days and 11% (CI 8%-13%) at 100 days posttransplant (Figure 1). The median day of bacterial infection development was 8 days (range: 0-100) posttransplant. Seventy-five percent of early infection in children occurred within 31 days post-UCBT. In the adult cohort, the cumulative incidence of early bacterial infection was 19% (CI 17%-21%) at 50 days and 21% (CI 19%-24%) at 100 days after UCBT. Early bacterial infection on median day 10 (0-97) posttransplant occurred in the 260 adult recipients (22%) with 75% of the events occurring within 25 days. Statistical analysis demonstrated that the cumulative incidence of early bacterial infection in adults was significantly higher than that in children ($P < .0001$).

The majority of early bacterial infections developed during neutropenia (in 80% of children and 80% of adults). The median day of early bacterial infection development during neutropenia was 7 days (range: 0-80 days) for children and 8 days (0-80 days) for adults respectively, whereas the corresponding figures for early bacterial infections after neutrophil recovery were 55 days (20-100 days) and 46 days (14-97 days), respectively.

Types of Infections

Of the total of 1872 patients, 337 (18%) suffered from bacterial infections between day 0 and day 100 after UCBT, with 12% of children and 22% of adults suffering from early bacterial infections. As shown in Table 2, bacteremia was the most common infection within the first 100 days. In the child cohort, 68 cases of bacteremia, 2 of pneumonia, and 4 of colitis (2 of *Clostridium difficile* colitis) developed, and in the adult cohort, 247 cases of bacteremia, 38 of pneumonia, 2 of colitis (one of *Clostridium difficile* colitis), 2 each of urinary infection and sinusitis, and 1 each of *Bacillus cereus* meningitis and catheter infection. Of the 218 adults with early bacteremia, 192 patients had 1 infection episode, 23 had 2, and 3 had 3 infection episodes.

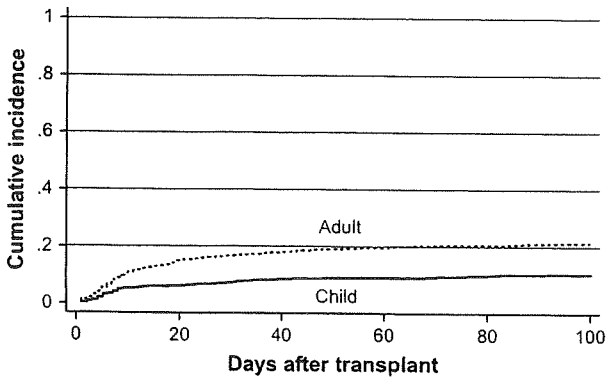

Figure 1. Cumulative incidence of early bacterial infection within 100 days following unrelated cord blood transplantation was 9% (95% CI 7%-11%) at 50 days and 11% (CI 8%-13%) at 100 days posttransplant for children. The corresponding values were 19% (CI 17%-21%) and 21% (CI 19%-24%) for adults.

Table 2. Patients with Early Bacterial Infections Who Received Cord Blood Transplantation

	Child (Age <16)		Adult (Age ≥16)	
	Patients	Episodes	Patients	Episodes
Bacteremia	65	68	218	247
Pneumonia	2	2	37*	38
Colitis	4	4	2	2
Urinary infection	0	0	2	2
Sinusitis	0	0	2	2
Meningitis	0	0	1	1
Catheter infection	0	0	1	1
Others	6	6	7	7

Total patients with infection in children and in adults were 77 and 260, respectively.
Number *Clostridium difficile* colitis 3.
*Ten patients with bacteremia developed pneumonia as the second bacterial infection.

Causative Micro organisms of Bacteremia

Of the 315 episodes of bacteremia, Gram-positive micro organisms accounted for 234 (74%), and Gram-negative micro organisms for 81 (26%) of the cases (Table 3). *Staphylococcus* species (spp) were the most common Gram-positive pathogens responsible for 147 of the bacteremia cases (47%), with coagulase-negative *Staphylococcus* (CNS) detected in 111 of these cases (76%). *Staphylococcus epidermidis* was the most

Table 3. Causative Micro organisms of the Early Bacteremia following Cord Blood Transplantation

	No. episodes		(%)
Bacteremia	315		
Gram-positives	234		(74)
Staphylococcus spp.		147	(47)
Enterococcus spp.		56	(18)
Sreptococcus spp.		19	(6)
Bacillius spp.		8	(3)
Corynebacterium spp.		2	
Clostridium spp.		1	
Mycobacterium tuberculosis		1	
Gram-negatives	81		(26)
Pseudomonas aeruginosa		34	(11)
Acinetobacter spp.		7	
Enterobacter cloacae		7	
E. coli		5	
Stenotrophomonas maltophilia		5	
Burkholderia cepacia		4	
Klebsiella pneumoniae		3	
Chyseobacterium spp.		3	
Alcaligenes xylosoxidans		2	
Salmonella spp.		2	
Serratia spp.		1	
Morganella morganii		1	
Leuconostoc spp.		1	
Micrococcus spp.		1	
Aeromonas hyrophila		1	
Capnocytophaga spp.		1	
Bacteroides fragilis		1	
Provotella oralis		1	
Fusobacterium necrophorum		1	

common CNS micro organism and was isolated in 94 cases. *Staphylococcus aureus* was isolated in 32 of the 147 cases, and was reported as methicillin-resistant *Staphylococcus aureus* (MRSA) in 25 of 32 (78%). *Enterococcus* spp was found in 56 cases (18% of all bacteremia cases), with 23 cases of *Enterococcus faecalis*, 21 cases of *Enterococcus faecium*, 1 case of *Enterococcus gallinarum*, and 11 unidentified cases. *Streptococcus* spp was the third most common Gram-positive pathogen (19 cases), with 15 cases of alpha-*Streptococcus*, 3 of *Streptococcus agalaciae*, and 1 unidentified case. *Streptococcus mitis* was found in 9 of the 15 alpha-*Streptococci* cases (60%). Of the 81 cases with Gram-negative micro-organisms, *Pseudomonas aeroginosa* was found in 34 cases, accounting for 11% of all bacteremia cases, and *Stenotrophomonas maltophillia* in 5 cases. Anaerobic Gram-negative organisms, such as *Bacteroides fragilis* (n = 1), *Provotella oralis* (n = 1), and *Fusobacterium necrophorum* (n = 1), were also isolated.

We also investigated causative micro organisms of early bacteremia that developed in children and adults either during neutropenia or after neutrophil recovery. The distribution of these micro organisms in all groups was similar except for that of *Stenotrophomonas maltophillia* and *Enterococcus* spp, with all 5 *Stenotrophomonas maltophilia* bacteremias that had developed in adults during neutropenia, the percentage of *Enterococcus* spp. bacteremia having developed in adults during neutropenia being 3.8 times higher than in children.

Causative Micro organisms of Pneumonia

Thirty-seven adults and 2 children developed bacterial pneumonia within the first 100 days after UCBT. Bacterial pneumonia developed as the first infection in 29 of these patients and 10 developed bacterial pneumonia following bacteremia, 4 of them because of the same micro organism that caused bacteremia and 6 because of a different micro organism. One patient developed secondary pneumonia of *Stenotrophomonas maltophilia* following MRSA pneumonia, which accounted for a total of 40 episodes of early bacterial pneumonia. Gram-positive and Gram-negative micro organisms accounted for 50% each of the cases of bacterial pneumonia. The causative micro organisms of pneumonia in adults were identified as *Staphylococcus aureus* (n = 6), CNS (n = 8), *Enterococcus* spp. (n = 3), *Corynebacterium* spp (n = 1), *Pseudomonas aeruginosa* (n = 5), *Burkholderia cepacia* (n = 2), and *Stenotrophomonas maltophilia* (n = 13). Eleven of the *Stenotrophomonas maltophilia* pneumonias developed during neutropenia.

Outcome of Patients with Early Bacterial Infection

Of the 43 patients who developed bacteremia with shock, 32 (74%) died as did 143 of 240 (60%) of those who developed bacteremia without shock. For patients who developed early bacteremia, bacterial infection was the main cause of death for 20 (47%) of the patients with bacteremia with shock, and 55 (23%) of the patients with bacteremia without shock. *Pseudomonas aeroginosa* bacteremia caused a higher mortality, because 73% of the patients with this type of bacteremia died. Bacterial infection was the main cause of death for 53% patients who developed *Pseudomonas aeroginosa* bacteremia. Twenty-six (70%) of the 37 adults who developed bacterial pneumonia died, as did 18 (49%) of the 37 adults who developed early bacterial pneumonia because of bacterial infection.

Risk Factors for Early Bacterial Infection after UCBT

Among the factors assessed as risk factors for early bacterial infection for children, older age group (6-10 years, and 11-15 years versus 0-5 years of age) at transplant (hazard ratio [HR] = 1.9 and 2.8, CI 1.1-3.3 and 1.6-5.1; $P = .024$ and $P < .0001$, respectively), presence of prior hematopoetic stem cell transplantation (HR = 1.8, CI 1.1-3.1; $P = .032$), infusion of $<5.10 \times 10^7$ nucleated cells per kilogram of patient's body weight (HR = 1.6, CI 1.0-2.6, $P = .049$), and use of nonmyeloablative conditioning regimen (HR = 1.8, CI 1.0-3.2; $P = .039$) were identified as significant in univariate analysis (Table 4). Use of prednisolone for GVHD prophylaxis was identified as a marginal risk factor (HR = 1.6, CI, 1.0-2.7; $P = .070$) in univariate analysis. Multivariate analysis identified older age group (6-10 years, and 11-15 years versus 0-5 years of age) at transplant (HR = 1.96 and 2.66, CI, 1.11-3.47 and 1.44-4.91; $P = .020$ and .002, respectively) as an independent risk factor of early bacterial infection for children. Use of prednisolone for GVHD prophylaxis was also identified as a marginal risk factor (HR = 1.63, CI 0.98-2.71; $P = .062$).

In the adult cohort, use of nonmyeloablative conditioning regimen was not significant. Univariate analysis results identified the use of tacrolimus for GVHD prophylaxis as a marginal risk factor (HR = 1.31, CI 1.0-1.7; $P = .055$) compared to the use of CsA for GVHD prophylaxis (Table 5). The cumulative incidence of early bacterial infection tended to be higher for patients in the adult cohort who received tacrolimus-based GVHD prophylaxis compared to those who received nontacrolimus GVHD prophylaxis (25%, 95% CI, 20%-30% versus 20%, 95% CI, 17%-24% at 100 days posttransplant, $P = .088$). No significant risk factor for early bacterial infection was identified in univariate analysis, so that multivariate analysis was not performed. The risk of early bacterial infection did not increase with age in the adult cohort (Table 5).

Effect of Early Bacterial Infections on Survival

The probability of survival of children 6 months and 2 years after UCBT was 70% (CI 66%-73%)

Table 4. Univariate Analysis for Risk of Early Bacterial Infection in Children

Factor	n	HR	95% CI	P value
Age at transplant (Years)				
0-5	392	1.00		
6-10	164	1.90	1.09-3.33	.024
11-15	107	2.84	1.60-5.05	<.0001
Prior HSCT				
≥1 prior HSCT	108/664	1.82	1.05-3.14	.032
Disease				
Acute myelogenous leukemia	151	1.00		
Acute lymphoblastic leukemia	279	0.89	0.49-1.62	.701
HLA disparity				
≥Two-antigens mismatch for GVHD direction	114/656	0.82	0.42-1.61	.574
≥Two-antigens mismatch for rejection direction	121/655	0.76	0.39-1.48	.415
Number of cells infused*				
$CD34^+$ cell $<1.42 \times 10^5$/kg	244/487	1.21	0.70-2.09	.494
Nucleated cell $<5.10 \times 10^7$/kg	332/664	1.62	1.00-2.63	.049
Conditioning regimen				
Myeloablative	545/664	1.00		
Nonmyeloablative	99/664	1.82	1.03-3.23	.039
Myeloablative condition with total body irradiation	331/664	1.24	0.78-1.99	0.368
Antithymocyte globulin/antilymphocyte globulin	75/664	1.58	0.83-3.01	.164
GVHD prophylaxis				
Cyclosporine based	401	1.00		
Tacrolimus based	199	1.23	0.72-7.08	.451
Prednisolone not used	469	1.00		
Prednisolone used	161	1.60	0.96-2.66	.070
Methotrexate not used	268	1.00		
Methotrexate used	362	0.87	0.53-1.42	.580
Disease status of malignant disease				
Standard disease†	215	1.00		
Advanced disease‡	308	1.03	0.61-1.76	.907

HSCT indicates hematopoietic stem cell transplantation; GVHD, graft-versus-host diseasel; CI, confidence interval; HR, hazard ratio.
*Number of cells at freezing.
†Standard disease means first complete remission or first chronic phase of maliganant disease.
‡Advanced disease means all others except standard disease.

Table 5. Univariate Analysis for Risk of Early of Early Bacterial Infection Adults

Factor	n	HR	95% CI	P Value
Age at transplant (years)				
16-30	270	1.00		
31-45	338	0.85	0.59-1.21	.355
46-60	445	1.04	0.75-1.43	.834
≥61	155	1.05	0.69-1.59	.838
Prior HSCT				
≥1 prior HSCT	294/1208	1.02	0.76-1.37	.881
Disease				
Acute myelologenous leukemia	490	1.00		
Aute lymphoblastic leukemia	211	0.90	0.63-1.29	.572
Lymphoma	188	1.23	0.87-1.77	.233
HLA disparity				
≥Two-antigens mismatch for GVHD direction	601/1187	0.99	0.77-1.27	.937
≥Two-antigens mismatch for rejection direction	623/1187	0.92	0.71-1.17	.485
Number of cell infused *				
$CD34^+$ cell $<0.80 \times 10^5$/kg	560/1130	1.18	0.90-1.51	.240
Nucleated cell $<2.53 \times 10^7$/kg	608/1208	1.17	0.91-1.49	.224
Conditioing regiman				
Myeloablative	579/1208	1.00		
Nonmyeloablative	621/1208	1.21	0.95-1.55	.125
Myeloablative condition with total body irradiation	504/1208	1.21	094-1.55	0.145
GVHD prophylaxis				
Cyclosporine based	846	1.00		
Tacrolimus based	312	1.31	0.99-1.72	.055
Methotrexate not used	590	1.00		
Methotrexate used	582	0.84	0.66-1.08	.182
Disease status malignant disease				
Standard disease†	427	1.00		
Advanced disease‡	739	1.10	0.58-1.44	.469

HSCT indicates hematopoietic stem cell transplantation; GVHD, graft-versus-host disease; CI, confidence interval; HR, hazard ratio.
*Number of cell at freezing.
†Standard disease means first complete remission or first chronic phase of maliganant disase.
‡Advanced disease means all other except standard disease.

and 52% (CI 48%-56%), respectively. The median follow-up of survivors was 2.1 years (range: 0.07-7.5). Bacterial infection was the main cause of death in 12 of the 77 pediatric recipients (16%) with early bacterial infection. Evaluation of early bacterial infection as a time-dependent covariate for patient's survival showed statistical significance (HR = 1.6, CI 1.2-2.2; $P = .005$) in univariate analysis. When adjusted for patient age, sex, disease status, presence of previous transplant, transplanted cell dose, HLA disparity, conditioning regimen, and GVHD prophylaxis, this factor showed no significance (HR = 1.5, CI 0.9-2.4; $P = .111$) for children.

In the adult cohort, the probability of survival 6 months and 1 year posttransplant was 50% (CI 47%-53%) and 41% (CI 38%-44%), respectively. The median follow-up of survivors was 1.0 year (range: 0.05-6.2). Bacterial infection was the main cause of death in 79 of the 260 adult recipients (30%) with early bacterial infection. The analysis of the effects of early bacterial infection showed statistical significance in univariate analysis (HR = 2.1, CI 1.7-2.5; $P < .0001$) as well as in multivariate analysis (HR = 2.1, CI 1.7-2.6; $P < .0001$) adjusted for the same variables as for the child cohort.

Overall survival (OS) rates of children who developed bacterial infection during neutropenia 100 and 365 days after infection were 58% (95% CI 44%-70%), and 40% (95% CI 27%-53%), respectively. The corresponding rates for children who developed bacterial infection after neutrophil recovery were 67% (95% CI,38%-85%) and 67% (95% CI 38%-85%), respectively. In the adult cohort, the corresponding rates were 40% (95% CI 33%-47%) and 27% (95% CI 20%-34%), for bacterial infection having developed during neutropenia and 49% (95% CI 34%-63%) and 38% (95% CI 23%-52%) for after neutrophil recovery.

Because early bacterial infection, neutrophil recovery, and aGVHD occur during the early phase after transplant, we performed multivariate analyses by treating these variables as time-dependent variables for the analysis of early bacterial infection in terms of status of neutrophil recovery and status of aGVHD.

Multivariate analyses revealed that early bacterial infection remained a significant risk factor for overall mortality of adults (HR = 2.05, CI 1.68-2.49; $P < .0001$). However, early bacterial infection did not affect child mortality (HR = 1.32, CI 0.81-2.15; $P = .27$). Neutrophil recovery was a significant risk factor for overall child mortality (HR = 0.43, CI 0.28-0.67; $P < .0001$) and adults (HR = 0.47, CI 0.38-0.59; $P < .0001$) after adjustment for patient and transplant characteristics. However, grade ii to iv aGVHD did not have an effect on child mortality (HR = 1.00, CI 0.72-1.38; $P = .98$) and adults (HR = 1.08, CI 0.89-1.32; $P = .43$). These findings suggest that early bacterial infection is an independent risk factor for overall mortality of adults.

DISCUSSION

Bacterial infections remain a major complication following UCBT. To the best of our knowledge, this study of 664 pediatric and 1208 adult patients represents the largest study reported to date for the examination of early bacterial infection following UCBT. The incidence of early bacterial infection for adult patients was significantly higher than that for pediatric patients. The median day of bacterial infection development was 8 days in children and 10 days in adults posttransplant, respectively.

Gram-positive organisms were predominant (74%) in the cases of early bacteremia examined in our study. Previous studies also reported that Gram-positive organisms were prominent in bacteremia following UCBT [8,12]. In our large-scale study, *Staphylococcus epidermidis* was the most common organism isolated in 94 of the 147 *Staphylococcus* spp. cases, whereas *Staphylococcus aureus* was isolated in 32 cases. Among patients with bacteremia of *Staphylococcus aureus*, MRSA was found in 78% of the patients. *Enterococcus* spp. was the second most common Gram-positive pathogen, with 23 cases of *Enterococcus faecalis* and 21 of *Enterococcus faecium*. The percentage of *Enterococcus* spp. bacteremia in adults was 3.8 times higher than that in children during neutropenia. Because carbapenems or vancomycin has been used in the past, vancomycin-resistant *Enterococcus faecalis* or *Enterococcus faecium* was found in some patients. *Streptococcus* spp. was the third most common Gram-positive pathogen (19 cases), 79% of which were accounted for by alpha-*Streptococcus*, with *Streptococcus mitis* being the most common pathogen. *Pseudomonas aeruginosa* was the most frequently occurring bacterium in Gram-negative organisms.

Stenotrophomonas maltophilia was found in 13 of 37 (35%) adults who developed early bacterial pneumonia, and the condition of 75% of these patients deteriorated in spite of intensive therapy. Eleven of 13 *Stenotrophomonas maltophillia* adult pneumonias developed during neutropenia. *Stenotrophomonas maltophilia* is naturally resistant to penicillins, cephems except ceftazidim and cefpiramide, aminoglycosides, and carbapenems, therefore, antibiotics must be carefully selected for the treatment of patients with bacteremia or pneumonia caused by this bacterium.

For children, use of nonmyeloablative conditioning regimen was identified to be significant in univariate analysis. It was somewhat surprising that the use of nonmyeloablative conditioning in children was associated with a higher frequency of infections than in the myeloablative treated patients. Because the standardized JCBBN 100-day CRF do not include items for identifying information on comorbidity such as the recently introduced comorbidity index by Sorror et al [16], we could not make adjustments for patients' comorbidity status at transplant. We therefore cannot rule out the possibility that high-risk patients with organ failure and poor infectious defense were more likely to have been treated with nonmyeloablative conditioning, increasing risk of bacterial infections.

Multivariate analysis identified older age (6-10 years, and 11-15 years versus 0-5 years of age) at transplant as an independent risk factor for early bacterial infection in children, whereas univariate analysis revealed that older age at transplant and infusion of $<5.10 \times 10^7$ nucleated cells per kilogram of patient's body weight were identified as significant. These findings suggest that older age of children was a stronger risk factor than the number of infused nucleated cells per kilogram of patient's body weight. Higher activity of cell reproduction in younger children may be associated with a low incidence of mucosal toxicity, thus contributing to a lower risk of bacterial infection.

In adults, the incidence of early bacterial infection was almost twice as high, 21% at 100 days posttransplant compared to 11% for children. Although no specific risk factor was identified in adults, the prognostic significance of early bacterial infection was clearly identified in our analysis, thus indicating the importance of the prevention of early bacterial infection. No tendency for risk to increase with age was observed in individuals 16 years or older.

In conclusion, we analyzed the incidence of early post-UCBT bacterial infection in pediatric and adult patients. The incidence of early bacterial infection for adult patients was significantly higher than that for pediatric patients. The risk of early bacterial infection increased with age for individuals younger than 16 years, but not for those 16 years or older. Early bacterial infection had a negative effect on survival, especially in adults for whom the incidence of early bacterial infection was 21% and the median day of development was 10 days post transplant. These findings suggest that the prevention of bacterial infection during conditioning and the very early post-UCBT phase is especially important. Prospective clinical

studies are needed to establish the better prophylaxis against early bacterial infection.

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ORIGINAL ARTICLE

Long-term outcome of cord blood transplantation from unrelated donors as an initial transplantation procedure for children with AML in Japan

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To assess the outcome of unrelated umbilical cord blood transplantation (UCBT), 141 children with AML who underwent UCBT (39 in first CR (CR1), 33 in CR2, 4 in CR3 and 65 at more advanced stages (not in CR)) were analyzed in a retrospective multicenter study in Japan. Short-term MTX was used for prophylaxis of acute GVHD in 80 cases (57%). The cumulative incidences of neutrophil recovery, platelet recovery and acute GVHD (grades 2–4) were 78.7, 62.4 and 40.1%, respectively, and the 100-day transplantation-related mortality (TRM) was 10.8%. Multivariate analysis showed that an infused $CD34^+$ cell dose of 1.35×10^5 cells per kg or more was associated with favorable neutrophil and platelet recovery, and that short-term MTX was associated with a lower 100-day TRM. The 6-year relapse rate was 38.8% and was associated with disease status. Six-year overall survival was 45.8% (70.4 ± 8.3% in CR1, 59.3 ± 11.3% in CR2, 75.5 ± 21% in CR3 and 20.6 ± 6.2% for children with non-CR). We conclude that the results of UCBT are particularly promising for children with a karyotype suggesting a poor prognosis, and for those who receive transplants in CR2 and CR3 after an early relapse.
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Introduction

With currently available treatment, 80–90% of children with AML achieve CR, but 30–40% of these patients subsequently suffer recurrence, reducing the long-term survival rate to only about 50%.[1–4] After recurrence, the likelihood of survival is poor, being 21–33% according to recent reports.[5–8] BMT from an HLA-matched sibling or unrelated donor has a major role in the treatment of children with high-risk or relapsed AML.[9–11] However, although there are currently more than 250 000 donors registered in the Japan Marrow Donor Registries Program, a substantial proportion of children who lack a sibling donor will never undergo BMT from an HLA-matched unrelated donor either because such a donor cannot be found or because the time required to identify a donor is too long. Moreover, for children who undergo unrelated BMT, the increased HLA disparity adversely affects survival because of the high risk of GVHD and opportunistic infections.

Hematopoietic stem cells from an unrelated cord blood (UCB) transplant can restore hematopoiesis and immune function after a myeloablative conditioning regimen, even if the graft is not perfectly HLA identical to the recipient.[12–15] This important medical advance led to the establishment of large cord blood (CB) banks that made possible the use of UCB to provide transplants for patients lacking a conventional related or unrelated BM (UBM) donor. In addition, when compared with the availability of UBM grafts, UCB offers the advantage of significantly faster availability of banked cryopreserved UCB units. A previous comparative study of children receiving UCB or UBM transplants for acute leukemia revealed that the relapse rate did not increase after umbilical cord blood transplantation (UCBT).[16] However, it was not possible to report specific

data for children with AML at different disease stages because of the relatively small number of patients in each subgroup.[17–21] Using data from the Japan Cord Blood Bank Network (JCBBN) registry, we are now able to report outcomes and their association with patient-, disease- and transplant-related factors for 141 children who underwent UCBT as an initial hematopoietic stem cell transplantation (HSCT) procedure for AML.

Patients, materials and methods

Patient selection criteria

All children reported to the JCBBN registry as having undergone UCBT for AML were included in this study, with the exception of those with either Down's syndrome or Fanconi anemia. A total of 141 children aged 15 years or younger who received transplants from August 1997 through March 2006 in 60 centers in Japan (see Appendix) were analyzed. Patients with AML (with high-risk karyotypes, secondary AML, megakaryoblastic leukemia or absence of remission after the first course of chemotherapy) in first CR (CR1), second CR (CR2), third CR (CR3) and no CR (non-CR) were candidates for UCBT. Approval for this study was obtained from the JCBBN institutional review board. Informed consent was obtained in accordance with the Declaration of Helsinki Principles.

Patient characteristics

The characteristics of the 141 children who received UCBT as their initial HSCT procedure are listed in Table 1. Four of them were considered to have secondary AML on the basis of a history of exposure to chemotherapy or radiotherapy, a history of myelodysplasia or Blackfan Diamond anemia. Abnormal karyotypes were classified into the favorable-risk group if t(8; 21), t(15; 17) or inv(16) was detected. In patients lacking these favorable karyotypes, the presence of monosomy 7, 11q23 abnormalities other than t(9;11), monosomy 5, del(5q), abnormal 3q, t(6; 9) or a complex karyotype involving the presence of five or more abnormalities were defined as a poor-risk group. The remaining abnormalities were classified as an intermediate-risk group. At the time of UCBT, 39 children were in their CR1; 14 patients with a poor-risk karyotype and 21 of 25 patients with a poor response to the first course of chemotherapy without a poor-risk karyotype were included, 32 were in their CR2, 4 were in their third or subsequent CR and 65 were non-CR.

Table 1 Feature at diagnosis

Age, median (range)	2.9 years (0–15.2 years)
Gender (M/F)	76/65
WBC count/ $\times 10^9$ *per liter*	
Median (range)	17.5 (0.6–675)
Disease status at UCBT (%)	
CR1	39 (27)
CR2	33 (23)
CR3	4 (3)
Non-CR	65 (47)
Cytogenetics (%)	
Favorable karyotype	39 (27)
Intermediate karyotype	45 (32)
Poor karyotype	33 (24)
Not detected	24 (17)
FAB subtype (%)	
Month 0	6 (4)
Month 1	15 (11)
Month 2	23 (16)
Month 3	2 (1)
Month 4	15 (11)
Month 5	30 (21)
Month 6	6 (4)
Month 7	26 (19)
Undetermined	18 (13)

Abbreviations: F = female; FAB = French–American–British classification; M = male; UCBT = umbilical cord blood transplantation.

Umbilical cord blood characteristics and the transplantation procedure

Eligibility. The clinical protocols for UCBT were approved by the institutional review board of each participating institution. Patients were eligible for enrollment if their disease was stable but had no HLA-identical related or unrelated donor; or if their disease was unstable with no related donors or an HLA-matched UBM donor could not be identified within 6–8 weeks. The disease status of those with hematological malignancies was categorized according to the criteria specified in the International Bone Marrow Registry.[22]

Donor registries and selection of grafts. Searches for unrelated CB donors were processed through the JCBBN, through which over 28 000 CB units were made available in August 2006. A preliminary search of umbilical CB banks was performed by using the patient's HLA phenotype, as determined by serologic typing for class I HLA-A and HLA-B antigens and low-resolution DNA typing for class II HLA-DRB1 alleles. High-resolution DNA typing of class I and/or II HLA alleles was performed on physician's request. Preferred UCB units were those matched at four or more of six HLA antigens and that contained a minimum count of 2×10^7 nucleated cells per kg of the recipient's body weight before freezing. Units of UCB were not depleted of T lymphocytes. Use of HLA-A and -B serology and low-resolution allelic DRB1 typing showed that most of the children had either one (65%) or two (13%) disparities with their graft (Table 2). The $CD34^+$ cell counts were analyzed by the two most commonly used $CD34^+$ cell quantification methods (ISHAGE protocol and ProCount; Becton Dickinson, Franklin Lakes, NJ, USA)[23] at 11 regional CB banks.

Preparative regimen and prophylaxis against GVHD. The conditioning regimen and acute GVHD prophylaxis varied according to the policy of each center, type of disease, prior treatment and disease status at the time of UCBT. The patients or their parents gave consent for UCBT after being informed of the potential risks and benefits of the procedure. The GVHD prophylaxis is summarized

Table 2 Feature at cord blood transplantation

Age, median (range)	4.0 years (0–15 years)
Recipient's body weight, median (range)	14.9 kg (5–65 kg)
Infusion cell dose (× 10^7per kg recipient)	
Median (range)	5.03 (1.85–15.1)
Infused CD34$^+$ cell (× 10^5 per kg recipient)	
Median (range)	1.43 (0.06–38.3)
Date of transplantation (%)	
Before December 1999	26 (18)
After January 2000	115 (82)
CMV serology (%)	
Negative	62 (44)
Positive	34 (24)
Not detected	45 (32)
Conditioning regimen (%)	
TBI containing	98 (70)
Non TBI	43 (30)
GVHD prophylaxis (%)	
CYA	92 (65)
Tacrolimus	35 (25)
Others	14 (10)
MTX containing	80 (56)
Not MTX containing	61 (44)
HLA compatibility (GVH direction, low-resolution typing) (%)	
HLA identical	32 (22)
1 HLA disparity	91 (65)
2 HLA disparities	17 (13)

in Table 2. Standard short-term MTX prophylaxis for GVHD was given at a dose of 10–5 mg/m^2 on day 1 and 7.5–10 mg/m^2 on days 3, 6 and 11. Supportive therapy differed among the transplant centers. Protocols for intensive myeloablative therapy and the use of UCBs for transplantation were reviewed and approved by the institutional review boards at the transplantation centers.

Statistical methods

For this analysis, 1 July 2007 was used as the reference date (that is, the day on which all centers locked data on patient outcomes). The median duration of follow-up was 31 months (range, 3–92 months). The outcome end points were neutrophil recovery, platelet recovery, GVHD, relapse, TRM, OS and disease-free survival (DFS). Categorical data in cross-tabulation tables were compared by using the Fisher's exact test. Neutrophil recovery was defined by an ANC of at least 0.5×10^9 per liter for 3 consecutive days, the first of which was used as the recovery day. Platelet recovery was defined by a nontransfused platelet count of at least 20×10^9 per liter for 7 consecutive days. Death occurring before day 90 or 180 was considered as a competing risk for neutrophil or platelet recovery, respectively. Graft failure rates for neutrophils or platelets were calculated for patients surviving without relapse or autologous infusion (competing events) for more than 90 or 180 days, respectively. Acute and chronic GVHD were diagnosed and graded at each center according to standard criteria.[24,25] Relapse was defined on the basis of morphological evidence of leukemia in the BM or other extramedullary organs. TRM was defined as all causes of nonleukemic death occurring after transplantation. OS was the time between transplantation and death due to any cause. DFS was defined as the time interval from UCBT to the first event, either relapse or death, during CR. These outcomes were all right censored. For OS and DFS, the Kaplan–Meier method provided an estimate of the incidences over time, whereas the Cox models were used to evaluate the joint influence of patient-, disease- and transplant-related variables on the outcome. However, the other end points shared a competing risk setting, if patients developed events that avoided the occurrence of the event of interest. For example, after death or a relapse before engraftment, no recovery or GVHD could occur. Therefore these end points (neutrophil and platelet recovery, acute and chronic GVHD, relapse and TRM) were analyzed by using cumulative incidence curves for estimating the incidence over time, and Fine and Gray models were used to assess prognostic factors.[26,27] For all models, we first fitted univariate models that contained each of the variables one at a time. Second, the values of all variables with a *P*-value below 0.1 by the likelihood ratio test were included in a multivariate model. Cause-specific hazard ratios were estimated with 95% confidence intervals. All statistical analyses were performed with STATA software version 8.0 (Stata Inc., College Station, TX, USA).

Results

Neutrophil and platelet recoveries

The cumulative incidence of neutrophil recovery at day 90 was 78.7 ± 3.4%. During the first 90 days after transplantation, competing risk for neutrophil recovery was death ($n = 11$). The graft failure rate for neutrophil recovery was 21% (30 of 141 patients). For those patients who recovered, the median time to achieve an ANC equal to or more than 0.5×10^9 per liter was 28 days (range, 13–90 days). In univariate analysis, factors associated with neutrophil recovery were as follows—(1) status of disease at transplantation: cumulative incidence of neutrophil recovery at day 90; the neutrophil recovery rate was 82.9 ± 4.3% for children who received transplants in remission (CR1, CR2 and CR3) vs 73.8 ± 5.5% for those with non-CR; $P = 0.04$; (2) the CD34$^+$ cell dose: the cumulative incidence of neutrophil recovery rate was 89.7 ± 3.9% when 1.35×10^5 per kg or more CD34$^+$ cells were infused vs 60.9 ± 7.2% when less than 1.35×10^5 per kg CD34$^+$ cells were infused; $P < 0.0001$; Figure 1a. There was a trend toward neutrophil recovery when prophylactic use of G-CSF was present (79.4 ± 3.9 vs 76.5 ± 7.3% when hematopoietic growth factor was not used, $P = 0.092$). No other factors affecting neutrophil recovery had a significant influence. In multivariate analysis, the factor associated with an improved neutrophil recovery was infusion of CD34$^+$ cells at a dose of 1.35×10^5 per kg or more (Table 3).

The day 180 cumulative incidence of platelet recovery was 62.4 ± 4.9%. During the first 180 days after transplan-

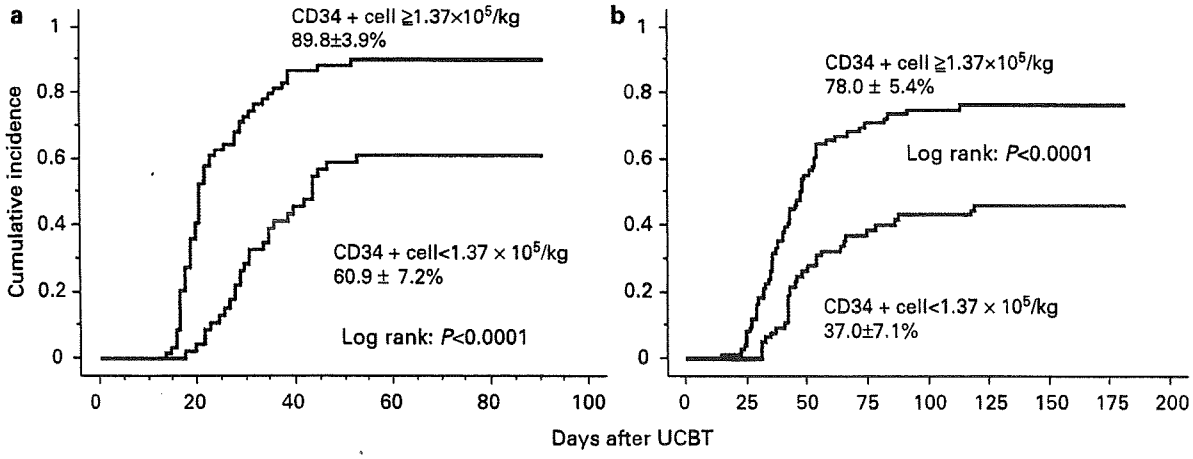

Figure 1 The cumulative incidence of neutrophil recovery. Cumulative incidence of neutrophil recovery according to the number of $CD34^+$ cell per recipient's body weight (**a**) and platelet recovery according to the number of $CD34^+$ cell per recipient's body weight (**b**).

Table 3 Multivariable analyses of risk factors for the main outcomes after UCBT for childhood AML

Factors	*Hazard ratio (95% confidence interval)*	P-*value*
Neutrophil recovery		
Infused CD34 cell dose 1.35×10^5 per kg or more	2.77 (1.73–4.45)	<0.0001
Platelet recovery		
CR1–3 at transplantation	2.24 (1.32–3.81)	0.0028
Infused CD34 cell dose 1.35×10^5 per kg or more	3.55 (2.02–6.25)	<0.0001
Relapse		
Advance status (no CR) at transplantation	0.25 (0.14–0.49)	<0.0001
Age under 4 years	0.46 (0.23–0.91)	0.028
Transplantation-related mortality at day 100		
Use of MTX for GVHD prophylaxis	0.21 (0.064–0.92)	0.035
Disease-free survival		
CR1–3 at transplantation	0.24 (0.15–0.4)	<0.0001
Overall survival		
CR1–3 at transplantation	0.269 (0.15–0.50)	<0.0001

tation, the competing risk for platelet recovery was death ($n=34$). The graft failure rate was 37.8% for platelet recovery (53 of 141 patients). For patients who recovered, the median time to achieve platelet recovery was 56 days (range, 14–180 days). In univariate analysis, the factors associated with platelet recovery were as follows—(1) status of disease at transplantation: the cumulative incidence of platelet recovery at day 180 was $76.3 \pm 4.9\%$ for children who received transplants in remission (CR1, CR2 and CR3), compared with $46.2 \pm 6.2\%$ for patients who received transplants in a more advanced phase (non-CR); $P<0.0001$; (2) $CD34^+$ cell dose: the cumulative incidence of platelet recovery rate was $78.0 \pm 5.4\%$ when 1.35×10^5 per kg or more $CD34^+$ cells were infused vs $37 \pm 7.1\%$ when less than 1.35×10^5 per kg $CD34^+$ cells were infused; $P<0.0001$; Figure 1b; (3) short-term MTX for GVHD prophylaxis: the cumulative incidence of platelet recovery rate was 70.0 ± 5.1 vs $52.5 \pm 6.4\%$ when MTX was not used; $P=0.016$. The use of prophylactic hematopoietic growth factor and period of transplantation were not significantly associated with the speed or incidence of platelet recovery. In multivariate analysis, the factors associated with an improved platelet recovery were CR at transplantation (CR1, CR2 and CR3) and $CD34^+$ cell dose (infusion of 1.35×10^5 per kg or more $CD34^+$ cells) (Table 3).

Acute and chronic GVHD

Acute GVHD (grade II or more) was observed in 54 patients (44 in grade II, 8 in grade III and 2 in grade IV). The 100-day cumulative incidence of acute GVHD was 40.1. No patient-, disease-, or transplant-related factors that could be associated with the incidence of acute GVHD were found. Notably, the number of HLA disparities of GVH direction between CB and recipient was not significantly associated with grades II–IV acute GVHD. Two years after UCBT, the cumulative incidence of chronic GVHD was $14.7 \pm 3.5\%$. Of the 123 patients at risk, 15 presented signs of chronic GVHD (10 with limited and 5 with extensive signs).

Early transplant-related mortality

During the first 100 days after UCBT, 15 patients succumbed to 29 episodes of transplantation-related complications (11 episodes of infection, 6 episodes of bleeding and 13 other events) and competing risks for TRM at day 100 (100-day TRM) was relapse ($n=21$). The main cause of CBT-related death was interstitial pneumonia. The cumulative incidence of 100-day TRM was $10.8 \pm 2.6\%$. In univariate analysis, the following factors were associated with an increased risk of death—(1) the use of short-term MTX for GVHD prophylaxis: the death rate was $19.9 \pm 5.1\%$ when MTX was not used vs $3.8 \pm 2.2\%$ when used, $P=0.0026$, Figure 2a; (2) date before December 1999: the death rate was 26.9 ± 8.7 vs $7.0 \pm 2.4\%$, $P=0.0031$,

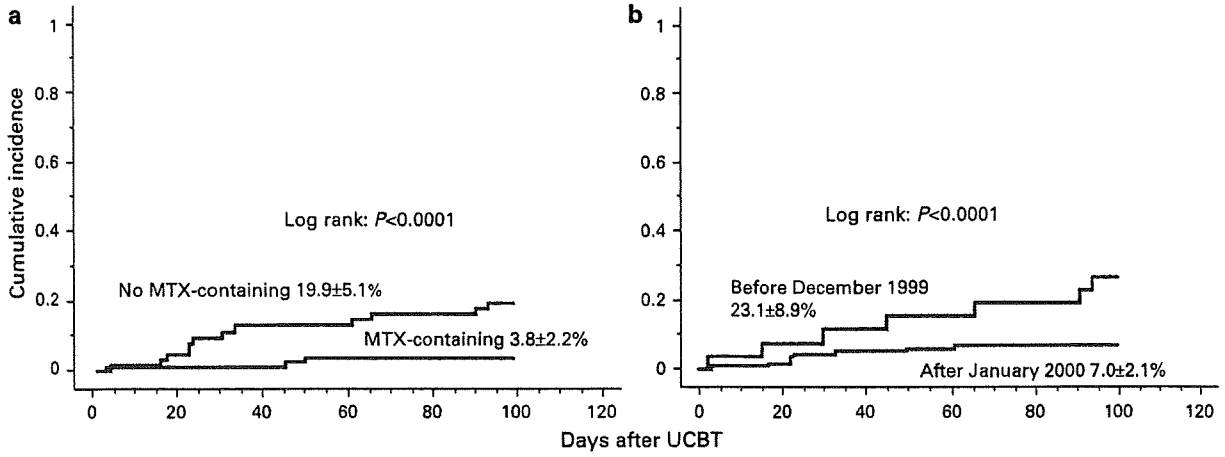

Figure 2 The cumulative incidence of 100-day transplantation-related mortality (100-day TRM). Cumulative incidence of 100-day TRM according to use of MTX for prophylaxis for GVHD (a) and 100-day TRM according to the date of unrelated cord blood transplantations (b).

Figure 2b. Fisher's exact test showed that proportional deviations between the use of short-term MTX for GVHD prophylaxis and the time of UCBT were significant (19 of 26 patients were not treated with short-term MTX in or before 1999, whereas 42 of 115 patients were not treated with MTX after 2000, $P=0.0009$). Multivariate analysis showed that the only factor associated with an increased early TRM was the non-use of short-term MTX as part of the GVHD prophylaxis (Table 3). There was marginal significant difference between a trend toward an increased risk of death when patients received infusion of $CD34^+$ cells at doses below 1.35×10^5 per kg (13.1 vs 5.2% for those receiving more than 1.35×10^5 per kg, $P=0.051$).

Relapse incidence

Hematological relapses occurred in 46 patients after UCBT, and 27 patients succumbed with experiencing disease recurrence. The 6-year cumulative relapse incidence (RI) was 41.3 ± 4.8%. In univariate analysis, the following two factors were associated with an increased risk of relapse: age under 4 years (51.0 ± 6.2 vs 26.5 ± 7.0%, $P=0.0027$); non-CR at transplantation (68.2 ± 7.3% for more advanced disease status vs 23.5 ± 5.4% for those in remission (CR1, CR2 and CR3, $P<0.00001$; Figure 3a). More precisely, the 6-year cumulative RI was 15.2 ± 6.3% for patients who received transplants in CR1, 33.7 ± 9.3% for those in CR2 and 68.2 ± 7.5% for those with non-CR at the time of UCBT. The RI after UCBT in children presenting poor-risk cytogenetic abnormalities was 25.4 ± 9% compared with 47.1 ± 7% in others ($P=0.12$). The absence of previous acute GVHD (grades II–IV) was not associated with an increased RI ($P=0.62$). The patients were divided into two groups for each of two separate categories: a poor-risk group and a favorable-risk and an intermediate-risk group, according to karyotype; and groups within and beyond the median period (330 days) of initial remission. However, no statistically significant differences were noted among these groups. In multivariate analysis, the two factors associated with an increased relapse rate were lack of CR at UCBT and age under 4 years at UCBT.

Disease-free survival, overall survival and causes of death

A total of 64 patients succumbed; causes of death were disease relapse in 27, transplantation-related events in 29 (Table 4), unknown etiology in 4 and other reasons in 6. The estimated 6-year OS and DFS were 46.1 ± 5.0 and 39.3 ± 4.7%, respectively. In univariate analysis, the most significant factor was disease status at the time of UCBT. The 6-year DFS was 53.8 ± 6.9% for children who received transplants during remission (CR, CR2 and CR3), in contrast to 0% for those with non-CR ($P<0.0001$; Figure 3b). The 6-year DFS was 67.7 ± 7.8% for children who received transplants during the CR1, 49.6 ± 11.3% for those in the CR2, 75 ± 21% for those in CR3 or beyond and 0% for non-CR at the time of UCBT. No other factors affecting DFS had a statistically significant influence. More precisely, the 6-year DFS were 53.3 ± 8.9% for children with a poor prognostic karyotypes, 41.6 ± 7.6% for those with intermediate karyotypes and 39.6 ± 9.0% for those with favorable karyotypes ($P=0.67$), 39.7 ± 5.7% for children received non-TBI-based conditioning regimen and 0% for those with TBI regimen ($P=0.66$). The 6-year DFS was not significantly affected by the frequency of exposure to HLA mismatches; 38.4 ± 9.3% for children who received transplants with HLA-identical donor, 19.6 ± 14.2% for those with HLA 1 mismatch, and 43.3 ± 13.6% for those with HLA 2 mismatch ($P=0.93$). In multivariate analysis, the only factor associated with DFS was CR at UCBT (Table 3).

The 6-year OS was 65.6 ± 6.8% for children who received transplants during remission (CR, CR2 and CR3) vs 20.6 ± 6.2% for those without CR ($P<0.0001$). More precisely, the 6-year OS was 70.4 ± 8.3% for those who received transplants in the first CR, 59.3 ± 11.3% for those in the second CR, 75 ± 21% for those in CR3 and 20.6 ± 6.2% for those who were non-CR at the time of UCBT (Figure 3c). There was marginal significant difference between prophylactic use of MTX for GVHD and no use of MTX for GVHD (51.7 ± 6.7 vs 39.7 ± 7.3%, $P=0.074$). No other factors affecting OS had a statistically significant influence. In multivariate analysis, the only factor associated with OS was CR at UCBT (Table 3).

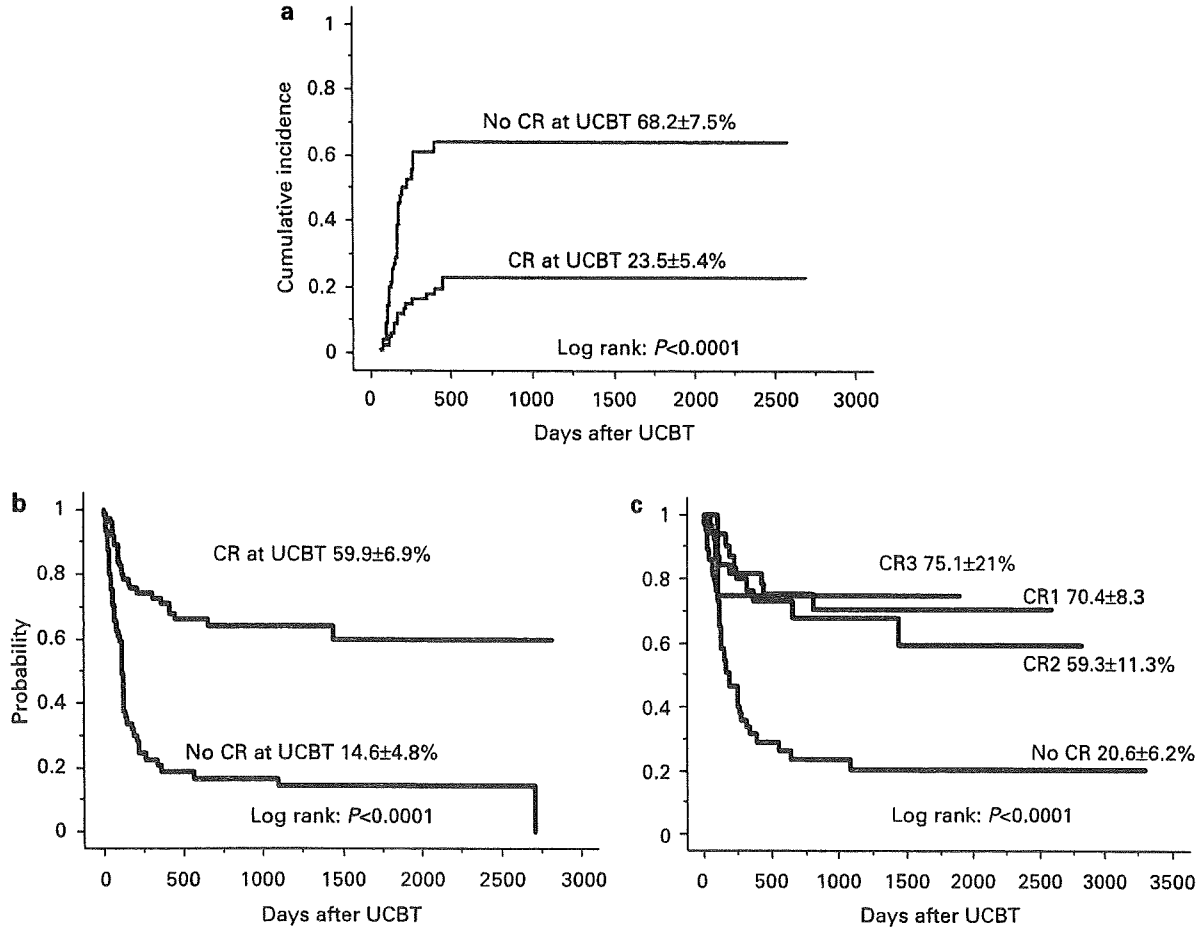

Figure 3 Six-year cumulative relapse incidence (RI). Cumulative incidence of relapse according to disease status at unrelated cord blood transplantations (**a**), 6-year disease-free survival. Probability of disease-free survival according to disease status at unrelated cord blood transplantations (**b**), and 6-year OS. Probability of overall survival according to disease status at unrelated cord blood transplantations (**c**).

Table 4 TRM after UCBT in 29 cases

Infection	16
IP	5
Bacterial infection	5
Fungal infection	4
Infection from unknown origin	1
Viral infection	1
Bleeding	4
ARDS	3
VOD	2
BO	2
TMA	1
GVHD	1

Abbreviations: ARDS = acute respiratory distress syndrome; BO = bronchitis obliterans; IP = interstitial pneumonia; TMA = thrombotic microangiopathy; VOD = veno-occulsive disease.

Discussion

To the best of our knowledge, this is the first 6-year retrospective registry-based analysis to have been specifically designed to clarify the results of UCBT for childhood AML in Japan. As expected, and described previously,[19] it was found that outcome was associated with disease status at the time of transplantation. UCBT applied during remission for pediatric AML is important, and gives a better outcome when conducted in children with active leukemia. Only four patients in the present series were in CR3. Future studies using a larger number of patients are therefore warranted.

Recently, in cases of pediatric AML during remission, there has been a tendency to base the indication for HSCT on the risk classification at the time of diagnosis.[28] In the Medical Research Council 12 study, the estimated probability of 5-year survival from CR in 51 high-risk patients (including 31 with BMT from sibling donors at first CR) was 47%, and DFS 41%.[1] In the BFM 1993 study, the 5-year event-free survival of 310 children with AML treated with HSCT was 50%, whereas their OS was 54%.[2] In our study, the DFS and OS in 39 patients with AML treated with UCBT from unrelated donors (conducted during the first remission) were 67.7 ± 7.8 and 70.4 ± 8.3%, respectively. Our results are therefore comparable to those of other studies. For some reasons, unclear at this time, survival rate in our study is higher than previously published.

The prognosis of AML after recurrence is poor, with a survival rate ranging from 21 to 33%, according to recent

reports.[5–8] The present study indicated that the DFS and OS among 33 children with AML treated with UCBT during the second CR were 49.6 and 59.3%, respectively. However, several recent reports have indicated that CR1 > 12 months is critical to salvage and improve OS rates in children with AML without the benefit of SCT;[5] the OS is around 50% in this group of patients. Further investigation is needed because our patients who suffered relapses comprised a heterogeneous group: among the 33 patients in our series who were in CR2, 24 had CR1 >12 months and were still responsive to chemotherapy, and 9 suffered recurrence less than 12 months after initial diagnosis. The DFS and OS of these nine patients were 45.5 ± 18.8 and 72.9 ± 16.5%, respectively. This indicates that UCBT at CR2 may achieve results similar to those reported by others, even in AML patients with early recurrence. The results of UCBT were particularly promising for children with high-risk AML at CR1, and the procedure also holds promise for those who are in CR2 and CR3 after early relapse. The outcome of UCBT for non-CR children with AML was poor in our study. For those patients, a haploidentical HSCT may be another stem cell source. However in non-CR children with AML, convincing studies are missing comparing stem cell sources, conditioning regimen or graft manipulation.[29] Therefore, no final judgment on these issues is possible to date. The karyotype of malignant cells is known to be one of the most relevant predictors of treatment outcome in childhood AML.[28] In our study, 24% of the patients for whom cytogenetic analysis was successful were classified into the poor-risk cytogenetic category. Interestingly, however, the 6-year DFS and the incidence of relapse among these children with a poor-risk karyotype were not significantly different from those in children with a favorable-risk karyotype (53.3 ± 8.9 vs 30.6 ± 9.9%, $P = 0.52$ and 30.7 ± 9.2 vs 39.1 ± 10.0%, $P = 0.94$, respectively).

In the present study, the 100-day cumulative TRM was 10.8%. This incidence is lower than those reported in other series of studies on children receiving UCBT.[17,19,30,31] Apparently, TRM constitutes a principal obstacle discouraging wider application of UCBT in children with high-risk AML, as it does to CBT in many other diseases. When short-term MTX was used for GVHD prophylaxis, the 100-day TRM was reduced to 3.8%. Short-term MTX has been demonstrated to be effective for GVHD prophylaxis when combined with a calcineurin inhibitor in patients receiving BM or PBSC transplantation.[32–34] Recently, it has been demonstrated that short-term MTX was associated with a lower rate of post transplantation immune reactions without compromising engraftment, resulting in better OS in adult UCBT recipients.[34] It has been suggested that the favorable impact of short-term MTX is due to attenuation of organ damage resulting from post-UCBT immune reactions and the avoidance of corticosteroids to reduce the risk of infectious complications. However, the effectiveness of short-term MTX in UCBT for children with AML remains unclear. In UCBT with short-term MTX for GVHD prophylaxis, the 100-day TRM was significantly reduced. This suggests that short-term MTX, when applied to pediatric AML as a single-disease entity, also suppresses any early immune reaction, as is the case in adults. It is likely that the use of short-term MTX ultimately affects the incidence of TRM. This is the first report describing the efficacy of short-term MTX in association with UCBT for treatment of AML in children. Univariate analysis showed that the incidence of 100-day TRM was 23.1% in transplantations carried out before December 1999 ($n = 26$), and 6.1% thereafter ($n = 115$). In addition, when the dose of infused $CD34^+$ cells exceeded the median (1.35×10^5 per kg), the 100-day TRM incidence tended to decrease (to 5.2%),[35] showing that the dose of infused $CD34^+$ cells was a more potent indicator of prognosis than that of nucleated cells. In that study, there was a threshold of 1.7×10^5 $CD34^+$ cells per kg, and the authors suggested that UCB units containing less than this dose of $CD34^+$ cells should be considered inadequate for routine use because of the very high TRM risk.[35] Our present study is the first reported study to have produced similar results for a single-disease entity. These issues are crucial in the choice of UCBT for any given patient, but would be more efficiently addressed in large registry studies than in a disease-specific study like ours. The rarity of use of short-term MTX for prevention of GVHD before 1999 may explain the high incidence of TRM at that time. Our findings indicate that to reduce the incidence of TRM shortly after UCBT for pediatric AML, it is essential to use a sufficient dose of $CD34^+$ cells to hasten the recovery of the neutrophil population, and to administer simultaneously short-term MTX to prevent early immune reactions.

The incidences of relapse are comparable to those reported after BMT from unrelated HLA-matched donors.[9–11] In the Seattle experience of 161 patients with AML who received unmanipulated BM transplants from unrelated donors, the cumulative incidences of relapse were 19% in CR1, 23% in CR2, 25% in subsequent CR, 44% during relapse and 63% during primary induction failure.[10] Among the 91 patients with AML who received CBT from unrelated donors in the analysis conducted by the Eurocord Group[19], the cumulative incidences of relapse were 10 ± 7% in CR1, 23 ± 7% in CR2, 1 of 4 patients in subsequent CR and 61 ± 11% during primary induction failure. The present study included an evaluation of the association of well-identified prognostic factors of childhood AML, such as the karyotype of the malignant cells,[28] and the duration of the first CR in children who received transplants during their second CR.[36] Our results suggest that these two prognostic factors, identified in patients with AML undergoing concomitant chemotherapy or standard allogeneic BMT, may not have the same predictive value in the context of unrelated UCBT. This potent antileukemic effect in poor-risk AML supports the hypothesis of an adequate GVL effect after UCBT. Our results revealed that transplantation during a period other than that of remission constitutes the sole risk factor for relapse.

The results of UCBT were particularly promising for children with a karyotype suggesting a poor prognosis, and in those who received transplants in CR2 and CR3 after an early relapse. In the absence of a randomized trial, we cannot definitively state the relative efficacy of BM and CB grafts, but the data support the use of CB grafts in children with acute leukemia.

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Appendix

Transplant Centers

Transplant centers that performed CBT by the JCBBN coordination and produced follow-up reports are: Division of Hematology/Oncology, Shizuoka Children's Medical Center, Shizuoka; Department of Pediatrics, Dokkyo Medical University, Mibu; Department of Pediatrics, Mie University School of Medicine, Mie; Department of Pediatrics, Kobe City Medical Center General Hospital, Kobe; Department of Pediatrics, Sapporo Medical University School of Medicine, Sapporo; Department of Pediatrics, Okayama University Graduate School of Medicine and Dentistry, Okayama; Department of Pediatrics, Ibaraki Children's Hospital, Mito; Department of Pediatrics, Yamagata University School of Medicine, Yamagata; Division of Haemato-Oncology/Regulation Medicine, Kanagawa Children's Medical Center, Yokohama; Division of Pediatric Hematology/Oncology, Nagoya Japanese Red Cross First Hospital, Nagoya; Department of Hematology/Oncology, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka; Department of Pediatrics, Kyoto University Graduate School of Medicine, Kyoto; Department of Pediatrics, Yokohama City University School of Medicine, Yokohama; Division of Pediatric Oncology, National Cancer Center Hospital, Tokyo; Department of Hematology, Toranomon Hospital, Tokyo; Department of Pediatrics, Hokkaido University Graduate School of Medicine, Sapporo; Department of Pediatric Hematology and Oncology, Tohoku University School of Medicine, Sendai; Department of Pediatrics, Niigata Cancer Center Hospital, Niigata; Department of Pediatrics, Yamaguchi University School of Medicine, Ube; Department of Pediatrics, Shizuoka Red Cross Hospital, Shizuoka; Department of Pediatrics, University of Occupational and Environmental Health, Kitakyushu; Department of Pediatrics, Osaka City University Graduate School of Medicine, Osaka; Department of Pediatrics, Nippon Medical School, Tokyo; Department of Pediatrics, Graduate School of Medicine, Fukui University, Fukui; Department of Pediatrics, Keio University School of Medicine, Tokyo; Department of Pediatrics, Osaka University Graduate School of Medicine, Suita; Department of Pediatrics, Tokushima University School of Medicine, Tokushima; Department of Pediatrics, Nagoya University Graduate School of Medicine, Nagoya; Department of Pediatrics, Shiga University of Medical Science, Otsu; Department of Pediatrics, Kagawa University Faculty of Medicine, Kagawa; Department of Pediatrics, Nihon University School of Medicine, Tokyo; Department of Pediatrics, Tokai University School of Medicine, Hatano; Division of Pediatrics, Iwate Prefectural Kitakami Hospital, Kitakami; Division of Hematology, National Center for Child Medical Health and Development, Tokyo; Division of Hematology/Oncology, Saitama Children's Medical Center, Iwatsuki; Department of Pediatrics, Matsushita Memorial Hospital, Moriguchi; Department of Pediatrics, Hamanomachi Hospital, Fukuoka; Department of Pediatrics, School of Medicine, University of Yamanashi, Yamanashi; Department of Pediatrics and Developmental Biology, Tokyo Medical and Dental University, Tokyo; Department of Pediatrics, Saga University, Saga; Department of Pediatrics, Faculty of Medical and Pharmaceutical Sciences, Kumamoto University, Kumamoto; Department of Pediatrics, Hyogo Children Hospital, Hyogo; Department of Pediatrics, Hirosaki University School of Medicine, Hirosaki; Department of Pediatrics, Hyogo Medical University, Hyogo; Department of Pediatrics, Graduate School of Medical Sciences, Kyushu University, Fukuoka; Department of Pediatrics, Ehime University, Toon; Department of Hematology and Oncology, Chiba Children's Hospital, Chiba; Department of Pediatrics, Iwate Medical University School of Medicine, Morioka; Division of Pediatric Hematology/Oncology, Japanese Red Cross Nagoya Second Hospital, Nagoya; Department of Pediatrics, Kobe University Graduate School of Medicine, Kobe; Department of Pediatrics, Faculty of Medicine, University of the Ryukyus, Nishihara; Division of Hematology and Oncology, Seirei Hamamatsu General Hospital, Hamamatsu; Department of Pediatrics, Kagoshima University Graduate School of Medicine, Kagoshima; Department of Pediatrics, Saitama Medical University, Moroyama; Department of Pediatrics, St Luke's International Hospital, Tokyo; Division of Pediatrics, Osaka City General Hospital, Osaka; Department of Pediatrics, National Hospital Organization Nagoya Medical Center, Nagoya; Division of Hematology/Oncology, Nagano Children's Hospital, Azumino; Division of Hematology, Saiseikai Maebashi Hospital, Maebashi.

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ORIGINAL ARTICLE

Improved outcome of refractory Langerhans cell histiocytosis in children with hematopoietic stem cell transplantation in Japan

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Langerhans cell histiocytosis (LCH) that is refractory to conventional chemotherapy has a poor outcome. Hematopoietic stem cell transplanta tion (SCT) is a promising approach for refractory LCH because of its immunomodulatory effect. In this study, the outcomes of children with refractory LCH undergoing SCT in Japan were analyzed. Between November 1995 and March 2007, 15 children younger than 15 years (9 males, 6 females) with refractory LCH underwent SCT. The patients' median age at diagnosis was 8 months (range, 28 days to 28 months), and all had failed conventional chemotherapy. The median age at SCT was 23 months (range, 13–178 months). Nine had risk organ involvement at diagnosis, including liver ($n=6$), spleen ($n=5$), lung ($n=5$), and/or hematopoietic system ($n=4$). For SCT, a myeloablative regimen was used for 10 patients, and a reduced-intensity conditioning regimen (RIC) was used for five. The donor source varied among the patients, but allogeneic cord blood was primarily used ($n=10$). Subsequently, 11 of 15 patients have survived with no evidence of disease, with a 10-year overall survival (OS) rate (median ± standard error) of 73.3 ± 11.4%. The 10-year OS rate of nine patients with risk organ involvement at diagnosis was 55.6 ± 16.6%, whereas six without risk organ involvement have all survived with no evidence of disease ($P=0.07$). These results indicate that SCT is promising as a salvage approach for children with refractory LCH.

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Keywords: Langerhans cell histiocytosis; refractory; stem cell transplantation; reduced intensity conditioning

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Introduction

Langerhans cell histiocytosis (LCH) is a rare disease with a wide variety of clinical presentations, from localized disease to disseminated disease.[1–3] Although the risk factors for LCH have not been fully elucidated, patients younger than 2 years of age at onset, with risk organ involvement, including the hematopoietic system, liver, spleen, or lung, and disease refractory to conventional chemotherapy have a very poor outcome, with survival rates of about 20%.[4–8]

The treatment strategy for these high-risk LCH patients has not yet been established. Recently, it was reported that a combination of 2-chlorodeoxyadenosine (2-CdA) and cytarabine (Ara-C) was effective for refractory LCH.[9] Allogeneic stem cell transplantation (SCT) has also been used because of its strong immunomodulatory effects for LCH.[10,11] This report describes the improved outcomes of 15 children with refractory LCH who underwent SCT in Japan.

Patients and methods

Data collection

The HLH/LCH committee of the Japanese Society of Pediatric Hematology (JSPH) sent the first questionnaires to all hospitals in Japan where pediatric hematologists (JSPH members) worked, asking for the number of children with LCH who underwent SCT between November 1995 and March 2007. The second questionnaires were then sent to 16 hospitals where SCT was done for LCH, asking about the clinical features at onset, treatment before SCT, donor source, conditioning regimen, complications, and outcome. Thirteen hospitals responded to the second questionnaires, with a total of 15 eligible patients. The registration data of the pediatric SCT program, independently managed by the SCT committee of the JSPH, were also available to confirm the profiles of the patients who underwent SCT.

Diagnostic criteria and definition of disease state
All patients were diagnosed as having LCH by histopathological examination of the affected organs, which were positive for either CD1a or S100 staining. Each patient was divided into one of three subsets at diagnosis: single system single-site (SS-s), single system multi-site (SS-m), and multi-system (MS). In the MS subset, patients with one of the following factors were classified as the high-risk group: younger than 2 years of age at onset; risk organ involvement, including the hematopoietic system (bone marrow, BM), liver, spleen, or lung; or disease refractory to conventional chemotherapy. Patients without these factors in the MS, SS-m, and SS-s subsets were classified as the low-risk group. The characteristics of these 15 patients are shown in Table 1. The disease state was evaluated at three time-points in all patients: (1) within 6 weeks after initial diagnosis, (2) within 12 or 14 weeks after diagnosis, and (3) before SCT. A good response (GR) was defined as the disappearance of signs or symptoms of disease; a partial response (PR) was defined as regression $>50\%$ of signs or symptoms of disease with no organ dysfunction and no new lesions; a non-response (NR) was defined as regression $<50\%$ of signs or symptoms of disease with or without organ dysfunction and no new lesions; and progressive disease (PD) was defined as progressive signs or symptoms of disease and/or the appearance of new lesions.[8]

Statistical analyses
Continuous variables were compared using the Mann–Whitney *U*-test. The overall survival (OS) rate with standard error (s.e.) was estimated using the Kaplan–Meier method and compared using the log-rank test.[12] The OS was calculated for the period from the day of diagnosis until the day of death from any cause. The outcome data were updated in December 2008.

Results

Clinical course of 15 patients before SCT
Between November 1995 and March 2007, 15 children (9 males, 6 females) with LCH refractory to conventional chemotherapy underwent SCT at 13 institutions. The characteristics of all 15 patients are summarized in Table 1, and the details of each patient are shown in Table 2. The median age at diagnosis was 8 months (range, 28 days to 28 months). At initial diagnosis, 12 patients had MS type LCH, and nine had risk organ involvement, including the liver ($n=6$), spleen ($n=5$), lung ($n=5$), and/or hematopoietic system ($n=4$). One patient had diabetes insipidus (DI) and pituitary gland involvement at diagnosis. No CNS disorders other than DI were found in any of the patients. Two patients had SS-m type LCH at diagnosis, with multiple bone lesions. One patient had SS-s type LCH, with thymus involvement and respiratory dysfunction at initial diagnosis.

Eleven patients had received conventional chemotherapy according to the study protocol JLSG-96 ($n=4$) or JLSG-02 ($n=4$) of the Japan LCH study group,[8] and DAL-HX 83 ($n=3$) of the Deutsche Arbeitsgemeinschaft fur Leukaemieforschung Histiocytosis X-83 study group.[4] Remaining four patients had received multi-drug chemotherapy following their institutional protocol. Two patients received the combination of 2-CdA and high dose Ara-C before SCT as salvage therapy and failed to achieve complete remission (patients 6 and 8). Six weeks after initial diagnosis, the disease state was PD ($n=6$), NR ($n=2$), PR ($n=5$), and GR ($n=2$). Twelve or 14 weeks after chemotherapy, the disease state was PD ($n=6$), NR ($n=0$), PR ($n=6$), and GR ($n=3$). Eleven patients with MS type, two with SS-m type, and one with SS-s type at diagnosis had risk organ involvement before SCT.

The median age at SCT of 15 patients was 23 months (range, 13–178 months). At SCT, 14 patients had risk organ involvement. The affected organs before SCT were liver ($n=10$), hematopoietic system ($n=7$), and lung ($n=4$). Five of six patients in the low-risk group had risk organ involvement at SCT.

The disease status of six patients (patients 2–6 and patient 8) in the high-risk group was PD with risk organ involvement at SCT. The disease status of patient 2 was PD despite chemotherapy according to the JLSG-02 protocol, and liver dysfunction developed gradually. BM involvement was observed in patients 3–6, patient 8, patient 9, and patient 13 despite multi-drug chemotherapy. Patient 3, patient 6, and patient 8 suffered from serious infections before SCT. All six of these patients underwent SCT during 8–11 months after diagnosis. The disease status of the other three patients (patient 1, patient 7, and patient 9) was PR or NR with risk organ involvement at SCT. These three patients underwent SCT for 12–30 months after diagnosis.

Three of the six patients in the low-risk group (patient 10, patient 12 and patient 14) had suffered from recurrent active disease for 6, 13, and 7 years, respectively, despite chemotherapy consisting of vincristine, pirarubicin, prednisone, Ara-C, vinblastine, etoposide, and cyclophosphamide with or without cyclosporine. At recurrence, lung involvement and left phrenic nerve palsy were observed in patient 10, who required oxygen for 2 months. Patient 12 had recurrence of multiple bone lesions, including the ear, and needed a hearing aid. She relapsed twice with multiple

Table 1 Summary of the clinical characteristics of the LCH patients who underwent SCT

No. of patients	15
Age, median (range)	8 months (28 days to 28 months)
Sex, male/female	9 males, 6 females
Stage at diagnosis	
MS	12
SS-m	2
SS-s	1
Age at SCT, median (range)	23 months (13–178 months)
Allo-SCT/Auto-SCT	13 allo, 2 auto
Time from diagnosis to SCT	
Median (range)	12 months (7–164 months)
Observation time	
From SCT ($n=11$), median (range)	100 months (20–158 months)
From Dx ($n=11$), median (range)	110 months (27–277 months)

Abbreviations: allo = allogeneic; auto = autologous; Dx = diagnosis; SCT = stem cell transplantation.

Table 2 Detailed characteristics of the 15 patients with refractory LCH who underwent SCT

Patient No.	*Sex*	*Onset*				*At SCT*					*Outcome*[b]
		Age	*Involved organ*	*Type of LCH*	*Initial response*	*Age*	*Involved organ*	*Disease status*	*Donor source*	*Conditioning regimen*[a]	
1	M	3 months	LIV, SPL, LU, skin, bone, LN	MS	PR	23 months	LIV, skin, bone, LN	PR	CB (sibling)	RIC (Flu, PAM)	Alive in CR (+73 months)
2	M	5 months	LU, skin	MS	PD	13 months	LIV	PD	PB (mother)	RIC (Flu, PAM)	Died (18 days)
3	F	5 months	LIV, SPL, LN	MS	NR	14 months	BM, LIV	PD	UCB	Myeloab (TBI, CY, ATG)	Alive in CR (+92 months)
4 (1st)	F	8 months	BM, LIV, skin, bone, LN, thymus	MS	PD	17 months	BM, LIV	PD	PB (father)	RIC (Flu, CY, TBI)	
4 (2nd)						19 months	BM, LIV, LU	PD	PB (father)	RIC (TBI)	Relapse and died (271 days)
5	M	8 months	BM, LIV, SPL, LU, skin, bone	MS	PD	17 months	BM, LIV	PD	UCB	Myeloab (TBI, VP16, PAM)	Alive in CR (+107 months)
6	M	8 months	LIV, SPL, skin, middle ear, LN	MS	NR	16 months	BM	PD	UCB	RIC (Flu, PAM, TBI)	Alive in CR (+20 months)
7	F	9 months	BM, LIV, SPL, LU, skin, bone, thymus, pancreas	MS	PD	21 months	LIV, SPL, skin, bone, thymus	NR	UCB	Myeloab (TBI, CY)	Relapse and died (47 days)
8	F	15 months	LU, skin, bone	MS	PR	27 months	BM, LIV	PD	UCB	Myeloab (TBI, CY, VP16)	Died (188 days)
9	M	21 months	BM, pituitary, DI	MS	PR	51 months	BM, pituitary, DI, bone	NR	BM (sibling)	Myeloab (CY, VP16)	Alive in CR (+158 months)
10	M	28 days	Skin, LN, bone, mediastinal mass	MS	PD	83 months	LU	PD	autoPB	Myeloab (VP16, TEPA, IFO)	Relapse and alive (+109 months)
11	F	6 months	Skin, intestine	MS	PD	16 months	LIV, LU	PD	UCB	Myeloab (Flu, PAM, BU)	Alive in CR (+39 months)
12	F	13 months	LN, middle ear	MS	GR	178 months	Bone, pituitary, DI	PD	autoPB	Myeloab (VP16, TEPA, IFO)	Relapse and alive (+113 months)
13	M	4 months	Bone	SS-m	PR	35 months	BM, LIV	PD	UCB	RIC (Flu, PAM, ALG, TLI)	Alive in CR (+38 months)
14	M	28 months	Bone	SS-m	GR	122 months	LIV	PD	UCB	Myeloab (Flu, PAM, BU)	Alive in CR (+144 months)
15	M	9 months	Thymus	SS-s	PR	22 months	LU, skin, thymus, LN, gingiva	PR	UCB	Myeloab (TBI, CY, VP16)	Alive in CR (+110 months)

Abbreviations: ATG = antithymocyte globulin; auto = autologous; BM = bone marrow; CR = complete remission; CY = cyclophosphamide; DI = diabetes insipidus; F = female; LIV = liver; LN = lymph node; PAM = melphalan; LU = lung; M = male; MS = multisystem; myeloab = myeloablative; NR = non-response; PB = peripheral blood; PD = progressive disease; PR = partial response; RIC = reduced-intensity conditioning; SCT = stem cell transplantation; SPL = spleen; SS-m = single system multisite; SS-s = single system single site; TBI = total body irradiation; TLI = total lymphoid irradiation; UCB = unrelated cord blood; VP16 = etoposide.

Nine patients (patients 1–9) are classified in the low-risk group, and six (patients 10–15) are in the low-risk group.

[a]Dose of TBI/TLI was 10–12 Gy in the myeloablative regimen and 2 Gy in the reduced conditioning regimen.

[b]Values in parentheses indicate the duration from SCT to the final observation.

skull lesions and occurrence of DI, despite chemotherapy. Patient 14 developed systemic xanthogranuloma 3 years after diagnosis. He suffered from liver dysfunction, ascites, pleural effusion, fever, and pancytopenia before SCT. Among the remaining three patients, patient 13, who relapsed during maintenance therapy, became refractory to more intensive chemotherapy, and BM and CNS involvement occurred at SCT. Patient 11 suffered from diarrhea, bloody stool, and protein-losing gastroenteropathy at initial diagnosis. After 2 months, skin rash, hepatosplenomegaly, and disseminated intravascular coagulation were seen and she was diagnosed based on a rectal biopsy. She failed to achieve remission after JLSG Induction regimen A and B and received cisplatin according to the neuroblastoma regimen after disease activation. Patient 15 failed to achieve remission after the JLSG Induction regimen and received chemotherapy according to the non-Hodgkin lymphoma regimen. Patient 15, who had thymus involvement and respiratory dysfunction at diagnosis, obtained only a PR clinically and radiographically, and the skin, lung, gingiva, and palpebral conjunctiva became involved 6 months after diagnosis.

Donor source and conditioning regimen

The donor source and conditioning regimen for SCT are also summarized in Table 2. A myeloablative conditioning regimen was used in 10 patients; total body irradiation was used in five, while the other five received a non-total body irradiation regimen. Five patients received a reduced-intensity conditioning regimen (RIC), which consisted of fludarabine, melphalan, or cyclophosphamide, and low-dose total body irradiation/total lymph node irradiation and/or antithymocyte globulin. Nine patients underwent unrelated cord blood transplantation. One patient received cord blood from an HLA-matched sibling. Overall, 10 of 15 patients received CBT with a median of 1.4×10^6/kg $CD34^+$ cells (range, 0.19–7.5×10^6/kg) or a median of 0.91×10^8/kg nucleated cells (range, 0.86–1.4×10^8/kg). Peripheral blood (PB) from haploidentical parental donors was used in two patients, and autologous PB was used in two patients, with a median of 10.9×10^6/kg $CD34^+$ cells (range, 7.5–13.0×10^6/kg). One patient received BM from an HLA 2 loci-mismatched sibling, with 3.0×10^8/kg nucleated cells. Prophylaxis for graft-versus-host disease and graft rejection consisted primarily of methotrexate and either cyclosporine or tacrolimus.

Clinical course of 15 patients after SCT

The clinical course of 15 patients after SCT is listed in Table 2. Engraftment with >500/μl absolute neutrophil count was seen in all patients except for one who died of multi-organ failure on day 18. Regimen-related toxicity was seen in six of 15 patients; mucositis of grade 1 to grade 4 according to the common terminology criteria for adverse events[13] was the most common, and three patients had liver dysfunction of grade 2 or grade 3. One patient had thrombotic microangiopathy, which resolved without long-term sequelae. Four patients had various infections, such as sepsis, herpes simplex virus, and cytomegalovirus. Three patients who underwent unrelated cord blood transplantation had acute graft-versus-host disease (grades I, III, IV). One of them and another patient developed chronic graft-versus-host disease extensive type.

After SCT, two patients never entered remission and died on day 18 and day 188, respectively (patient 2 and patient 8). Patient 7 relapsed on day 20 and died on day 47 after SCT, due to sepsis, veno-occlusive disease, and gastrointestinal bleeding. Patient 4 underwent a second SCT on day 49 after the first SCT because of graft failure. She relapsed on day 194 and died on day 271 after the second SCT due to liver dysfunction, pancytopenia, and sepsis. Two patients (patient 10 and patient 12) relapsed at 8 months and 4 months after auto-PBSCT, respectively. Patient 10 had recurrence involving the cervical spine and received prednisone, vinblastine, and cyclosporine for 4 years. Patient 12 had recurrence of multiple bone lesions, including the femur, skull, and scapula in turn, and was treated with radiation therapy to control the bone lesions for 2 years. Patient 14 had macrophage activating syndrome after SCT, and TNF-α blocker and dexamethasone palmitate were administered for several months. Finally, 11 of 15 patients remain alive with no evidence of disease. The 10-year OS rate (median ± s.e.) in these patients was 73.3 ± 11.4% (Figure 1). The 10-year OS rate of nine patients who had risk organ involvement at diagnosis was 55.6 ± 16.6%, whereas six patients who had no risk organ involvement at diagnosis have all survived with no evidence of disease. There was no significant difference in outcome between the two groups ($P = 0.07$), because of the small number of patients.

Late toxicities associated with SCT included short stature with body height <−2.0 s.d. in five patients, whereas DI and hearing disturbance were seen in two patients each. One patient, who had a hip fracture and hearing disturbance, was evaluated as being intellectually retarded. Another patient, who suffered from a CNS lesion before SCT, had speech delay. Except for one infant who was too young to evaluate, the Karnofsky score[14] of the remaining eight survivors was 100%.

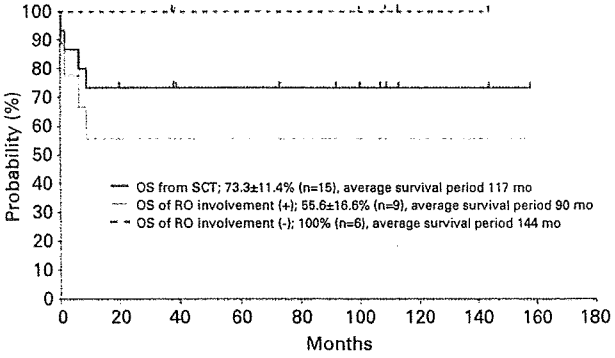

Figure 1 Overall survival (OS) of patients with LCH who underwent SCT. The 10-year OS rate of the 15 patients with refractory LCH who underwent SCT was 73.3 ± 11.4%. The 10-year OS rate of the nine patients who had risk organ involvement at diagnosis was 55.6 ± 16.6%, whereas all six patients who had no risk organ involvement at diagnosis remain alive with no evidence of disease. There was no significant difference in outcome between the two groups ($P = 0.07$). RO, risk organ.

Discussion

More than 40 patients with refractory LCH have been reported to have received SCT since 1987.[15] The OS of the 29 patients who underwent myeloablative SCT was 48% (14/29 patients), and the transplant-related mortality was 45%. However, in a recent study reported from Europe,[10] seven (78%) of nine patients survived with no signs of disease activity after RIC-SCT. The conditioning regimen consisted of fludarabine, melphalan, low-dose total lymph node irradiation (2 Gy), and either of antithymocyte globulin or MabCampath (anti-CD52 antibody). In Japan, four patients with LCH underwent SCT between 1994 and 1997, and two of them (50%) survived.[16] In this study, 11 of the 15 patients have survived with no evidence of disease; 8/10 (80%) with myeloablative conditioning and 3/5 (60%) with RIC-SCT, and the 10-year OS rate was superior (73.3 ± 11.4%). In particular, two patients with RIC-SCT had organ dysfunction before SCT, suggesting that a less toxic conditioning regimen is also effective for patients with organ dysfunction.

In the recent report of 22 patients who underwent SCT, the donor source was a sibling in 17 (77%) and a matched unrelated donor in five (23%).[11] The stem cell source was BM in 12 (55%), PB in six (27%), and CB in four (18%).[11] In this study, 10 of 15 patients received UCB, and eight of these have survived with no evidence of disease, including three with RIC-SCT. Therefore, our results support the use of UCB as an alternative donor source when neither a sibling donor nor a matched unrelated donor is available.

The appropriate timing of SCT for LCH is unclear. In this study, nine of 15 patients underwent SCT within 12 months after initial diagnosis, including seven with risk organ involvement at diagnosis. This group had a life-threatening clinical course, and they required prompt SCT as the only potentially curative treatment. On the other hand, three patients without risk organ involvement at initial diagnosis underwent SCT 7 years or later after diagnosis, resulting in no evidence of disease. A total of six patients in the low-risk group underwent SCT because of PD or disease refractory to conventional chemotherapy. Although it is not known which LCH patients really require SCT, our findings suggest that SCT should also be considered in patients in the low-risk group at diagnosis who develop active or PD even after long-term chemotherapy. A large-scale prospective study could provide useful information to select subsets of LCH that definitely need SCT.

It has recently been shown that various cytokines have an important role in the pathogenesis of LCH, suggesting that eradication of the pathologic cells associated with cytokine production could be effective for refractory LCH. In the previous report, two patients with refractory LCH who underwent RIC-SCT showed resolution of disease after SCT.[10] Kinugawa *et al.*[16] reported one patient who failed engraftment, followed by complete autologous recovery and resolution of disease activity.[16] In this study, one patient who underwent allo-BMT (patient 9) had a similar clinical course. Two patients who relapsed after auto-PBSCT showed resolution of disease following conventional chemotherapy (patients 10 and 12). Although the disease state of the two patients at 12 or 14 weeks after chemotherapy was GR, multiple recurrences had occurred, and their disease state at SCT was PD. These two patients were rescued by a myeloablative conditioning regimen with infusion of donor T lymphocytes, which prevented deterioration of the LCH. Steiner *et al.*[17] also reported one patient who achieved complete remission after RIC-SCT, despite post transplant mixed chimerism, in which only a T-cell subset proved to be of donor origin. He emphasized that a strong immunomodulating influence, mainly exerted by allogeneic T-cells, rather than eradication of the LCH cell clone, may be potentially curative in LCH. Correction of inappropriate immunological crosstalk by the replacement of allogeneic donor cells may be pivotal.

Bernard *et al.*[9] reported that 7 of 10 patients with refractory LCH had achieved sustained complete remission after treatment with 2-CdA and Ara-C. In this study, two patients (patients 6 and 8), who failed to respond to the combination of 2-CdA and Ara-C, underwent SCT, and one has been alive with no disease after RIC-SCT. Prior utilization of 2-CdA may help tailor the indications for SCT.

In conclusion, the improved outcomes of SCT for refractory LCH show that it is a promising new salvage approach. RIC-SCT is desirable for young children, especially with non-malignant disease. Further investigations are required to establish the SCT strategy for refractory LCH.

Conflict of interest

The authors declare no conflict of interest.

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