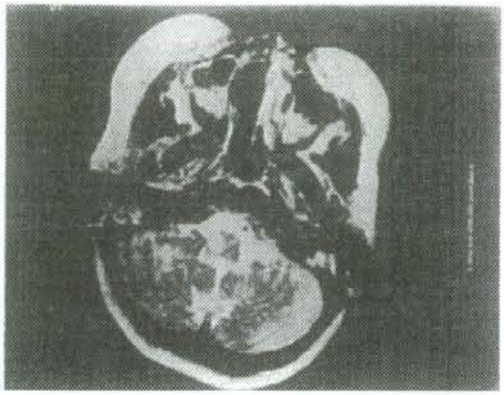

Fig. 1 High signal intensity in the right occipital lobe, cerebellum and pons (arrow) on T2-weighted magnetic resonance imaging.

in the nuclei. We detected three regulated regions of JCV from autopsy specimens obtained the cerebellum, occipital lobe and brainstem, which had abnormal signal intensity on MRI.

Discussion

We describe PML in a patient with WAS syndrome after allogeneic BMT. JCV is a member of the Parvoviridae polyomaviruses, to which BK virus and SV40 also belong. In 1971 JCV was isolated from brain tissue of a PML patient for the first time.[4] The seroconversion rate of JCV is estimated to be approximately 70% in healthy adults. After the first contact infection, JCV is usually kept latent in the kidney. When profound immunosuppression occurs, JCV invades the central nervous system and destroys oligodendrocytes, which form the myelin sheath, and causes progressive demyelination. PML is a type of slow viral infection.

Multiple lesions appear in the cortex of cerebri and cerebelli and various cerebral symptoms develop, headache, hemiparesis, loss of memory, character change, visual dysfunction, allophasis and dysbasia. The present patient had intention tremor, muscle hypotonia and dyspnea, which correlated with the demyelination lesions spreading to the cerebellum and brainstem.

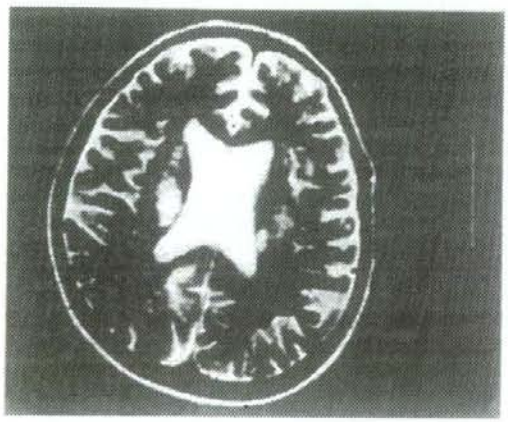

Fig. 2 High signal intensity in the right corona radiata and left basal ganglia on T2-weighted magnetic resonance imaging.

Most PML patients are immunocompromised, including malignant lymphoma, leukemia, transplant recipients and systemic lupus erythematosus. Recently, PML has increased among HIV-infected patients,[1] but there have been a few reports of PML patients receiving stem cell trasplantation.[3,5]

The PML lesions have multiple demyelination in the white matter. Phagocyte invasion is scanty in the demyelinated lesions, and a local lymphocytic immunoreaction is not usually found on histology. In the present patient the autopsy findings were compatible with PML.

Progressive multifocal leukoencephalopathy should be considered in the differential diagnosis if progressive symptoms of cerebri and MRI findings are present in immunocompromised patients. The diagnosis of PML can be confirmed by brain biopsy, but it is not routinely done. PCR for identification of JCV is a useful method. It is reported that PCR has an overall sensitivity of 75% in the diagnosis of PML,[6] but false positivity remains a difficult problem.

The genome of JCV is circular 5130 bp DNA. It is composed of the early region coding for T antigen, the latter region coding for composed constituents, and the regulated region coding for the starting point of DNA replication and the promoter/enhancer.[7] The conventional PCR method for JCV produces false positivity because it amplifies a target for T antigen or capsid protein. But we used a PCR method that amplified a target for the regulated region in the present patient.[8] It is well known that the regulated region in JCV differs between healthy individuals and PML patients, and that the regulated region of JCV in each PML patient each has its own mutation.[9] Therefore the risk of false positivity can be minimized by testing the regulated region. We examined two components of the regulated regions of JCV, TK-1a and TK-1b, which were detected in the CSF of the present patient. We could detect three regulated regions, TK-1a, TK-1c and TK-1d, from his cerebellum, occipital lobe and brainstem, which had abnormal signal intensity on MRI and pathological changes in autopsy specimens. The structure of TK-1a and TK-1c are formed only from deletion of a part of the prototype region, and the structure of TK-1d is formed by deletion and repetition of the prototype region. Each deletion of the three regulated regions is all on its own, therefore each of them is formed from the prototype separately. These results suggest that three regulated regions originate from a single strain of JCV; regulated regions of JCV are extremely protean in PML patients (Fig. 3).

There is currently no effective therapy for PML. Therefore immunity should be recovered or enhanced, if possible, by cessation of immunosuppressive therapy, for example. There are several reports that antiviral drugs such as cytarabine, acyclovir, interleukin-2 (IL-2) and IFN-α are efficacious to a certain extent in treating PML.[10,11] We administered antiviral drugs, acyclovir, ribavirin, and IFN-α, expecting the recovery of lymphocyte function. Furthermore, we tried autologous activated lymphocyte therapy; the patient's lymphocytes were activated by IL-2

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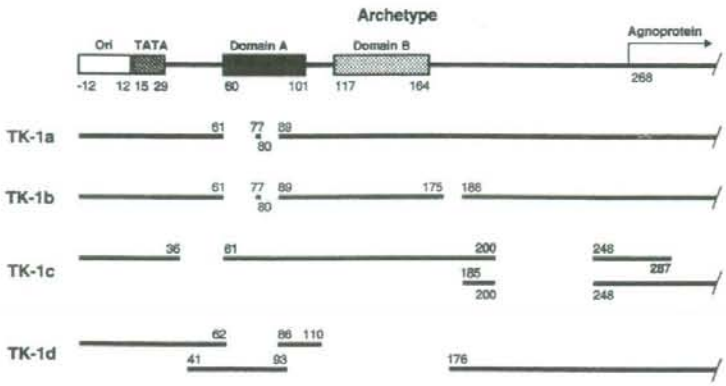

Fig. 3 Amplified regulated regions of JC virus.

in vitro and returned to the patient. This therapy had been efficacious in several infection episodes of acyclovir-resistant herpes simplex 1 infection before BMT in the present patient. All of these attempts, however, could not alter the disease course.

Progressive multifocal leukoencephalopathy is a fatal disease, and survival is generally <6 months, although a longer clinical course has been reported.[12] The disease progressed very quickly in the present patient and became fatal at approximately 6 weeks after the onset. PCR to detect the regulated region of JCV was an effective tool to diagnose PML in the present case. Protean regulated regions of JCV testify to the battle between the virus and the host. PML is uncommon as a complication of stem cell transplantation, and transplant physicians should include it as a differential diagnosis in patients who develop progressive CNS symptoms.

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Brief report

Chronic graft-versus-host disease following umbilical cord blood transplantation: retrospective survey involving 1072 patients in Japan

Hiroto Narimatsu,[1] Shigesaburo Miyakoshi,[2] Takuhiro Yamaguchi,[3] Masahiro Kami,[1] Tomoko Matsumura,[2] Koichiro Yuji,[2] Naoko Murashige,[4] Eiji Kusumi,[5] Yuko Kodama,[1] Tsunehiko Komatsu,[6] Hisashi Sakamaki,[7] Yasushi Kouzai,[8] Masaya Okada,[9] Yuko Osugi,[10] Ryoji Kobayashi,[11] Masami Inoue,[12] Satoshi Takahashi,[13] Shunro Kai,[2] Koji Kato,[2] Tokiko Inoue-Nagamura,[2] Shuichi Taniguchi,[2] and Shunichi Kato,[2] for the Japan Cord Blood Bank Network

[1]Divison of Exploratory Research, Institute of Medical Science, The University of Tokyo, Tokyo; [2]Japan Cord Blood Bank Network, Tokyo; [3]Department of Clinical Trial Data Management, Graduate School of Medicine, The University of Tokyo, Tokyo; [4]Office for Life-Style Related Diseases Control, Ministry of Health, Labor and Welfare, Tokyo; [5]Department of Hematology, Toranomon Hospital, Tokyo; [6]The Third Department of Internal Medicine, Teikyo University School of Medicine, Tokyo; [7]Division of Hematology, Tokyo Metropolitan Komagome Hospital, Tokyo; [8]Department of Transfusion, Tokyo Metropolitan Fuchu Hospital, Tokyo; [9]Division of Hematology, Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya; [10]Department of Pediatric Oncology/Hematology, Osaka City General Hospital, Osaka; [11]Department of Paediatrics, Hokkaido University Graduate School of Medicine, Sapporo; [12]Department of Hematology/Oncology, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka; and [13]Division of Molecular Therapy, Advanced Clinical Research Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan

We have little information on chronic graft-versus-host disease (GVHD) after cord blood transplantation (CBT). We investigated its clinical features in 1072 Japanese patients with hematologic malignancies who received a transplant through the Japan Cord Blood Bank Network. The primary end point was to investigate the incidence of any chronic GVHD. Median age of the patients was 33 years (range, 0-79 years). The cumulative incidence of chronic GVHD 2 years after transplantation was 28%. Chronic GVHD was fatal in 29 patients. Multivariate analysis demonstrated that development of chronic GVHD was favorably associated with both overall survival and event-free survival. Multivariate analysis identified risk factors of chronic GVHD: higher patient body weight, higher number of mismatched antigens for GVHD direction, myeloablative preparative regimen, use of mycophenolate mofetil in GVHD prophylaxis, and development of grades II to IV acute GVHD. Although chronic GVHD is a significant problem after CBT, it is associated with improved survival, perhaps due to graft-versus-malignancy effects. (Blood. 2008;112:2579-2582)

Introduction

Chronic graft-versus-host disease (GVHD) is a significant concern in allogeneic hematopoietic stem cell transplantation (HSCT). Many studies have been published on clinical features of chronic GVHD following bone marrow transplantation (BMT) and peripheral blood stem cell transplantation (PBSCT). In contrast, we have limited information on chronic GVHD following cord blood transplantation (CBT).

Methods

Informed consent was obtained in accordance with the Declaration of Helsinki. According to local policy, the study was approved by Japan Cord Blood Bank Network. We had no direct contact with human subjects during our study; data on patients who underwent CBT were obtained from the Japan Cord Blood Bank Network. The primary end point of this study was to investigate the incidence of any chronic GVHD after CBT. Cord blood units are provided with written informed consent. Between June 1997 and August 2006, 2713 cord blood transplant recipients were registered with the Japan Cord Blood Bank Network. All recipients received a single cord blood unit. We included those with hematologic malignancies who underwent CBT without T-cell depletion. We excluded patients with a history of any type of allogeneic HSCT prior to CBT. A total of 2015 patients met the criteria. Of those, we excluded 943 with disease progression, death without progression, and graft failure within 100 days after transplantation. In this study, we retrospectively investigated clinical features of chronic GVHD in the remaining 1072 patients. Cytomegalovirus-seropositive cord blood unit was not provided to transplantation centers. Acute and chronic GVHD were diagnosed and graded according to standard criteria.[1,2] GVHD that developed after day 100 was defined as chronic GVHD. If the mode of presentation for chronic GVHD was progressive, the date of onset was defined as day 100. SAS version 9.1.3 (SAS Institute, Cary, NC) was used for all statistical analyses.

Results and discussion

The median age of the 1072 patients was 33 years (range, 0-79 years) and the median patient body weight was 50.0 kg (range, 4.0-96.1 kg). The median follow up of the surviving patients was 18.1 months (range, 3.3-110.1 months). Of the 1072 patients, 492 (46%) developed grades II to IV acute GVHD within 100 days of transplantation. Chronic GVHD was diagnosed in 312, and the cumulative incidence of chronic GVHD 2 years after transplantation was 28% (95% confidence interval [CI], 25%-31%; Figure 1).

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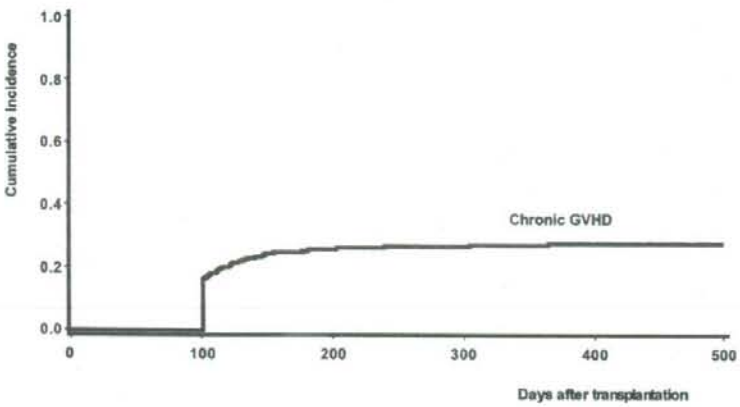

Figure 1. Cumulative incidence of chronic GVHD. The cumulative incidence of chronic GVHD 2 years after transplantation was 28% (95% confidence interval [CI], 25%-31%). Cumulative incidence curves were used to calculate the probability of chronic GVHD to take account of competing risks: disease progression, death without disease progression, and onset of graft failure.

Of the 299 patients in whom the information on types of chronic GVHD was available, 184 and 115 patients developed limited and extensive diseases, respectively.

The onset of chronic GVHD was quiescent (n = 225), de novo (n = 48), progressive (n = 18), and unknown (n = 8). Information on involved organs of chronic GVHD was available in 145 patients. Their involved organs were skin (n = 94), oral cavity (n = 35), liver (n = 32), lung (n = 24), gastrointestinal tract (n = 20), and eye (n = 16). Of 224 patients from whom information on both the treatment of chronic GVHD and its response was available, 93 patients (42%) received systemic immunosuppressive agents including corticosteroids (n = 63), calcineurin inhibitors (n = 15), both (n = 13), and others (n = 2). Of those 93 patients, 64 patients (68%) responded to the treatment. Chronic GVHD was refractory in the remaining 29, and fatal in 5.

Multivariate analysis identified prognostic factors of chronic GVHD: higher patient body weight (hazard ratio [HR], 1.01; 95% CI, 1.01-1.02; $P = .001$), higher number of mismatched antigens for graft-versus-host direction (HR, 1.31; 95% CI, 1.07-1.60; $P = .01$), reduced-intensity preparative regimen (HR, 0.69; 95% CI, 1.11-3.28, $P = .02$), use of mycophenolate mofetil in GVHD prophylaxis (HR, 1.91; 95% CI, 1.11-3.28, $P = .02$), and development of grades II to IV acute GVHD (HR, 1.51; 95% CI, 1.18-1.93; $P = .001$; Table S1, available on the *Blood* website; see the Supplemental Materials link at the top of the online article).

Of the 1072 patients, 307 died during follow up due to disease progression (n = 189), chronic GVHD (n = 29), infection (n = 23), and others (n = 66). Prognostic factors of transplantation outcomes are shown in Table 1. Overall survival (OS) and event-free survival (EFS) at 3 years after CBT were 63% (95% CI, 36%-90%) and 54% (95% CI, 27%-80%), respectively.

The present study is the first larger report addressing features of chronic GVHD in CBT. The incidence of chronic GVHD after CBT was 28% in our study, which was comparable with the previous smaller reports on CBT conducted in Western countries[3-6] and was lower than that after BMT from unrelated donors (UR-BMT) in Japanese patients.[7,8] Since the genetic backgrounds of the Japanese people are relatively homogeneous compared with those of the Western people, the incidences of chronic GVHD following UR-BMT are reportedly low in Japanese patients; our results in CBT contrast with such previous investigations.[7,8] The present study suggests that the mechanism of GVHD development is possibly affected by factors other than the genetic backgrounds of the patients' ethnicities. There are few previous studies on treatment responses of chronic GVHD after CBT. The present study suggests a higher response rate and lower mortality in chronic GVHD patients after CBT than that after UR-BMT and PBSCT.[9-16] The low overall incidence despite HLA mismatches, relatively low need for systemic immunosuppression, and positive survival impact indicate milder nature of chronic GVHD after CBT. This may be related to the immature lymphocytes in cord blood that are immunologically tolerant,[17,18] although the basic mechanism is unknown and awaits further investigations.

The present study demonstrated that chronic GVHD after CBT improved EFS and reduced the risk of disease progression. Although graft-versus-malignancy (GVM) effects of chronic GVHD after CBT have not been reported, our results were comparable with previous studies in BMT and PBSCT.[2,19,20] Chronic GVHD following CBT probably has clinically significant GVM effects. Interestingly, chronic GVHD after CBT improved OS as well. These findings contrasted with the unclear effects of chronic GVHD on OS improvement in conventional allogeneic HSCT.[2,19,20] The observations are probably associated with the possible favorable impact of chronic GVHD after CBT on nonrelapse mortality.

The present study provided useful information on chronic GVHD after CBT, although some issues remain to be discussed. First, since our study was retrospective, unrecognized bias may have affected the results. Second, we used the classical diagnostic criteria of chronic GVHD[2] and did not distinguish the late onset acute GVHD that occurs after day 100 from the classic chronic GVHD as the National Institutes of Health consensus project proposed.[21] Third, suggestions by our study on future strategies for cord unit selection and GVHD prophylaxis would be informative. Our multivariate analysis suggested an association of mycophenolate mofetil with development of chronic GVHD, whereas methotrexate was associated with better nonrelapse mortality. Although the mechanism is uncertain, clinicians may prefer to use methotrexate as GVHD prophylaxis. Since GVM effects of chronic GVHD were suggested, risk stratification may be possible in cord blood selection. Using cord blood with higher HLA mismatches for patients with high-risk primary diseases might be permissible. Finally, the applicability of our results to patients with higher body weight with heterogeneous genetic backgrounds in Western countries remains to be discussed. All of our patients received single cord blood units, whereas the majority of cord blood transplants are double cord blood units in Western countries. However, the incidence of chronic GVHD is lower and response to immunosuppressants is

Table 1. Risk factors of overall survival and event-free survival in multivariate analysis

	Overall survival			Event-free survival†			Disease progression			Nonrelapse mortality‡		
Multivariate factors*	**HR 95%**	**CI**	***P***	**HR 95%**	**CI**	***P***	**HR 95%**	**CI**	***P***	**HR 95%**	**CI**	***P***
Patient age, 40 y or older vs younger than 40 y	1.80	1.32-2.44	.0002	1.72	1.36-2.17	<.001	‡‡			2.76	1.86-4.10	<.001
Primary diseases§												
Acute myeloid leukemia	1.00			1.00			1.00			‡‡		
Acute lymphoblastic leukemia	1.42	1.03-1.97	.03	1.30	0.99-1.70	.06	1.14	0.84-1.54	.40	‡‡		
Adult T-cell leukemia/lymphoma	1.38	0.72-2.64	.33	1.54	0.87-2.72	.14	1.83	0.91-3.67	.09	‡‡		
Myelodysplastic syndrome	0.76	0.52-1.11	.16	0.65	0.46-0.91	.01	0.58	0.39-0.86	.01	‡‡		
Chronic myelogenous leukemia	0.50	0.24-1.04	.07	0.64	0.38-1.11	.11	0.61	0.32-1.14	.12	‡‡		
Non-Hodgkin lymphoma	0.82	0.53-1.26	.36	0.86	0.61-1.23	.42	0.84	0.55-1.29	.42	‡‡		
Hodgkin lymphoma	2.44	0.73-8.16	.15	3.41	1.35-8.58	.01	3.58	1.11-11.51	.03	‡‡		
Multiple myeloma	0.59	0.28-1.25	.17	0.79	0.42-1.49	.46	0.77	0.34-1.78	.54	‡‡		
Disease status at transplantation‖												
Early¶	1.00			1.00			1.00			‡‡		
Intermediate#	1.15	0.78-1.71	.49	1.09	0.79-1.52	.60	1.11	0.76-1.62	.60	‡‡		
Advanced**	2.44	1.78-3.35	<.001	2.32	1.78-3.02	<.001	2.65	1.94-3.62	<.001	‡‡		
ABO mismatch												
Match	1.00						‡‡			‡‡		
Major mismatch	0.69	0.50-0.94	.02	‡‡			‡‡			‡‡		
Minor mismatch	0.88	0.63-1.22	.44	‡‡			‡‡			‡‡		
Major/minor mismatch	1.10	0.79-1.54	.56	‡‡			‡‡			‡‡		
Higher no. of serologic HLA disparity for host-versus-graft direction, linear by 1 antigen increase	‡‡			0.80	0.69-0.92	.002	0.79	0.67-0.92	.004	‡‡		
Sex of the donor, female vs male	‡‡			‡‡			1.35	1.06-1.72	.02	‡‡		
Preparative regimen, reduced-intensity vs myeloablative regimen	1.25	0.92-1.70	.15	‡‡			‡‡			‡‡		
Methotrexate use in GVHD prophylaxis	‡‡			‡‡			‡‡			0.58	0.39-0.86	.01
Acute GVHD††												
Grades 0-I	1.00			1.00			‡‡			1.00		
Grade II	0.95	0.72-1.26	.73	0.85	0.67-1.08	.18	‡‡			1.26	0.79-2.02	.33
Grades III-IV	1.77	1.29-2.43	.001	1.37	1.03-1.81	.03	‡‡			3.66	2.31-5.81	<.001
Chronic GVHD	0.71	0.54-0.94	.02	0.64	0.50-0.82	.001	0.65	0.48-0.87	.003	0.66	0.42-1.03	.07

Associations between potential prognostic factors and outcomes were evaluated using Cox proportional hazard regression models. Occurrence of chronic GVHD was included into models as a time-dependent covariate. We used a stepwise procedure at a significance level of 5% to construct prognostic models. The proportional hazards assumption of Cox model was assessed mainly by a graphic approach.

HR indicates hazard ratio; 95% CI, 95% confidence interval; and GVHD, graft-versus-host disease.

*The following variables were considered in univariate analysis: recipient age at transplantation; recipient body weight; HLA mismatch; blood type mismatch; sex mismatch; infused nuclear cell dose and CD34+ cell dose; primary diseases; stage of primary diseases at transplantation; previous history of hematopoietic transplantation; preparative regimen and GVHD prophylaxis; and previous acute GVHD.

†EFS was defined as the time from transplantation to the following events: first progression of the primary diseases, death without progression, and graft failure. Progressions of leukemia and myelodysplastic syndrome were defined as relapse of primary diseases on the basis of morphologic evaluation in the bone marrow or other sites. Progression of malignant lymphoma was defined as involvement of new sites, recurrence in originally involved sites, or increase of more than 25% in original tumor masses. Progression of multiple myeloma was defined as an increase in serum or urine M-component, development of new soft tissue plasmacytomas, and/or increase in the size of soft tissue plasmacytomas.

‡ Nonrelapse mortality was defined as death without progression of the primary diseases.

§Those included acute lymphoblastic leukemia (n=347), acute myeloid leukemia (n=323), adult T-cell leukemia (n=28), myelodysplastic syndrome (n=171), chronic myelogenous leukemia (n=50), non-Hodgkin lymphoma (n=117), Hodgkin lymphoma (n=7), and multiple myeloma (n=29).

‖Disease status at CBT was classified into 3 groups according to the standardized report forms of the International Bone Marrow Transplant Registry (IBMTR).

¶The early group including first complete remission of acute leukemia and lymphoma, first chronic phase of chronic myeloid leukemia, first complete or partial remission of multiple myeloma, and myelodysplastic syndrome refractory anemia.

#The intermediate group including second or subsequent complete remission of acute leukemia and lymphoma, accelerated phase of chronic myeloid leukemia, and second or subsequent complete or partial remission of multiple myeloma.

**The advanced group including refractory disease, relapse or partial response of acute leukemia and lymphoma, blastic crisis of chronic myeloid leukemia, and other malignancies.

††Those included grade 0 (n=252), grade I (n=297), grade II (n=328), grade III (n=148), and grade IV (n=16).

‡‡Those factors were not included in the multivariate prognostic models because those *P* values did not exceed .05 in univariate analysis.

better in CBT than in UR-BMT in Western countries[22]; hence, the milder nature with lower incidence in chronic GVHD following CBT is probably common among Japanese and Westerns patients. The features of chronic GVHD following CBT in Western adult patients require further investigations.

In conclusion, the present study demonstrated that the incidence of chronic GVHD after CBT is probably lower than BMT or PBSCT, and is associated with improved survival. More intensive studies on the biology of chronic GVHD in CBT are warranted for our understanding and better management of chronic GVHD.

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Authorship

Contribution: H.N., S.M., and M.K. designed the research and wrote the paper; T.Y. analyzed data; T.M., K.Y., N.M., E.K., Y. Kodama, T.K., H.S., Y. Kouzai, M.O., Y.O., R.K., M.I., and S. Takahashi performed research; S. Kai, K.K., T.I.-N., and S. Taniguchi managed the data; and S. Kato took leadership of the authors.

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Correspondence: Hiroto Narimatsu, Division of Exploratory Research, Institute of Medical Science, the University of Tokyo, 4-6-1, Shirokanedai, Minato-ku 180-8639, Tokyo, Japan; e-mail: narimt54@med.nagoya-u.ac.jp.

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ORIGINAL ARTICLE

Unrelated cord blood transplantation in CML: Japan Cord Blood Bank Network analysis

T Nagamura-Inoue[1,2], S Kai[3,4], H Azuma[5,6], M Takanashi[7,8], K Isoyama[9,10], K Kato[11,12], S Takahashi[13], S Taniguchi[14], K Miyamura[15], K Aoki[16], M Hidaka[17], F Nagamura[18], A Tojo[1,2,13], XM Fang[19] and S Kato[20,21], for Japan Cord Blood Bank Network

[1]*Department of Cell Processing and Transfusion, Research Hospital, The Institute of Medical Science, University of Tokyo, Tokyo, Japan;* [2]*Tokyo Cord Blood Bank, Tokyo, Japan;* [3]*Department of Transfusion Medicine, Hyogo Medical University, Nishinomiya, Japan;* [4]*Hyogo Cord Blood Bank, Nishinomiya, Japan;* [5]*Hokkaido Red Cross Blood Center, Sapporo, Japan;* [6]*Hokkaido Cord Blood Bank, Sapporo, Japan;* [7]*Japanese Red Cross Tokyo Metropolitan Blood Center, Tokyo, Japan;* [8]*The Metro Tokyo Red Cross Cord Blood Bank, Tokyo, Japan;* [9]*Department of Paediatrics, Showa University Fujigaoka Hospital, Yokohama, Japan;* [10]*Kanagawa Cord Blood Bank, Yokohama, Japan;* [11]*Department of Paediatric Haematology/Oncology, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan;* [12]*Tokai Cord Blood Bank, Nagoya, Japan;* [13]*Department of Hematology/Oncology, Research Hospital, The Institute of Medical Science, University of Tokyo, Tokyo, Japan;* [14]*Department of Hematology, Toranomon Hospital, Tokyo, Japan;* [15]*Department of Haematology/Oncology, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan;* [16]*Department of Hematology, Kitakyusyu City Medical Center Hospital, Kitakyusyu, Japan;* [17]*Department of Hematology and Collagen Disease, National Hospital and Organization Kumamoto Medical Center, Kumamoto, Japan;* [18]*Division of Clinical Trial Safety Management, Research Hospital, The Institute of Medical Science, University of Tokyo, Tokyo, Japan;* [19]*Division of Biostatistics, Kitasato University Graduate School, Tokyo, Japan;* [20]*Department of Cell Transplantation and Regenerative Medicine, Tokai University School of Medicine, Isehara, Japan and* [21]*Tokai University Cord Blood Bank, Isehara, Japan*

We analysed 86 patients with CML who received unrelated cord blood transplantation (UCBT), identified through a registry of the Japan Cord Blood Bank Network. At transplantation, the median patient age was 39 years (range, 1–67 years); 38 patients were in chronic phase (CP), 13 in the accelerated phase (AP) and 35 in blast crisis (BC). Median duration from diagnosis to UCBT was 1.5 years (range, 0.2–14.6 years). A nucleated cell (NC) dose of more than 3.0×10^7 per kg was sufficient to achieve neutrophil (91%) and platelet recovery (86%), whereas the lower dose of NC achieved only 60 and 61%, respectively. The duration and type of pre-transplant treatment did not affect neutrophil or platelet recovery. Results of multivariate analysis indicated that older patients (>50 years) had a higher incidence of transplant-related mortality. Advanced-disease stage and lower doses of NCs were significantly associated with lower leukaemia-free and event-free survival. At 2-year survival for patients in CP, AP and BC was 71, 59 and 32%, respectively ($P = 0.0004$). A pre-transplant European Group for Blood and Marrow Transplantation scoring system was effective in predicting the outcome of UCBT. We conclude that UCBT is a reasonable alternative therapy for patients with CML.

Correspondence: Dr T Nagamura-Inoue, Department of Cell Processing and Transfusion, Research Hospital, The Institute of Medical Science, University of Tokyo, 4-6-1, Shirokanedai, Minato-ku, Tokyo 108-8639, Japan.
E-mail: tokikoni@ims.u-tokyo.ac.jp
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Introduction

Recent clinical research on unrelated cord blood transplantation (UCBT) has encouraged the use of human umbilical cord blood (CB) as a source of haematopoietic SCT (HSCT) in patients with haematopoietic malignancies.[1–5] In Japan, more than 3500 UCBTs have been performed through 11 CB banks in the Japan Cord Blood Bank Network (JCBBN). As a component of quality management and promotion, the JCBBN established a common registry for studying the results of UCBT. However, the clinical application of UCBT for CML has not been established because treatment with interferon (IFN)-α and molecular-targeting reagents, such as imatinib mesylate (imatinib), have induced complete cytogenetic remission and improved long-term survival without the need for allogeneic haematopoietic transplantation.[6–8] Imatinib has now replaced IFN as the first-line therapy for CML and induces a molecular remission (MR) as well as a complete cytogenetic response (CCyR) in the vast majority of patients newly diagnosed with CML who are in the chronic phase (CP) of the disease.[8–10] Imatinib induces complete haematological responses not only in patients in CP but also in those in the accelerated phase (AP) of the disease and in blastic crisis (BC).[11] Although its long-term

efficacy and feasibility were confirmed in the International Randomized Study of Interferon and STI571 (IRIS),[12] sustained administration of IFN is recommended for preventing disease progression and recurrence from minimal residual disease, which cannot be readily eradicated by imatinib alone.[13,14] Some patients who show point mutations in the BCR-ABL oncogene are resistant to imatinib,[15] and might also be resistant to the new derivatives, dasatinib and nilotinib.[16–19]

Patients who are resistant to IFN or imatinib may be candidates for HSCT. Both BMT and PBSC transplantation have provided reasonable results in the patients, even in those who were treated with IFN or imatinib before transplantation.[20,21] Furthermore, minimal residual clones of Ph1 were eradicated by post-HSCT donor lymphocyte infusion.[22,23] Use of UCBT in patients with CML was first reported by Rubinstein *et al.*[3] in the context of haematopoietic malignancies. CML appeared to fall into the group of diseases deemed unfavourable for the application of UCBT because of relatively higher graft failure; however, Sanz *et al.*[24] reported in 2001 that all seven evaluable cases engrafted and four patients with CML-CP remained alive with MR for more than one and a half years.

To address whether patients with CML can be considered applicants for UCBT and to clarify the prognostic factors for UCBT, we analysed 86 registry-based patients with CML who underwent UCBT as the initial HSCT through JCBBN. Our results suggest that UCBT, using an adequate dose of nucleated cells (NCs), may be considered a reasonable therapeutic option for patients with CML, regardless of the duration of treatment.

Patients, materials and methods

Patient selection criteria

Only patients recorded at the JCBBN registry as having undergone UCBT for CML were included in this study. Eighty-six patients who received UCBT as the initial HSCT were analysed. They received UCBT from 1997 to 2006 at CBT centres in Japan. Informed consent was provided according to the Declaration of Helsinki.

Patient characteristics

The characteristics of the 86 patients are listed in Table 1. Median age was 39 years (range, 1–69 years), including 9 children (1–15 years), 58 young adults (16–50 years) and 19 older adults (51 or older). Median weight was 58.3 kg (range, 9–96.1 kg). At UCBT, 38 patients were in CP (7 in first CP, 29 in second CP, 2 in third or subsequent CP), 13 patients were in AP (9 in first AP, 3 in second AP and 1 in third or subsequent AP) and 35 patients were in BC (23 in first BC, 11 in second BC and 1 in third or subsequent BC). A higher percentage of the older adults had advanced stages of the disease compared to the children ($P = 0.03$). In the 19 older adults, only 4 (21%) were in CP, 5 (26%) were in AP and 10 (53%) were in BC; in the 58 young adults, 29 (50%) were in CP, 5 (8.6%) were in AP and 24 (41.4%) were in BC. In contrast, of the nine children, five (56%) were in CP, three (33%) were in AP and one (11%) was in BC at CBT. Patients received transplants at a median time of 1.96 years (range, 0.18–17 years) after they were diagnosed with CML. Sixteen had received chemotherapy

Table 1 Characteristics of patients with CML given an unrelated cord blood transplant

Patients characteristics	n = 86
Gender	
M:F	55:31
Age, years	
Median (range)	39 (1–67)
Children (1–15 years), young adult (16–50 years) and older adult (51–67 years)	9:58:19
Weight, kg	
Median (range)	58.3 (9–96.1)
Duration from diagnosis to CBT, year	
Median (range)	1.96 (0.18–17)
Stage at CBT	
CP (n = 38)	
First	7
Second	29
Third or subsequent	2
AP (n = 13)	
First	9
Second	3
Third or subsequent	1
BC (n = 35)	
First	23
Second	11
Third or subsequent	1
In 38 patients with CP at UCBT	
Haematological response	
Complete	30
Partial	2
No response	2
Unknown	4
Cytogenetic response	
Complete (0%)	16 (7/16 molecular CR)
Partial (<35%)	9
No response (35–100%)	9
Unknown	4
Previous treatment	
Hydrea/BU/Ara-C, other chemotherapy	16
IFN-α-based therapy	22
Imatinib-based therapy	28
IFN-α and imatinib	18
Unknown	2
Pre-transplant risk scoring system of EBMT	
1	1
2	1
3	10
4	18
5	25
6	21
7	10

Abbreviations: AP = accelerated phase; BC = blastic crisis; CBT = cord blood transplantation; CP = chronic phase; EBMT = European Group for Blood and Marrow Transplantation; F = female; Imatinib = imatinib mesylate, M = male.

with BU and hydroxyurea, 22 had received IFN, 28 had received imatinib, 19 had received IFN and imatinib-based treatment and 2 had received an unknown treatment. Of the 38 patients in CP at UCBT, 30 patients showed a complete haematological response; 2, a partial response; 2, no response and 4 had an unknown response. Furthermore, of the 30 patients showing a complete haematological response, 16 had achieved a CCyR, including seven complete MRs; 9 had achieved a partial response; 9 had no response and 4 had an unknown response. Sixteen patients who achieved a CCyR, included only 1 patient in first CP complicated with colon cancer and 15 patients in second or third CP.

Umbilical cord blood characteristics and transplantation procedure

The CB cells were processed and cryopreserved in the cell processing and cryopreservation facilities of 11 public CB banks in Japan. The CB cells were initially processed using hydroxyl ethyl starch (HES)[25] and cryopreserved according to the technical and quality management guidelines of the JCBBN.

The CB characteristics are shown in Table 2. NC dose before cryopreservation was performed for all patients, but the number of GM colony-forming units (GM colonies) and $CD34^+$ cells dose were available only for 78 and 77 patients, respectively. HLA antigen disparities were categorized as either GVHD or rejection direction (Table 2). Low-resolution antigens of HLA-A, -B and -D were identified for all patients by serologic typing. HLA-DRB1 alleles were determined by high-resolution molecular typing using the sequencing-based HLA typing method in 81 of 86 cases. Patients without either an available family donor or an unrelated bone marrow donor were eligible for CBT, when two or less than two antigen mismatched HLA CB units were available.

Conditioning and GVHD prophylaxis regimens varied among the centres. Briefly, the conditioning regimen consisted of myeloablative therapy in 66 cases, reduced intensity in 18 cases and miscellaneous chemotherapy in 2 cases. The myeloablative-conditioning regimen included TBI-containing regimens (TBI $\geqslant$ 8 Gy) or conventional high-dose chemotherapies with CY ($\geqslant 120\,mg\,kg^{-1}$). G-CSF was administered until engraftment in 80 cases, whereas no cytokine was administered in 6 cases. For GVHD prophylaxis, patients received CsA-, tacrolimus- or mycophenolate mofetil-based regimen, except one patient, who received no prophylaxis (Table 2).

Statistical methods

The median duration of follow-up was 24 months (range, 3–67 months). The outcome end points were neutrophil recovery, platelet recovery, GVHD, relapse, transplant-related mortality (TRM), overall survival (OS), leukaemia-free survival (LFS) and event-free survival (EFS). The definitions of the statistical models used were in accordance with the Statistical guidelines for European Group for Blood and Marrow Transplantation (EBMT, http://www.ebmt.org/1WhatisEBMT/whatisebmt2.html). Neutrophil recovery was defined by an ANC of at least 0.5×10^9 per liter for 3 consecutive days, the first being used as the recovery day. Platelet recovery was defined by a non-transfused platelet count of at least 20×10^9 liter for 3 consecutive days. Deaths occurring before day 90 or 180 were considered as competing risks for neutrophil or platelet recovery, respectively. The graft failure rate for neutrophils was calculated for patients living without

Table 2 Transplant characteristics of patients with CML given an unrelated cord blood transplant

Unrelated cord blood characteristics		
No. of evaluable		$n = 86$
Nucleated cells per kg recipient	Median (range)	2.5 (1.1–11.6) $\times 10^7$ per kg
$CD34^+$ cells per kg recipient*	Median (range)	0.88 (0.15–7.3) $\times 10^5$ per kg
GM colonies per kg recipient**	Median (range)	18.5 (0.65–102.1) $\times 10^3$ per kg
HLA compatibility with the recipient (%)		
Low-resolution HLA A, B and high-resolution of DRB1 (rejection direction)		
	Identical	10 (12)
	1 Ag mismatch	13 (15)
	2 Ag mismatches	40 (47)
	3 or more Ag mismatches	17 (20)
	Unknown	6 (7)
Low-resolution HLA A, B and high-resolution of DRB1 (GVHD direction)		
	Identical	5 (6)
	1 Ag mismatch	17 (20)
	2 Ag mismatches	42 (49)
	3 or more Ag mismatches	16 (19)
	Unknown	6 (7)
ABO compatibility with the recipient		
	Matched	20 (23)
	Minor incompatibility	32 (37)
	Major incompatibility	34 (40)
Transplantation characteristics		
Conditioning regimen (%)		
	Myeloablative	66 (77)
	Reduced intensity	18 (21)
	Miscellaneous	2 (2)
Post transplantation growth factor (%)		
	G-CSF administration	80 (93)
	No growth factor	6 (7)
GVHD prophylaxis (%)		
	CsA alone	13 (15)
	CsA + sMTX	39 (45)
	CsA + steroids	5 (6)
	CsA + MMF	1 (1)
	Tacrolimus	15 (17)
	Tacrolimus + sMTX	11 (13)
	Tacrolimus + steroids	1 (1)
	No prophylaxis	1 (1)

Abbreviations: Ag = antigen; FK = tacrolimus; MMF = mycophenolate mofetil; sMTX = short-term MTX.
Myeloablative conditioning regimen includes TBI-containing regimens with TBI at 8 Gy or more or conventional high-dose chemotherapies with CY 120 mg kg^{-1} or more.
*$n = 77$, **$n = 78$.

relapse for more than 90 days. Acute and chronic GVHD were diagnosed and graded at each centre according to the standard criteria.[26] Relapse was defined on the basis of the reappearance of the blast or Ph1 chromosome or BCR-ABL transgene by cytogenetic and/or molecular analysis, including PCR and FISH. TRM was considered a sole cause of non-leukaemic deaths occurring after transplantation; OS was defined as the time between transplantation and death due to any cause; LFS was defined as the time interval from UCBT to a first event, either relapse or death, in patients achievingCR; and EFS was defined as the time interval from UCBT to a first event, either diagnosis of graft failure, relapse or death, in patients achieving CR.

Kaplan–Meier estimates provided approximations of incidence over time for OS, LFS and EFS, whereas Cox models were used to evaluate the combined influence of patient-, CB cell dose-, disease- and transplant-related variables on the outcome. The end points of neutrophil and platelet recovery, acute and chronic GVHD, relapse and TRM were analysed using cumulative incidence curves that estimated incidence according to the Fine and Gray models, in which we first used univariate models that contained each of the variables one at a time. Then all variables with a $P<0.05$ by the likelihood-ratio test were included in a multivariate model. Cause-specific hazard ratios were estimated with 95% confidence intervals (CIs).[27] Statistical analysis was performed with the R Foundation version 2.5.1 (http://www.r-project.org/) and JMP version 6.0.2 software packages (SAS Institute, Cary, NC, USA).

Results

Neutrophil and platelet recovery

The cumulative incidence of neutrophil recovery on day 90 was 68% (95% CI, 58–78%; Figure 1a). During the first 90 days after transplantation, the competing risk for neutrophil recovery was death ($n=16$). The graft-failure rate for neutrophil recovery was 17% (12 of 70 living patients). For those patients who recovered, the median time to achieve an ANC equal to or greater than 0.5×10^9 per liter was 24 days (range, 6–78 days). The cumulative incidence of neutrophil recovery on day 90 was 91% (95% CI, 77–100%) for the patients who received more than 3.0×10^7 per kg NCs before cryopreservation, and 60% (95% CI, 48–72%) for those who received a lower dose of NCs ($P=0.00005$; Figure 1b). With respect to the dose of GM colonies before cryopreservation, the cumulative incidence of neutrophil recovery was 83% (95% CI, 70–100%) for patients who received more than 25×10^3 per kg of GM colonies and 62.0% (95% CI, 49–75%) for those who received a lower dose ($n=79$, $P=0.025$; Figure 1c). The association of neutrophil recovery with the dose of $CD34^+$ cells before cryopreservation, the rejection-directed disparities of HLA antigens in low and high resolution, disease status at UCBT and type of conditioning regimen (that is, myeloablative or not) was not statistically significant. Note that no statistical significance was found in the association of neutrophil recovery with type of pretreatment (IFN vs imatinib), or with the duration from diagnosis to UCBT (1 year or less vs

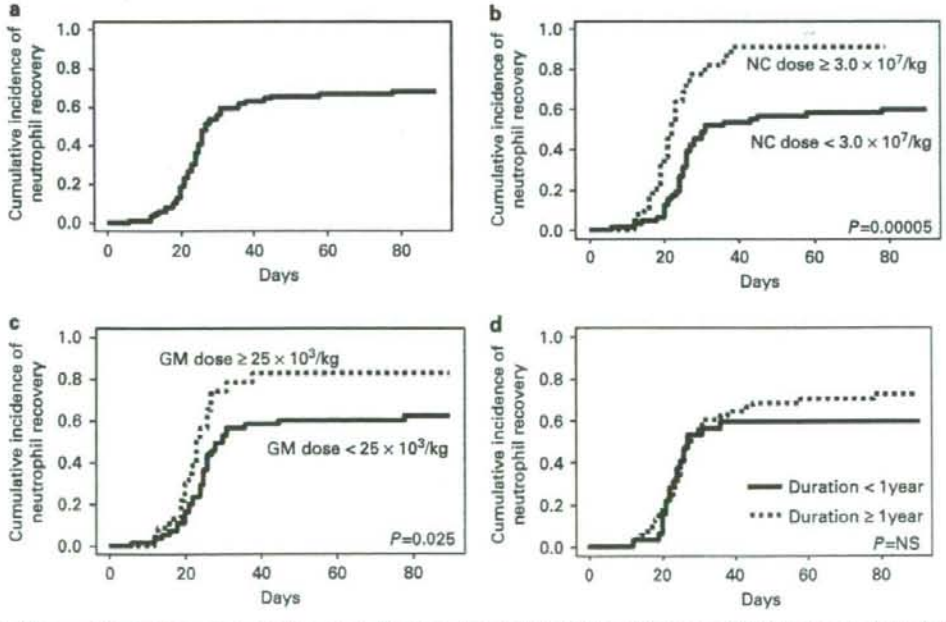

Figure 1 The cumulative incidence of neutrophil recovery. (**a**) Cumulative incidence of neutrophil recovery (**b**) by the dose of nucleated cells (NCs) and (**c**) by the dose of GM colonies before cryopreservation. (**d**) Cumulative incidence of neutrophil recovery by the duration from diagnosis to unrelated cord blood transplantation (UCBT).

longer), as shown in Figure 1d. In a multivariate analysis, only dose of NCs before cryopreservation at greater than 3.0×10^7 per kg was associated with improved neutrophil recovery (Table 3).

On day 180, cumulative incidence of platelet recovery, as indicated by more than 2×10^{10} per liter of platelets in blood, was 69% (95% CI, 59–79%; Figure 2a). During the first 180 days after transplantation, the competing risk for platelet recovery was death ($n = 16$). For those patients who recovered, the median time to achieve platelet recovery was 42 days (range, 26–148 days). In the univariate analysis, the factors statistically associated with platelet recovery were the dose of NC and GM colonies before cryopreservation (Figures 2b and c). On day 180, the incidence of platelet recovery was 86% (95% CI, 76–100%) for the patients who received more than 3.0×10^7 per kg NCs compared to 61% (95% CI, 49–73%) for those who received a lower dose ($P = 0.00002$). Additionally, the incidence of platelet recovery was 83% (95% CI, 66–100%) for patients who received more than 25×10^3 per kg of GM colonies, whereas it was 63% (95% CI, 50–76%) in those who received fewer ($P = 0.0062$). No statistical significance was found in the association of platelet recovery with dose of $CD34^+$ cells, HLA disparities, type of pretreatment or duration from diagnosis to UCBT. Multivariate analysis demonstrated that NC dose was the only factor significantly influenced by platelet recovery (Table 3).

Acute and chronic GVHD

Acute GVHD was observed in 38 of the 58 living patients with neutrophil engraftment (13 had grade I; 18, grade II; 5, grade III; 2, grade IV), whereas 20 had no acute GVHD. The cumulative incidence of acute GVHD at grade II or higher before day 100 was 47% (95% CI, 36–58%). We did not find a statistical association of HLA (AB-DRB1) disparities in a GVHD direction with the incidence of acute GVHD, although a trend towards a lower incidence of severe, acute GVHD was observed in less mismatched transplants. Chronic GVHD was found in 15 of the 57 patients who were alive and obtained engraftment (13 with limited disease; 2 with extensive disease).

TRM and cause of death

The respective 100-day and 1-year cumulative incidences of TRM were 20% (95% CI, 11–29%) and 25% (95% CI, 16–34%), respectively. When we separated the three age groups including children, young adults and older adults, the incidence of TRM in children was comparable to that in the young adults. The cumulative incidence of TRM at 1 year was 11% (95% CI, 0–33%) in children, 20% (95% CI, 9–30%) in young adults and 49% (95% CI, 25–73%) in older adults ($P = 0.026$). No significant difference was observed in TRM incidence by regimen (myeloablative (23%) vs reduced-intensity regimen (26%), $P = 0.5$). Even though the older-adults group received a greater percentage

Table 3 Multivariate analyses of risk factors for the main outcomes after unrelated cord blood transplant for CML

Factors		*Hazard ratio*	*(95% CI)*	P-*value*
Neutrophil recovery				
NC dose ($\times 10^7$ per kg)	<3.0	1		
	>3.0	2.27	1.69–2.84	0.0055
Platelet recovery				
NC dose ($\times 10^7$ per kg)	<3.0	1		
	>3.0	2.4	1.83–2.98	0.0029
TRM				
Age (years)	<50	1		
	>50	4.15	2.89–5.21	0.017
GM colony dose ($\times 10^3$ per kg)	<25	1		
	>25	0.06	0–1.98	0.0052
Disease stage at UCBT	CP to AP	1		
	BC	2.28	1.18–3.38	0.085
LFS				
NC dose ($\times 10^7$ per kg)	<3.0	1		
	>3.0	0.32	0.14–0.78	0.012
Disease stage at UCBT	CP/AP	1		
	BC	1.97	1.09–3.56	0.024
EFS				
NC dose ($\times 10^7$ per kg)	<3.0	1		
	>3.0	0.29	0.12–0.68	0.0045
Disease stage at UCBT	CP/AP	1		
	BC	1.81	1.05–3.12	0.032
Overall survival				
Disease stage at UCBT	CP/AP	1		
	BC	3.23	1.66–6.29	0.00058

Abbreviations: AP = accelerated phase; BC = blastic crisis; CP = chronic phase; CI = confidential interval; EFS = event-free survival; LFS = leukaemia-free survival; NCs = nucleated cells; TRM = transplantation-related mortality; UCBT = unrelated cord blood transplantation.

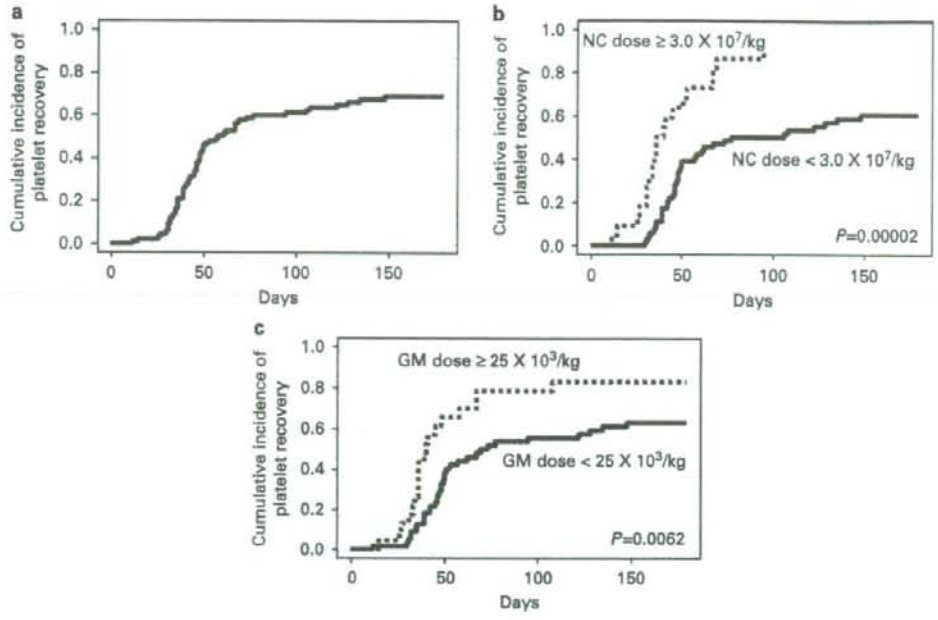

Figure 2 The cumulative incidence of platelet recovery. (a) Cumulative incidence of platelet recovery (b) by the dose of nucleated cells (NCs) and (c) by the GM colonies before the cryopreservation.

of the reduced-intensity regimen (12 patients with reduced-intensity and 7 patients with the myeloablative regimen), our data showed that old age (51 years or older) had a significant influence on the TRM. The cumulative incidence of TRM in older adults at 1 year was 49 ± 24, and $18 \pm 10\%$ in those younger than 50 years of age ($P = 0.0086$; Figure 3a). The cumulative incidence of TRM by disease stage at UCBT was $34 \pm 16\%$ in BC, $16 \pm 21\%$ in AP and $8 \pm 9\%$ in CP ($P = 0.019$; Figure 3b). Results of the multivariate analyses showed that older age and the dose of GM colonies were factors associated with a significantly increased risk of TRM. The disease stage at UCBT was only marginally associated with an increased risk of TRM (Table 3).

A total of 38 patients died and their causes of death are shown in Table 4. Five patients died of GVHD-related complications, two of veno-occlusive disease, two of bleeding, nine of infectious complications (bacterial, five; bacteria plus fungus, one; fungus, one; tuberculosis, one; viral encephalitis, one), one of tacrolimus-related encephalopathy and seven of graft failure-related complications. In total, ten patients died of relapse or induction failure, and one died of TRM after a second UCBT to treat the relapse. One patient died of the original malignancy, colon cancer.

Relapse incidence

In total, 29 patients had cytogenetic, molecular and/or haematological relapse after UCBT, as defined in Patients, materials and methods. The 2-year cumulative relapse incidence was 37% (95% CI, 26–48%). The only factor significantly associated with increased relapse incidence was NC-dose before cryopreservation of less than 3.0×10^7 per kg (44 ± 13 vs $9 \pm 12\%$; $P = 0.01$; Figure 3c). Patients with an advanced stage (AP and BC) of disease at transplantation showed a trend towards earlier relapse compared to those in CP at transplantation; however, no significant difference was observed 1 year after UCBT (Figure 3d).

In 29 patients who relapsed after CBT, 21 relapsed haematologically, whereas 8 patients had a molecular/cytogenetic relapse. In total 3 of the 21 patients with haematological relapse received the second HSCT; others received chemotherapy and/or STI or IFN. Five patients achieved haematological remission while still alive, but 15 died of relapse or of the second HSCT-related mortality. Among eight patients with molecular/cytogenetic relapse (one in first CP, six in second CP and one in second AP at the time of CBT), three patients received STI, two chemotherapy and one STI and chemotherapy, when minimal residual disease was found. Only two patients developed into haematological relapse, whereas the others continued in remission.

Event-free, leukaemia-free and overall survival

Estimated 2-year EFS, LFS and OS were 34 ± 6, 38 ± 6 and $53 \pm 6\%$, respectively (Figure 4a). Results of univariate and multivariate analyses showed that the disease stage at UCBT significantly influenced the LFS, EFS and OS (Table 3). Two-year LFS was 52% (95% CI, 37–73%) for patients in CP at the time of transplantation, 38% (95%