anaemia or refractory anaemia with ringed sideroblasts of myelodysplastic syndrome and with non-malignant diseases were defined as being at standard risk. All other patients were defined as being at high risk.

The overall survival (OS) of all of the patients was measured from the date of transplantation to the date of death from any cause.

Definition of haemophagocytic syndrome following haematopoietic stem cell transplantation

We modified the criteria proposed by others for diagnosing HPS after transplantation (Henter *et al*, 1991; Imashuku, 1997; Tsuda, 1997) and selected two major and four minor criteria. A diagnosis of HSCT–HPS required both major criteria, or one major and all four minor criteria. The first major criterion comprised engraftment failure, delayed engraftment, or secondary engraftment failure after HSCT and the second was histopathological evidence of haemophagocytosis. The four minor criteria comprised high grade fever, hepato-splenomegaly, elevated ferritin and elevated serum lactate dehydrogenase (LDH). Although progressive cytopenia has formed the backbone of the previous criteria, we excluded it considering the post-HSCT setting.

Statistical analysis

The cumulative incidences were estimated for neutrophil engraftment and the development of HSCT–HPS (Gooley *et al*, 1999). The probability of OS was estimated from the time of transplantation according to the Kaplan–Meier product limit method and outcomes were compared using the log-rank test. The following patient or transplant characteristics (baseline factors) were analysed using the Cox regression model to determine their impact on the development of HSCT–HPS: patient age, gender (matched or mismatched), blood type (matched or mismatched), disease (lymphoma or not), disease risk (standard or high), preparative regimen (reduced-intensity or myeloablative), GVHD prophylaxis (TAC alone or others), disparity of HLA-A, -B, -DR antigen (one or two mismatched antigens), and numbers of infused nucleated and $CD34^+$ cells. A value of $P < 0{\cdot}05$ was considered statistically significant. All data were statistically analysed using Stat-View 5.0 and S-Plus 2000 (Mathsoft, Seattle, WA, USA).

Results

Patient's characteristics

Table I summarizes the characteristics of the 119 patients and cord blood grafts. The median age was 55 years (range, 17–69); 103 patients (87%) had high risk diseases. The preparative regimen comprised fludarabine phosphate, melphalan and TBI in 91 patients (76%) and 106 patients (89%) received TAC alone as GVHD prophylaxis. MMF was administered in addition to TAC for eight patients (7%). The median numbers of infused total nucleated and $CD34^+$ cells were $2{\cdot}52 \times 10^7$ (range, 1·85–5·13) and $0{\cdot}766 \times 10^5$ cells/kg (range, 0·110–3·16), respectively. The donor–recipient pairs had serological

Table I. Patients' characteristics and transplantation procedures.

Characteristic	Number
Age (years), median (range)	55 (17–69)
Gender (male/female)	78/41
Primary diseases	
Acute lymphoblastic leukaemia	10
Acute myeloid leukaemia	52
Chronic myeloid leukaemia	5
Adult T-cell leukaemia/lymphoma	11
Myelodysplastic syndrome	6
Malignant lymphoma	32
Aplastic anaemia	1
Chronic idiopathic myelofibrosis	1
Acute leukaemia of ambiguous lineage	1
Preparative regimens	
Flu (125–180 mg/m²)/Mel (80–140 mg/m²)/TBI (2–8 Gy)	91
Flu (125–180 mg/m²)/Mel (80–140 mg/m²)	7
Flu (125–180 mg/m²)/BU (8–16 mg/kg)/TBI (4–8 Gy)	14
Flu (150–180 mg/m²)/BU (8–16 mg/kg)	3
Others	4
GVHD prophylaxis	
CsA alone	5
TAC alone	106
TAC and MMF	8
Cord blood cells	
Number of infused nuclear cells, median (range), $\times 10^7$/kg	2·52 (1·85–5·13)
Number of infused $CD34^+$ cells, median (range), $\times 10^5$/kg	0·766 (0·110–3·16)
Sex match	
Matched	24
Mismatched	95
HLA match	
6/6	2
5/6	14
4/6	103
ABO-blood type match	
Matched	36
Minor mismatched	31
Major mismatched	38
Bidirectional mismatched	14
Disease risk	
Standard/high	16/103

GVHD, graft-*versus*-host disease; BU, busulfan; CsA, ciclosporin; Flu, fludarabine phosphate; Mel, melphalan; MMF, mycophenolate mofetil; TAC, tacrolimus; TBI, total body irradiation; HLA, human leucocyte antigen.

mismatches at two HLA loci, a gender mismatch and an ABO blood-type mismatch in 103 (87%), 95 (80%) and 83 (70%) patients, respectively. Among 103 patients who survived beyond 28 d after CBT, neutrophil engraftment was achieved in 89 of them at a median of day 20 (range, 11–45). The cumulative incidence of neutrophil engraftment at day 60 was 85·6%. Secondary engraftment failure occurred in four of these 89 patients. Eleven patients were diagnosed with 'delayed engraftment' according to our definition. The direct causes of death in 16 patients who died within 28 d of CBT included sepsis ($n = 10$), haemorrhage ($n = 2$), relapse of primary disease ($n = 2$), thrombotic microangiopathy (TMA) ($n = 1$), and central nervous system complication ($n = 1$). Chimaerism data was obtained from 111 patients. Chimaerism analysis was performed in 58 patients in the peripheral blood and in 53 patients in the bone marrow. One hundred (90·1%) of them had achieved complete donor chimaerism by day 60. The median length of time required to complete donor chimaerism was 18 d (range, 9–93). Chimaerism was analysed in 10 of 16 patients who died within 28 d of CBT. All except one had complete donor chimaerism before neutrophil engraftment. Seventy-three (61·3%) of the 119 patients developed PIR. By day 100 after CBT, 55 patients had developed bacteraemia at a median of 10 d (range, 3–89 d). Of these 55 patients, 33 developed bacteraemia within 14 d of transplantation. Cytomegarovirus (CMV) was reactivated in 60 patients at a median of 33 d (range, 3–101 d). Ten patients developed histologically confirmed CMV enterocolitis. Eleven patients developed limbic encephalitis caused by human herpes virus 6 (HHV-6) at a median of 20 d of transplantation (range, 13–33 d).

HSCT–HPS patients' characteristics

Table II shows the characteristics of the 20 of 119 patients who had clinical features of HPS according to our diagnostic criteria. The cumulative incidence of HPS after CBT was 16·8% (Fig 1). HPS occurred within 4 weeks of transplantation and the median day of diagnosis was 15 d post-transplant (range, 10–27 d). The 20 patients comprised 13 men and seven women, with a median age of 52 years (range, 23–69 years); 17 patients had high risk disease. None of them had evidence of HPS before transplantation. The preparative regimen comprised fludarabine phosphate, melphalan and TBI for 15 patients and 19 patients received TAC alone as GVHD prophylaxis. MMF was administered in addition to TAC for one patient. The median numbers of infused total nucleated and CD34$^+$ cells were $2·40 \times 10^7$ cells/kg (range, 1·98–5·13) and $0·52 \times 10^5$ cells/kg (range, 0·18–3·10), respectively. The

Table II. Characteristics of HSCT–HPS patients.

Patient	Age (years)/ gender	Disease	Status	TNC ($\times 10^7$/kg)	CD34$^+$ ($\times 10^5$/kg)	Gender match	HLA match	Blood type match	Preparative regimen	GVHD prophylaxis
117	68/M	ALL	RL1	2·64	0·74	Match	4/6	BD MM	F125/M80/TBI4	TAC
157	38/M	AML	PIF	2·39	0·31	MM	4/6	Minor MM	F125/M80/TBI4	TAC
161	69/M	NHL	RL1	2·54	0·99	Match	4/6	Major MM	F125/M80/TBI4	TAC
164	48/F	ATLL	PR	5·13	3·10	MM	4/6	Minor MM	F125/M80/TBI4	TAC
171	23/M	AML	RL2	2·30	0·52	MM	5/6	Minor MM	F180/B8/TBI8	TAC
181	62/M	AML	CR2	1·94	0·18	MM	4/6	Major MM	F125/M80/TSP	TAC
194	61/M	CML	BC	2·25	1·47	MM	5/6	Match	F125/M80/TBI4	TAC
198	56/F	ATLL	PR	3·99	0·20	MM	4/6	BD MM	F125/B8/TBI4	TAC
208	52/M	NHL	PD	2·41	0·52	MM	4/6	Minor MM	F125/M80/TBI4	TAC
209	52/M	AML/MDS	CR1	2·52	0·58	Match	4/6	Major MM	F125/M80/TBI4	TAC
212	57/M	AML/MDS	NT	2·08	0·57	MM	4/6	Minor MM	F125/M80/TBI4	TAC
215	47/F	NHL	PD	3·16	0·45	MM	4/6	Major MM	F180/B8	TAC
239	50/F	AML/MDS	PIF	2·34	0·31	MM	6/6	Match	F180/M140/TBI4	TAC
240	39/M	AML	RL1	2·62	0·29	MM	4/6	Minor MM	F125/M140/TBI4	TAC
242	33/F	AML	RL1	2·57	0·39	MM	4/6	Minor MM	F125/M160/TBI4	TAC
246	66/M	AML/MDS	PIF	2·37	0·65	MM	4/6	Major MM	F125/M80/TBI4	TAC
274	31/F	NHL	RL pASCT	2·72	0·22	MM	4/6	Match	F180/M140	TAC
278	59/M	AML/MDS	PIF	1·98	0·50	MM	4/6	Major MM	F125/M80/TBI4	TAC
280	40/F	NHL	RL1	2·35	0·90	Match	4/6	Minor MM	F125/M80/TBI4	TAC
282	62/M	AML	CR2	2·05	0·70	Match	4/6	Minor MM	F125/M80/TBI4	TAC/MMF

ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; AML/MDS, acute myeloid leukaemia with multilineage dysplasia; ATLL, adult T-cell leukaemia/lymphoma; B, oral busulfan, mg/kg; BC, blastic crisis; BD, bidirectional; CML, chronic myeloid leukaemia; CR, complete response; F, fludarabine; GVHD, graft-*versus*-host disease; HLA, human leucocyte antigen; M, melphalan, mg/m^2; MDS, myelodysplastic syndrome; MM, mismatch; MMF, mycophenolate mofetil; NHL, non-Hodgkin lymphoma; NT, not treated; pASCT, post autologous stem-cell transplantation; PD, progressive disease; PIF, primary induction failure; PR, partial response; RL, relapse; TAC, tacrolimus; TBI, total body irradiation; TNC, total nucleated cell count; TSP, tespamine.

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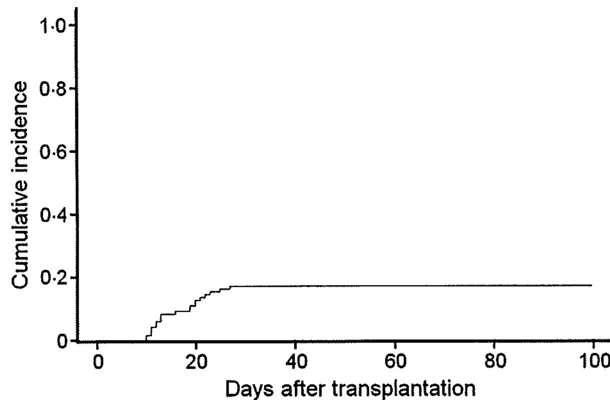

Fig 1. Cumulative incidence of HPS following CBT.

donor–recipient pairs had serological mismatches at two HLA loci, a gender mismatch and an ABO blood type mismatch in 17, 15 and 17 patients, respectively.

Clinical features of HSCT–HPS patients

Table III shows the clinical features and outcome of HSCT–HPS patients. All patients, except for one, presented with high grade fever. Hepatosplenomegaly was found in four patients and 11 had clinical manifestations of PIR. Serum aminotransferases (predominantly aspartate, rather than alanine aminotransferase) and bilirubin were elevated in 12 patients each. None of them had acute hepatic failure. Serum LDH and ferritin levels were elevated in 16 and 19 patients respectively [median value (range) of highest LDH, 340 (65–2444) i/u per litre and ferritin, 9397 (1423–568500) μg/l]. The highest values of serum ferritin by day 30 after CBT significantly differed between patients with and without HPS ($P < 0{\cdot}0094$) (Fig 2). The diagnosis of HPS was confirmed by cytological or pathological assessment of all patients, except for one with extremely elevated serum ferritin who rapidly developed secondary engraftment failure, which was strongly indicative for HSCT–HPS. Bone marrow aspirates from 18 of 19 patients exhibited haemophagocytosis (the remaining one was diagnosed by a bone marrow biopsy post-mortem). This test was performed between day 10 and 27 d after transplantation to determine the cause of delayed neutrophil recovery or to predict the development of HPS. Bone marrow aspiration smear showed very hypocellular marrow with a prominent increase of activated macrophages phagocytosing red cells and myeloid precursors.

Engraftment and chimaerism of HSCT–HPS patients

Of 14 patients with HPS who failed to engraft (primary engraftment failure), eight died within 28 d of CBT. Three patients achieved engraftment after day 29 of CBT (delayed

Table III. Clinical features and outcome of HSCT–HPS patients.

Patient	Engraftment (d)	M in BM (%)	Day of Dx	Chimaerism (% donor)	PIR	Fever	HSM	LDH (i/u per litre)	Ferritin (μg/l)	Intervention	Response
117	Not engrafted	29·0	19	NA	No	Yes	No	65	NA	None	Not engrafted
157	Not engrafted	66·0	19	98·4	Yes	Yes	Yes	1255	1423	CS/CsA	Not engrafted
161	Day 19, sEF	NA*	NA*	NA*	Yes	Yes	No	1372	9397	CS	Not engrafted
164	Day 13, sEF	1·0	25	96·2	No	Yes	Yes	2444	568500	CS/CsA	Engrafted
171	Not engrafted	43·0	27	0·2	No	Yes	No	166	6434	CS	Not engrafted
181	Not engrafted	53·0	12	94·0	No	Yes	Yes	587	18150	CS	Not engrafted
194	Not engrafted	24·0	13	98·8	Yes	Yes	No	664	34200	CS	Not engrafted
198	Not engrafted	17·0	20	94·6	Yes	Yes	No	208	2719	CS	Not engrafted
208	Not engrafted	21·5	13	99·6	Yes	Yes	No	994	18640	CS/VP16	Not engrafted
209	Not engrafted	51·0	12	64·0	No	Yes	No	174	1946	IVIG/second CBT	Engrafted
212	Day 30	30·5	11	63·6	Yes	Yes	NE	261	9339	IVIG	Engrafted
215	Day 33	18·5	21	99·8	Yes	Yes	No	216	9808	CS	Engrafted
239	Day 30	25·0	22	96·4	Yes	Yes	NE	313	5212	CS	Engrafted
240	Not engrafted	15·0	11	99·0	Yes	Yes	NE	268	58824	CS	Not engrafted
242	Not engrafted	10·0	10	96·4	Yes	Yes	No	143	3439	IVIG/second CBT	Engrafted
246	Not engrafted	15·0	21	18·2	No	Yes	No	800	7740	Second CBT	Engrafted
274	Not engrafted	90·0	11	68·4	Yes	Yes	NE	367	20304	Second CBT	Engrafted
278	Not engrafted	34·0	10	99·4	Yes	Yes	No	891	111800	None	Not engrafted
280	Not engrafted	11·5	13	100	No	Yes	Yes	1634	67600	CS/VP16	Not engrafted
282	Day 24, sEF	58·0	20	92·6	No	No	No	276	2464	CS	Not engrafted

BM, bone marrow; CBT, cord blood transplantation; CS, corticosteroid; CsA, ciclosporin; Dx, diagnosis; HSM, hepatosplenomegaly; IVIG, intravenous immunoglobulin; M, macrophage; NA, not available; NE, not evaluated; PIR, pre-engraftment immune reactions; sEF, secondary engraftment failure.
*Haemophagocytosis confirmed by post-mortem bone marrow biopsy.

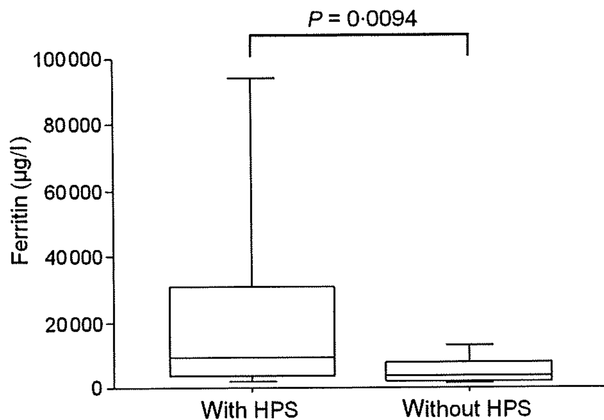

Fig 2. Comparison of highest value of serum ferritin by day 30 of CBT (with *versus* without HPS).

engraftment). Secondary engraftment failure occurred in three patients. Chimaerism data were obtained from 18 out of 20 patients. Donor chimaerism was complete at the time of HPS diagnosis in 13 patients. Three and two patients had donor- and recipient-dominant chimaerism, respectively. An examination of bone marrow clot specimens using XY-FISH method (Ishida *et al*, 2007) confirmed that the activated macrophages in two patients with HPS who achieved complete donor chimaerism (patients 157 and 181; Table II) were donor-derived.

Concomitant clinical conditions of HSCT–HPS patients

Concomitant clinical conditions might be relevant to the development of HPS. Twelve of 20 patients had extant infections, most of which were bacteraemia (n = 10). The pathogens in eight patients were Gram-positive cocci, namely coagulase-negative *Staphylococcus* (n = 5), *Enterococcus faecalis* (n = 2) and *Enterococcus faecium* (n = 1), and Gram-negative rods, *Stenotrophomonous maltophilia* (n = 1) and *Pseudomonous aeruginosa* (n = 1) in two. Three patients were infected with CMV. Two had simultaneous bacteraemia and HHV-6 infection was found in one patient who developed limbic encephalitis. Among eight patients who had no documented infections, five developed transient atypical lymphocytosis soon after transplantation, two had PIR, and the remaining patient developed HPS without any concomitant clinical conditions.

Therapeutic interventions for HSCT–HPS and outcome

Corticosteroid (CS) was administered in 13 of 20 patients to reduce macrophage activation, CsA was administered in addition to CS in two patients and etoposide (VP-16) was also administered in addition to CS in two others. Four patients underwent a second rescue CBT, two of which were after the administration of high-dose intravenous immunoglobulin (IVIG). One patient was treated with IVIG alone. Two patients could not undergo specific treatments due to severe infections and/or severe organ damage. These efforts finally resolved the failed engraftment in eight patients. The prognosis was poor; 17 of 20 patients died (85%) and eight had died by 28 d after CBT. The causes of death were sepsis (n = 7), relapse of primary disease (n = 3), haemorrhage (n = 2), TMA (n = 2), GVHD (n = 2) and central nervous system complication (n = 1). As of December 2007, the median follow-up after CBT for surviving patients was 598 d (range, 26–1426 d). The Kaplan–Meier probability of overall survival at 4 years was 31·4% (95% confidence interval, 20·0–42·8%). The overall survival was significantly poorer for patients with HPS than without HPS (15·0% vs. 35·4%; P = 0·0002, Fig 3).

Risk factors for HSCT–HPS

Univariate and multivariate analysis identified having fewer infused $CD34^+$ cells as a significant risk factor for the development of HPS (P = 0·01 and 0·006 respectively, Table IV). Patients were subdivided into two groups according to the intensity of preparative regimen; those who received 16 mg/kg of BU or 8 Gy of TBI were categorized as 'myeloablative' (n = 18), and the others who received less intensive regimens were classified as 'reduced-intensity' (n = 101). The incidence of HPS was higher in the 'reduced-intensity' group, although it did not reach statistical significance (P = 0·17).

Discussion

This study of clinical manifestations, therapeutic management, outcome and risk factors for HPS after CBT is the largest to date. Our results demonstrated that HPS is an aggressive and devastating complication after CBT that closely correlates with delayed engraftment or failure, resulting in a poor OS. As far as we understand from the English medical literature (Table V), only 23 patients in 16 case reports appear to have developed HPS after autologous (n = 5) and allogeneic (n = 18) HSCT

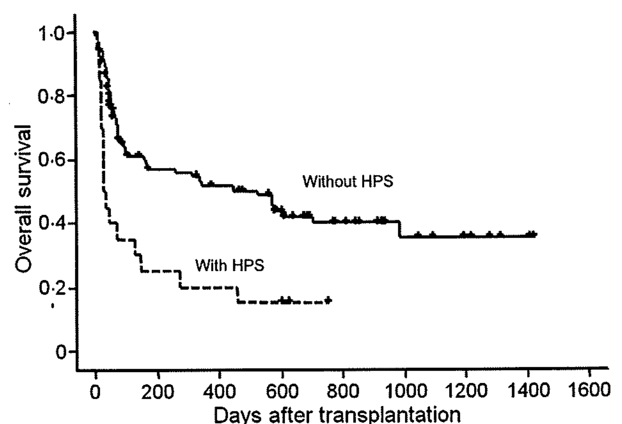

Fig 3. Comparison of overall survival (with *versus* without HPS).

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Table IV. Risk factors of HPS development.

Univariate factors		Cumulative incidence	*P* value
Age (<55 vs. ≥55 years)		19·3% vs. 14·5%	0·50
Gender (mismatch *versus* match)		18·3% vs. 11·5%	0·41
Blood type (mismatch *versus* match)		20·8% vs. 8·1%	0·09
Underlying disease (non-lymphoma *versus* lymphoma)		17·1% vs. 16·3%	0·85
Risk of underlying disease (standard *versus* high)		18·8% vs. 16·5%	0·80
Preparative regimen (reduced-intensity *versus* myeloablative)		19·4% vs. 5·6%	0·17
GVHD prophylaxis (TAC alone *versus* others)		18·4% vs. 7·7%	0·35
Disparity of HLA-A, -B, -DR antigen (1 or 0 vs. 2-antigen mismatch)		18·8% vs. 16·5%	0·89
GVH vector (2 vs. 1 or 0-mismatch)		18·4% vs. 12·5%	0·41
HVG vector (1 or 0 vs. 2-antigen mismatch)		21·1% vs. 15·1%	0·53
Number of infused total nucleated cells (<2·52 vs. ≥2·52 × 10^7/kg)		18·6% vs. 15·0%	0·60
Number of infused $CD34^+$ cells (<0·766 vs. ≥0·766 × 10^5/kg)		27·1% vs. 6·8%	0·01
Multivariate factors	**Hazard ratio**	**95% Confidence interval**	***P* value**
Blood type (mismatch *versus* match)	2·80	0·79–9·86	0·11
Preparative regimen (reduced-intensity *versus* myeloablative)	2·76	0·43–17·8	0·29
GVHD prophylaxis (TAC alone *versus* others)	2·71	0·41–17·9	0·30
Number of infused $CD34^+$ cells (<0·766 vs. ≥0·766 × 10^5/kg)	4·48	1·54–13·1	0·006
GVH vector (1 or 0 vs. 2-antigen mismatch)	1·48	0·52–4·21	0·46

GVH, graft-*versus*-host; HVG, host-*versus*-graft.

(Sokal *et al*, 1987; Levy *et al*, 1990; Reardon *et al*, 1991; Nagafuji *et al*, 1998; Sato *et al*, 1998; Takahashi *et al*, 1998; Ishikawa *et al*, 2000; Fukuno *et al*, 2001; Abe *et al*, 2002; Tanaka *et al*, 2004, 2007; Kishi *et al*, 2005a; Boelens *et al*, 2006; Ostronoff *et al*, 2006; Ishida *et al*, 2007; Koyama *et al*, 2007). Among 18 patients who received allogeneic HSCT, reduced-intensity preparative regimens were employed in nine patients and three underwent CBT. Thus, HPS has been considered a rare event after HSCT. The incidence of HPS following CBT in our study, however, was strikingly higher than previous reports have indicated.

Multivariate analyses identified the dose of $CD34^+$ cells as the only statistically significant risk factor. Given that a low dose of $CD34^+$ cells can negatively affect the rate of engraftment and duration to neutrophil recovery, more infectious complications accompanying low $CD34^+$ cell counts might be directly related to the onset of HPS. In our study cohort, the incidence of infectious complications arising during the early phase after transplant (by day 28) was higher in those with HPS than those without HPS (12/20 vs. 37/99; $P = 0{\cdot}027$), suggesting that infections are associated with the likelihood of developing HPS. The high prevalence of elderly patients and of those with high-risk disease status might explain this high incidence of infections. Consequently, the poor outcome following development of HPS was mainly due to the engraftment failure and following exacerbation of infections.

The intriguing finding of our chimaerism analysis of patients with HPS was that of donor-dominancy in 16 of 18 patients. XY-FISH determined that the phagocytosing macrophages were also of the donor type in the two evaluated patients. These findings indicated that HPS after CBT might be mediated by donor-derived macrophages rather than host-derived, and that engraftment failure is not due to a simple rejection mechanism, but to factors and events that activates donor-derived macrophages and leads to the cascade of HPS. The incidence of HPS may have been underestimated in previous reports, as the reason for graft failure after transplantation had often not been described, especially for graft failure with donor-dominant chimaerism.

The postulated pathophysiology of HPS is that excessive cytokine production from T cells activate macrophages, leading to a substantial loss of haematopoietic cells. Although of great interest, the role of cytokine levels in the precise mechanism of HPS needs further study in the future. We previously described unique early immune reactions after CBT and termed them PIR (pre-engraftment immune reactions), i.e. non-infectious high-grade fever concomitant with eruption, diarrhoea and weight gain, starting on a median of day 9 after CBT (Kishi *et al*, 2005b). In the present study, 61% of the patients developed this syndrome, suggesting that immune cells became activated soon after transplantation. We regarded this syndrome as early onset of acute GVHD, where activated donor T cell secreted various cytokines (Reddy & Ferrara, 2003).

We also recently reported that the degree of HLA mismatch in the graft-*versus*-host direction was inversely associated with engraftment kinetics after RI-CBT (Matsuno *et al*, 2009). Paradoxically to the former notion of graft failure, the degree of HLA mismatch in the host-*versus*-graft direction had no impact on the engraftment kinetics. These findings propose a novel mechanism responsible for

Table V. Occurrence of haemophagocytic syndrome among autologous and allogeneic haematopoietic stem cell transplantation reported in English medical literature.

Ref.	Age (years)/ gender	Disease	Stem cell HLA match	Preparative regimen	GVHD prophylaxis	Day of Dx	Principal cause	Intervention	Response
(A) After autologous haematopoietic stem cell transplantation									
Levy *et al* (1990)	6/F	Wilm tumour	Auto BM	Local RT/Mel/ADM	–	28	ADV-11	IVIG	Not engrafted
Nagafuji *et al* (1998)	52/F	AML	Auto PBSC	BU/VP16/Ara-C	–	25	CMV	CS/IVIG	Not resolved
Takahashi *et al* (1998)	43/F	NHL	Auto PBSC	CY/VP16/MCNU/CBDCA	–	130	Lymphoma	CS/IVIG	Not resolved
Fukuno *et al* (2001)	67/F	NHL	Auto PBSC	CY/VP16/MCNU	–	12	MRSA	CS/CsA	Not engrafted
Ostronoff *et al* (2006)	54/F	MM	Auto PBSC	Mel	–	16	ND	CS/IVIG	Engrafted
(B) After allogeneic haematopoietic stem cell transplantation									
Sokal *et al* (1987)	8/M	FA	ur-BM, 6/6	CY/TBI 4	CsA	300	HSV-1	–	Resolved
Reardon *et al* (1991)	8/F	ALL	r-BM, 6/6	BU/CY	CsA/CS	38	ADV	–	Not resolved
Sato *et al* (1998)	40/F	AML	ur-BM, 6/6	VP16/TBI 12	CsA/sMTX	59	CMV	IVIG/VP16	Not resolved
Ishikawa *et al* (2000)	40/M	AML	r-BM, 6/6	CY/TBI 12	CsA/sMTX	16 (D)	ND	CS	Engrafted
Abe *et al* (2002)	39/M	NHL	r-PBSC, 6/6	TBI 2	CsA/MMF	15 (D)	ND	CS/VP16	Not engrafted
Abe *et al* (2002)	50/F	NHL	r-PBSC, 5/6	TBI 2	CsA/MMF	56 (D)	ND	CS	Not engrafted
Tanaka *et al* (2004)	7/F	AML/MDS	ur-CB, 5/6	CY/TBI 12/Ara-C	CsA/sMTX	20 (D)	MRCNS	CS/second PBSCT	Engrafted
Kishi *et al* (2005a)	30/M	AML	r-PBSC, 5/6	BU/CY	TAC	11	ND	CS	Not resolved
Boelens *et al* (2006)	2/F	HS	r-BM/PBSC, 3/6	Flu/Mel/TSP/ATG	NR	35, sEF (D)	EBV-LPD	CS	Resolved
Ishida *et al* (2007)	2/M	JMML	ur-BM, 6/6	Flu/Mel/BU	TAC/sMTX	39, sEF (R)	NR	IVIG/second CBT	Engrafted
Ishida *et al* (2007)	2/M	JMML	ur-CB, –	Flu/Mel/VP16	TAC	11 (R)	NR	IVIG/VP16	Engrafted
Tanaka *et al* (2007)	54/M	AML	ur-CB, 5/6	CY/TBI 12/Ara-C	CsA/sMTX	33, sEF	NR	CS/second CBT	Engrafted
Koyama *et al* (2007)	9/–	ID	ur-BM, 6/6	Mel/TBI 6/ATG	TAC/sMTX	10	NR	CS/IVIG/VP16	Engrafted
Koyama *et al* (2007)	3/–	AML	r-BM, 4/6	Flu/TBI 12/Ara-C/VP16	TAC/sMTX	10	NR	CS/IVIG	Engrafted
Koyama *et al* (2007)	2/–	ALL	r-PBSC, –	TBI 10/TSP	TAC	8	NR	IVIG/VP16	Not engrafted
Koyama *et al* (2007)	16/–	EBV-LPD	r-PBSC, –	Flu/Mel/ATG	TAC	7	NR	CS/VP16	Not engrafted
Koyama *et al* (2007)	9/–	AML	ur-BM, 6/6	CY/TBI 12/TSP	TAC/sMTX	12	NR	CS/VP16	Engrafted
Koyama *et al* (2007)	3/–	NHL	r-BM, 3/6	TBI 12/VP16/TSP	TAC/sMTX/CS	5	NR	CS/VP16	Engrafted

ADM, adriamycin; ADV, adenovirus; ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; AML/MDS, acute myeloid leukaemia with multilineage dysplasia; Ara-C, cytosine arabinoside; ATG, anti-thymoglobulin; Auto, autologous; BM, bone marrow; BU, busulfan; CB, cord blood; CBDCA, carboplatin; CMV, cytomegalovirus; CS, corticosteroid; CsA, ciclosporin; CY, cyclophosphamide; Dx, diagnosis; (D), donor-derived; EBV-LPD, Epstein–Barr virus associated lymphoproliferative disorder; F, female; FA, Fanconi anaemia; Flu, fludarabine; HS, Hurler syndrome; HSV, herpes virus; ID, immunodeficiency; IVIG, intravenous immunoglobulin; JMML, juvenile myelomonocytic leukaemia; M, male; MCNU, ranimustine; Mel, melphalan; MM, multiple myeloma; MMF, mycophenolate mofetil; MRCNS, methicillin-resistant coagulase negative *Staphylococcus*; MRSA, methicillin-resistant *Staphylococcus aureus*; ND, not detected; NHL, non-Hodgkin lymphoma; NR, not referred; PBSC, peripheral blood stem cell; r, related; (R), recipient-derived; Ref, reference; RT, radiation therapy; ur, unrelated; sEF, secondary engraftment failure; sMTX, short-term methotrexate; TAC, taclorimus; TBI, total body irradiation; TSP, tespamine; VP16, etoposide.

engraftment failure after CBT and HPS might be one of the relevant mechanisms. HLA disparity in the graft-*versus*-host direction may augment allo-immune reactions, which evoke hypercytokinaemia, macrophage activation, and occasionally result in establishment of HPS. Indeed, most of our patients received cord blood units with an HLA mismatch due to the limited availability of cord blood units with a sufficient cell dose, and received relatively less intensive GVHD prophylaxis using calcineurin inhibitor alone. Thus, the donor T cells in the grafts were more susceptible to stimuli of cytokines triggered by infections and tissue damage from preparative regimens. In most of the other reported series, methotrexate (MTX), anti-thymocyte globulin (ATG), steroid, or MMF was used along with calcineurin inhibitor for GVHD prophylaxis and there are little reports about HPS. More intensive immunosuppression may have a positive effect on preventing post-transplant immune reactions (Narimatsu *et al*, 2007b) and the development of HPS.

An optimal strategy has not been established to treat HPS, especially after HSCT. Although CS was administered at the discretion of the primary physician to 13 HPS patients to reduce macrophage activation, HPS was resolved in only three patients and all four who could tolerate a second rescue CBT achieved durable engraftment.

In conclusion, HPS is a significant complication associated with engraftment delay and failure following CBT. The development of HPS increased mortality rates after CBT, worsening the prognosis. The precise mechanism of HPS development after HSCT remains unknown, although several lines of evidence suggest that donor immune cells are critically involved and therefore a key. The identification of high risk patients, more intensified GVHD prophylaxis, close and careful follow-up and prompt differential diagnosis are important for managing HSCT–HPS and avoiding engraftment failure. More detailed data from patients who have undergone CBT as well as other types of transplantation are warranted to further understand the mechanisms behind the development of HSCT–HPS and to develop more effective prophylaxis and treatment for this complication.

Acknowledgements

The authors also wish to thank all physicians, nurses, pharmacists, data-managers and support personnel for their care of patients in this study. This work was also supported (in part) by a Research Grant for Tissue Engineering (H17-014) and a Research Grant for Allergic Disease and Immunology (H20-015) from the Japanese Ministry of Health, Labour and Welfare.

Authors' contribution

S. Takagi and K.M. performed research and extracted data; Y.O., K.O. and A.Y. reviewed histopathological findings; N.M. and S. Takagi performed statistical analysis; N.U. and S. Taniguchi reviewed study design and methods; K.I., A.H., M.T., H.Y., D.K., Y.M., E.K., S.S., T.M., S. Miyakoshi and S. Makino contributed to writing the paper.

Conflict-of-interest disclosure

The authors declare no competing financial interests.

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blood

2009 114: 1689-1695
Prepublished online Jun 17, 2009;
doi:10.1182/blood-2008-12-194696

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Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published semimonthly by the American Society of Hematology, 1900 M St, NW, Suite 200, Washington DC 20036.
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Impact of HLA disparity in the graft-versus-host direction on engraftment in adult patients receiving reduced-intensity cord blood transplantation

Naofumi Matsuno,[1] Atsushi Wake,[1] Naoyuki Uchida,[1] Kazuya Ishiwata,[1] Hideki Araoka,[2] Shinsuke Takagi,[1] Masanori Tsuji,[1] Hisashi Yamamoto,[1] Daisuke Kato,[1] Yoshiko Matsuhashi,[1] Sachiko Seo,[1] Kazuhiro Masuoka,[1] Shigesaburo Miyakoshi,[1] Shigeyoshi Makino,[3] Akiko Yoneyama,[2] Yoshinobu Kanda,[4] and Shuichi Taniguchi[1]

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Delayed engraftment or graft failure is one of the major complications after cord blood transplantation (CBT). To investigate factors impacting engraftment, we conducted a retrospective analysis of adult patients who underwent reduced-intensity CBT at our institute, in which preparative regimens mainly consisted of fludarabine, melphalan, and total body irradiation with graft-versus-host (GVH) disease prophylaxis using single calcineurin inhibitors. Among 152 evaluable patients, the cumulative incidence of neutrophil engraftment was 89%. High total nucleated cell and CD34+ cell dose were associated with the faster speed and higher probability of engraftment. In addition, the degree of human leukocyte antigen (HLA) mismatch in the GVH direction was inversely associated with engraftment kinetics, whereas no statistically significant association was observed with the degree of HLA mismatch in the host-versus-graft direction. Similarly, the number of HLA class I antigens mismatched in the GVH direction, but not in the host-versus-graft direction, showed a negative correlation with engraftment kinetics. HLA disparity did not have significant impact on the development of GVH disease or survival. This result indicates the significant role of HLA disparity in the GVH direction in the successful engraftment, raising the novel mechanism responsible for graft failure in CBT. (Blood. 2009;114:1689-1695)

Introduction

Recent studies have demonstrated cord blood transplantation (CBT) as a safe and feasible alternative to bone marrow (BM) or peripheral blood (PB) stem cell transplantation (SCT) in adults when no suitable related donor is available.[1-4] The incidence and severity of acute graft-versus-host disease (GVHD) after CBT have been low compared with those after unrelated donor BM transplantation,[1-4] permitting use of a mismatched unit as a graft. The use of CBT has also been increasing because of the potential advantage of rapid availability and the lower risk to donors. The development of reduced-intensity (RI) conditioning regimens for transplantation, which results in less toxicity and depends largely on graft-versus-tumor effects rather than high-dose therapy to eliminate malignant cells, has been shown to allow elderly patients to undergo allogeneic transplantation.[5,6] We and other groups have reported the feasibility of RI-CBT for adult patients with advanced hematologic diseases.[7-12]

Despite the obvious advantage of CBT, high treatment-related toxicity has been observed, which precludes the application of CBT as a primary graft source. One of the major complications of CBT is delayed engraftment or graft failure. Thus far, several factors have been found to impact engraftment, including total nucleated cell (TNC) dose, CD34+ cell dose, and human leukocyte antigen (HLA) disparity.[13-15] Here, we report the results of a retrospective analysis of 163 adult patients who underwent RI-CBT at our institute, which revealed, for the first time, the importance of HLA disparity in the graft-versus-host (GVH) direction, adding a new viable factor in choosing cord blood (CB) units as transplantable grafts.

Methods

Study patients

This study included adult patients with hematologic malignancies who underwent RI-CBT as their first allogeneic SCT at Toranomon Hospital between January 2002 and December 2006 consecutively. Twenty-nine patients who had active serious infection or showed an Eastern Cooperative Oncology Group performance status of 3 or 4 before transplantation were not eligible for this study because of differences in transplantation procedures or supportive care resulting from serious organ dysfunction and active infection. Then, the remaining 163 consecutive patients were reviewed. All patients had diseases that were incurable with conventional treatments, lacked suitable sibling or unrelated donors, and were considered inappropriate for conventional allo-SCT as they were older than 50 years and/or had organ dysfunction (often attributable to previous intense chemotherapy and/or radiotherapy). Characteristics of the 163 patients are summarized in Table 1.

For disease status, those with hematologic malignancies in the first or second complete remission at the time of transplantation, those in the chronic phase or accelerated phase of chronic myeloid leukemia, and those with refractory anemia of myelodysplastic syndrome were defined as being at standard risk (n = 32), whereas those in other situations were defined as being at high risk (n = 131). All patients received a single CB unit. All patients provided written informed consent in accordance with the Declaration of Helsinki, and the study was conducted in accordance with the requirements of the Institutional Review Board of Toranomon Hospital.

Donor selection

CB units were obtained from the Japanese Cord Blood Bank Network. All CB samples, as well as the patient's blood samples, were serologically typed for HLA-A, -B and -DR antigens before transplantation. Alleles at the HLA-A, -B,

Submitted December 15, 2008; accepted June 5, 2009. Prepublished online as *Blood* First Edition paper, June 17, 2009; DOI 10.1182/blood-2008-12-194696.

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Table 1. Patient and cord blood characteristics

Variable	Value
No. of patients	163
Median age, y (range)	55 (17-79)
Sex: male/female, no. of patients	98/65
Primary diseases, no. of patients	
Acute lymphoblastic leukemia	20
Acute myeloid leukemia	63
Chronic myelogenous leukemia	5
Myelodysplastic syndrome	12
Malignant lymphoma	39
Adult T-cell leukemia/lymphoma	18
Multiple myeloma	2
Others	4
Risk of underlying disease, no. of patients: standard/high	32/131
Preparative regimens, no. of patients	
Flu + Mel + TBI 2-8 Gy	135
Flu + BU + TBI 4-8 Gy	18
Flu + Mel	6
Flu + BU	4
Median no. of infused nucleated cells, 10^7/kg (range)	2.68 (1.82-4.83)
Median no. of infused CD34$^+$ cells, 10^5/kg (range)	0.76 (0.05-4.40)
Blood-type mismatch, no. of patients: match/mismatch	47/116
HLA antigen mismatch, no. of patients	
0	3
1	24
2	136
GVHD prophylaxis, no. of patients	
Cyclosporine A alone	73
Tacrolimus alone	90

Flu indicates fludarabine; Mel, melphalan; TBI, total body irradiation; and BU, busulfan.

and -DRB1 loci were identified by high-resolution DNA typing in 107 pairs because HLA typing of alleles was not routinely performed in Japanese CB banks. In 127 pairs, HLA-A and -B antigens were identified by serologic typing and HLA-DRB1 alleles were determined by high-resolution DNA typing. CB grafts had at most 2 mismatches for HLA-A, -B, and -DR antigens and had a cryopreserved cell dose of at least 1.8×10^7 nucleated cells per kg of recipient body weight. Mismatch was counted separately in the GVH and host-versus-graft (HVG) direction, respectively. HLA mismatch in the GVH direction was defined when the recipient's antigens or alleles were not shared by the donor, whereas HLA mismatch in the HVG direction was defined when the donor's antigens or alleles were not shared by the recipient.

Transplantation procedures

Pretransplantation conditioning regimens varied and were determined by each attending physician according to the patient's disease, disease status, and history of prior therapy. All patients received purine analog-based preparative regimens. The majority of patients (n = 119) received preparative regimens consisting of fludarabine 125 mg/m^2, melphalan 80 mg/m^2, and 4 Gy total body irradiation (TBI). Patients in relatively poor performance status were conditioned with busulfan to avoid severe gastrointestinal tract toxicity induced by the use of melphalan. GVHD prophylaxis was carried out using a continuous infusion of cyclosporine A 3 mg/kg or tacrolimus 0.03 mg/kg from day −1 until the patients could tolerate oral administration.

Supportive care

All patients were treated in reverse isolation in laminar airflow-equipped rooms and received trimethoprim/sulfamethoxazole for *Pneumocystis jirovecii* prophylaxis. Fluoroquinolone, azole, and acyclovir were administered to prevent bacterial, fungal, and herpes virus infection, respectively. Cytomegalovirus pp65 antigenemia was monitored weekly. Hemoglobin and platelet counts were maintained at more than 7 g/dL and at 10×10^9/L, respectively. Granulocyte colony-stimulating factor was administered intravenously from day 1 until neutrophil recovery became durable.

Definition of engraftment, GVHD, and survival

Date of engraftment was defined as the first of 3 consecutive days when the neutrophil counts exceeded 0.5×10^9/L. Patients who did not achieve this criterion at any time after transplantation were considered as primary graft failure. Chimerism was assessed using fluorescent in situ hybridization in sex-mismatched donor-recipient pairs. In sex-matched pairs, polymerase chain reaction for variable numbers of tandem repeats was used with donor cells detected at a sensitivity of 10%. Acute and chronic GVHD was diagnosed and graded according to standard criteria.[16,17] Overall survival was calculated from the day of transplantation until death from any cause or last follow-up. Event-free survival was defined as the duration of survival after transplantation without disease progression, relapse, graft failure, or death. Final follow-up was conducted in December 2007, with a median follow-up of surviving patients being 29.0 months (range, 3.7-58.9 months).

Statistical methods

Cumulative incidence of neutrophil engraftment was calculated using the Gray method, treating death before engraftment or second transplantation as competing events.[18] Similarly, in the analysis of GVHD, death resulting from other causes or relapse leading to early withdrawal of immune suppression was considered competing risk. The probabilities of survival were estimated using the Kaplan-Meier method. Multivariate analysis was performed using the proportional hazards model. P values $< .05$ were considered statistically significant.

Results

Engraftment

Eleven of the 163 patients reviewed were not evaluable for the analyses of donor engraftment resulting from early death (before 28 days after

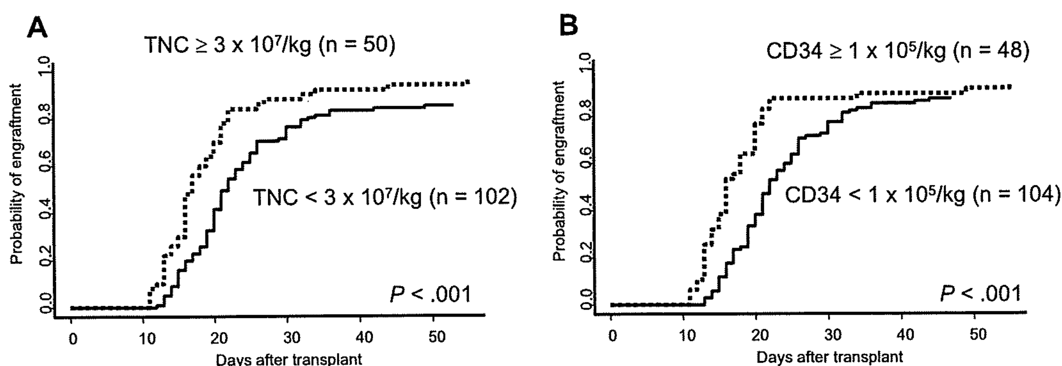

Figure 1. Cumulative incidence of neutrophil engraftment. (A) Effect of TNC dose. (B) Effect of CD34$^+$ cell dose.

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transplantation) from disease progression (n = 1), infection (n = 6), and multiple organ failure (n = 4). Of 152 evaluable patients, 135 patients achieved neutrophil engraftment. The cumulative incidence of engraftment at day 60 was 89%, and the median time to engraftment was 20 days (range, 11-55 days). Chimerism analyses were performed in 125 of 135 patients who achieved engraftment using either PB or BM samples at the time of neutrophil recovery. All patients except for one who had residual leukemic cells in PB at the time of engraftment showed complete donor chimerism (> 90%). The median length of time required to donor chimerism was 22 days (range, 11-55 days).

Age, recipient sex, risk of underlying disease, blood type mismatch, and GVHD prophylaxis did not affect engraftment kinetics (data not shown). TNC more than or equal to 3×10^7/kg was associated with a significantly higher probability of engraftment ($P < .001$), with the median time to engraftment of 16.5 days (range, 11-55 days) compared with 21 days (range, 12-49 days) for those who received less than 3×10^7/kg (Figure 1A). Similarly, $CD34^+$ cell dose more than or equal to 10^5/kg was associated with a significantly faster engraftment ($P < .001$) than those who received less than 10^5/kg (Figure 1B).

The cumulative incidence of engraftment and the time to engraftment according to the degree of HLA mismatch are shown in Table 2. Patients who had 0 and 1 antigen mismatch with the grafts were combined, considering the small number of patients in 0 mismatch group and comparable rate of engraftment and time to neutrophil recovery between 0 and 1 antigen-mismatched group (Figure 2A-B), and were compared with those of 2 antigens mismatched. Although patients with 0 or 1 antigen mismatch showed a trend toward superior engraftment kinetics compared with patients with 2 antigens mismatched, the differences did not reach statistical significance (Figure 2A; Table 2). We further analyzed the influence of HLA disparity on engraftment in both the HVG and GVH direction. In the HVG direction, the cumulative incidence of engraftment at day 60 was 93% in 0 or 1 antigen mismatch and 87% in 2 antigens mismatched ($P = .4$, Table 2). In the GVH direction, however, the cumulative incidence of engraftment was 96% in 0 or 1 antigen mismatch and 85% in 2 antigens mismatched ($P < .001$, Figure 2B; Table 2), demonstrating that HLA antigen disparity in the GVH direction was significantly associated with engraftment kinetics. As shown in Figure 2C, HLA antigen disparity in the HVG direction did not contribute to engraftment kinetics in patients with 0 or 1 antigen mismatch in the GVH direction, as was also observed in those with 2 antigens mismatched in the GVH direction. Although the number of patients in each group was small, patients with 0 or 1 mismatch in the GVH direction but 2 mismatches in the HVG direction (n = 28) showed a trend toward superior engraftment kinetics compared with patients with 0 or 1 mismatch in the HVG direction but 2 mismatches in the GVH direction (n = 18; $P = .07$). This finding may indicate that HLA disparity in the GVH direction plays a greater role in engraftment than that in the HVG direction.

In addition to the degree of mismatch, we analyzed the significance of class I (HLA-A, -B) or class II (HLA-DR) mismatch (Table 2). The number of class I antigens mismatched in the GVH direction showed a negative correlation with the probability and the speed of engraftment ($P = .006$, Figure 2D), but not in the HVG or both directions. More specifically, the presence of HLA-B antigens mismatched in the GVH direction was significantly associated with inferior engraftment kinetics ($P = .04$). To the contrary, HLA-DR antigen mismatch did not influence engraftment kinetics in either the HVG or the GVH direction.

The cumulative incidence of engraftment was also assessed using 120 pairs who had HLA-A, -B antigens and -DRB1 allele information available (Table 2). Patients with 0 or 1 mismatch showed better engraftment kinetics compared with those with 2, 3, or 4 mismatches in the GVH direction, which was about to be significant statistically ($P = .05$), whereas HLA mismatch in the HVG direction did not show significant impact on engraftment.

HLA allele mismatch at the HLA-A, -B, and -DR was examined in 102 pairs. In the GVH direction, the cumulative incidence of engraftment was 94% in 0 or 1 allele mismatch, 88% in 2 alleles mismatched, and 80% in 3 to 5 alleles mismatched ($P = .05$), showing that alleles mismatched in the GVH direction could be inversely associated with engraftment kinetics (Table 2). In contrast, allele disparity in the HVG direction did not affect engraftment (Table 2). When HLA-A, -B, and -DR alleles were analyzed independently, no statistically significant differences were observed in any allele tested in either the GVH or HVG direction (data not shown).

Multivariate analyses revealed that low TNC dose ($< 3 \times 10^7$/kg) and HLA antigens mismatched in the GVH direction (0 or 1 vs 2 antigens mismatched) were significantly associated with inferior engraftment kinetics, when age, recipient sex, risk of underlying disease, GVHD prophylaxis, and blood type mismatch were included as covariates ($P = .002$ and $P = .004$, respectively).

Clinical features of graft failure

There were 17 patients who failed to achieve engraftment: 8 males and 9 females, median age of 55 years (range, 17-68 years), high-risk diseases in 12 patients. Median TNC dose of CB grafts was 2.36×10^7/kg (range, $2.01\text{-}3.40 \times 10^7$/kg), and median $CD34^+$ cell dose was 0.59×10^5/kg (range, $0.30\text{-}1.38 \times 10^5$/kg). Nine of them died before engraftment because of disease progression (n = 2), infection (n = 5), multiple organ failure (n = 1), and idiopathic pneumonia syndrome (n = 1). The remaining 8 patients received a second RI-CBT at a median of 34 days (range, 28-49 days) after first RI-CBT, and 3 of them were alive in remission.

Among those who did not achieve engraftment, chimerism analyses in the BM early after transplantation were performed on 8 patients (median, 12 days; range, 10-17 days). Of those, 4 achieved complete donor chimerism, one had mixed chimerism (60% donor type), and 3 patients showed recipient chimerism. Four of 5 patients with donor dominant chimerism showed hemophagocytosis in the BM. On the other hand, all 3 patients with recipient chimerism did not show hemophagocytosis.

GVHD and survival

Among 134 evaluable patients, the cumulative incidence of acute GVHD of grade II to IV was 43%. The incidence of acute GVHD according to HLA disparity in the GVH direction was summarized in Table 3. Patients with 2 antigens mismatched showed a trend toward higher incidence of acute GVHD II-IV ($P = .08$). The number of class I or class II antigens mismatched had no correlation with the incidence of acute GVHD. Similarly, HLA disparity in the allele level was not significantly associated with the incidence of acute GVHD. Among 66 evaluable patients, the cumulative incidence of chronic GVHD was 51%. The degree of HLA mismatch was not significantly associated with the incidence of chronic GVHD (data not shown). Other pretransplantation factors, including age, infused cells, and GVHD prophylaxis, did not affect the incidence of GVHD. Overall survival and event-free survival at 2 years were 35% and 30%, respectively. HLA disparity in the GVH direction, as well as in the HVG direction, did not influence overall survival and event-free survival (Table 3; and data not shown).

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Table 2. Univariate analyses of engraftment kinetics according to HLA disparity

No. of HLA mismatches	Neutrophil engraftment				
	n	Cumulative incidence, %	Median day	Range	*P*
HLA-A, -B, -DR (antigen)					.09
0 + 1	23	91	17	11-30	
2	129	89	20	11-55	
HLA-A, -B, -DR (antigen, HVG)					.4
0 + 1	43	93	19	11-55	
2	109	87	20	11-49	
HLA-A, -B, -DR (antigen, GVH)					< .001
0 + 1	53	96	19	11-36	
2	99	85	20	11-55	
HLA-A, -B (class I antigen)					.1
0	13	92	17	12-30	
1	86	91	20	11-44	
2	53	85	20	11-55	
HLA-A, -B (class I antigen, HVG)					.4
0	22	96	18	12-36	
1	86	89	20	11-55	
2	44	84	20	11-49	
HLA-A, -B (class I antigen, GVH)					.006
0	23	95	17.5	11-36	
1	88	91	20.5	11-44	
2	41	81	20	12-55	
HLA-A (antigen)					.7
0	87	89	19	11-44	
1 + 2	65	89	20	11-55	
HLA-A (antigen, HVG)					.8
0	96	89	20	11-55	
1 + 2	56	89	20	11-49	
HLA-A (antigen, GVH)					.2
0	103	90	19	11-44	
1 + 2	49	86	20	13-55	
HLA-B (antigen)					.07
0	36	94	19	12-34	
1 + 2	116	87	20	11-55	
HLA-B (antigen, HVG)					.06
0	45	95	19	12-36	
1 + 2	107	86	20	11-55	
HLA-B (antigen, GVH)					.04
0	42	95	18.5	11-36	
1 + 2	110	86	20	11-55	
HLA-DR (antigen)					.4
0	70	87	20	11-55	
1 + 2	82	90	19.5	11-44	
HLA-DR (antigen, HVG)					.7
0	76	88	20	11-55	
1 + 2	76	89	20	11-44	
HLA-DR (antigen, GVH)					.8
0	83	88	20	11-55	
1 + 2	69	90	20	11-44	
HLA-A, -B (antigen), -DR (allele)					.5
0 + 1	13	92	18	14-30	
2	63	84	20	11-47	
3 + 4	44	86	20	11-49	
HLA-A, -B (antigen, HVG), -DR (allele, HVG)					.2
0 + 1	25	96	18	11-32	
2	54	80	20	11-44	
3 + 4	41	90	20	11-49	
HLA-A, -B (antigen, GVH), -DR (allele, GVH)					.05
0 + 1	26	96	18	11-36	
2	57	84	19.5	11-49	
3 + 4	37	84	20	11-34	

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Table 2. Univariate analyses of engraftment kinetics according to HLA disparity (Continued)

No. of HLA mismatches	Neutrophil engraftment				
	n	Cumulative incidence, %	Median day	Range	*P*
HLA-A, -B, -DR (allele)					.4
0 + 1	10	90	18	14-30	
2	36	86	20	11-44	
3 + 4 + 5	56	84	19	11-49	
HLA-A, -B, -DR (allele, HVG)					.3
0 + 1	19	94	19	11-32	
2	34	79	20	13-44	
3 + 4 + 5	49	86	21	11-49	
HLA-A, -B, -DR (allele, GVH)					.05
0 + 1	16	94	17	11-30	
2	40	88	20	11-44	
3 + 4 + 5	46	80	20	11-49	

Discussion

Delayed hematopoietic recovery and graft failure are significant concerns in adult CBT. In the present study, median time to engraftment was 20 days, which was comparable with that reported in previous studies.[1,4,7,19] These data indicate that our pretransplantation conditioning regimens, consisting mainly of fludarabine, melphalan, and 4 Gy TBI, along with single calcineurin inhibitors for GVHD prophylaxis, can exert reasonable immunosuppressive effects that allow rapid hematopoietic recovery after CBT. The engraftment was durable except for disease progression.

Almost all reports on CBT have demonstrated the profound impact of infused cell dose on engraftment.[13,14,20] We showed that both high numbers of TNCs and $CD34^+$ cells were favorably associated with time to engraftment and the probability of engraftment, confirming previous findings on the association of cell dose with neutrophil recovery. Considering that $CD34^+$ cell dose reflects stem cell contents in the CB unit, stem cell dose is one of the major determinants of successful engraftment, as has been observed in the xenogeneic transplantation model.[21-23]

Although our results, demonstrating that HLA disparity in the GVH direction affected engraftment kinetics more than HLA disparity in the HVG direction, may seem paradoxical to the former notion of graft failure that results from graft rejection in most cases, they suggest a novel mechanism of graft failure in CBT. Previously, we have reported that a high incidence of noninfectious high-grade fever often coexisted with eruption, diarrhea, and weight gain, starting on a median of day 9 in more than 50% of the patients receiving CBT.[8,24] We regarded this reaction as early onset of acute

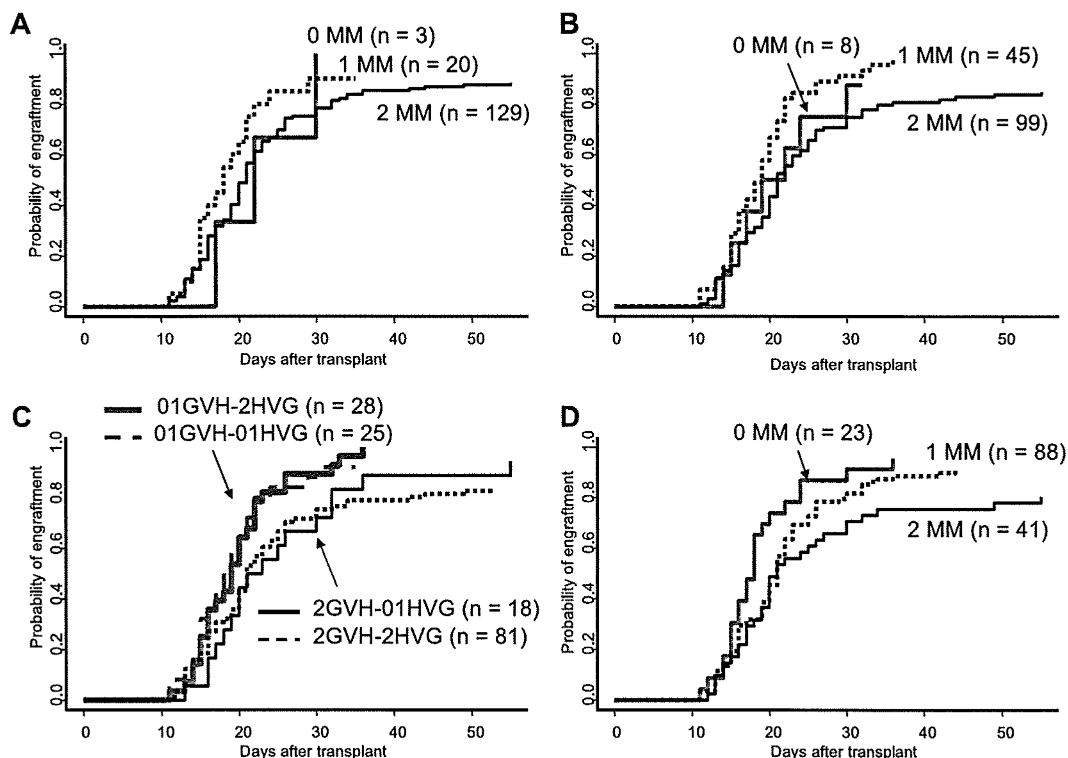

Figure 2. Cumulative incidence of neutrophil engraftment. MM indicates mismatch. (A) Effect of HLA antigen mismatch. (B) Effect of HLA antigen mismatch in the GVH direction. (C) Effect of HLA antigen mismatch according to mismatch both in the GVH and the HVG directions. 2GVH indicates 2 antigens mismatch in the GVH direction; 2HVG, 2 antigens mismatch in the HVG direction; 01GVH, 0 or 1 antigen mismatch in the GVH direction; 01HVG, 0 or 1 antigen mismatch in the HVG direction. (D) Effect of HLA class I antigen mismatch in the GVH direction.

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Table 3. Univariate analyses of acute GVHD and survival according to HLA disparity in the GVH direction

	Acute GVHD II-IV			2-year overall survival		
No. of HLA mismatches in the GVH direction	n	Cumulative incidence, %	P	n	Survival rate, %	P
HLA-A, -B, -DR (antigen)			.08			.5
0 + 1	50	33		59	36	
2	84	48		104	35	
HLA-A, -B (class I antigen)			.5			.2
0	22	36		24	54	
1	80	42		96	32	
2	32	46		43	32	
HLA-DR (class II antigen)			.5			.9
0	71	38		91	32	
1 + 2	63	47		72	38	
HLA-A, -B (antigen), -DR (allele)			.4			1.0
0 + 1	25	32		29	38	
2	48	51		60	38	
3 + 4	30	44		38	39	
HLA-A, -B, -DR (allele)			.3			.4
0 + 1	15	27		16	56	
2	35	49		41	37	
3 + 4 + 5	36	51		50	35	

GVHD in which activated donor T cells secreted various cytokines.[25] HLA disparity in the GVH direction may augment alloimmune reactions, which evoke hypercytokinemia and macrophage activation and occasionally result in establishment of hemophagocytic syndrome, one of the major complications directly related to graft failure in recipients.[26-28] Indeed, a considerable number of patients showed hemophagocytosis in the BM with donor dominancy, leading to graft failure, even though we cannot exclude the possibility of graft rejection caused by recipient lymphocytes in some cases. In addition, among those who achieved donor cell engraftment, delayed neutrophil recovery was prominent for those with more HLA mismatch in the GVH direction rather than in the HVG direction. Myelosuppression is commonly observed during acute or chronic GVHD, indicating that GVHD can negatively affect hematopoietic function of the graft, possibly because of an attack on the hematopoiesis-supporting recipient stromal cells[29] or production of cytokines from immune cells, such as transforming growth factor-β, known to regulate hematopoiesis negatively.[30] The delayed engraftment observed in our study may have been caused by similar mechanisms during the recovery of donor cells. Furthermore, our results demonstrated that HLA class I antigen mismatch in the GVH direction was associated with inferior engraftment. Higher impact of HLA class II disparity on the development of acute GVHD has been reported in National Marrow Donor Program data.[31] On the contrary, the Japan Marrow Donor Program registry data showed that mismatch in class I had higher impact than that in class II.[32] The discrepancy may be explained by unique ethnic background of the Japanese population. The observation shown here may further strengthen our hypothesis that GVH reactions play a crucial role in engraftment process. In the analysis using allele data, the statistical power of HLA disparity in the GVH direction on engraftment had decreased. This discrepancy probably results from the small sample size in each mismatched category but may be suggestive of more powerful immunogenicity of mismatch in antigen rather than allele level.

In the Eurocord registry data, which includes 550 CBTs, HLA disparity was shown to have a negative impact on engraftment, although the effect of direction of mismatch was not described.[14,33] More specifically, it was reported from the Düsseldorf Cord Blood Bank and Eurocord-Netcord Registry that HLA-A locus high-resolution typing in the HVG direction was associated with reduced cumulative incidence of engraftment in 122 patients receiving CBT.[34] Several reasons may explain this discrepancy from our observations. First, patients included in our study received relatively uniform pretransplantation conditioning regimens consisting mainly of fludarabine, melphalan, and TBI, whereas those in the Eurocord database had more variable pretransplantation conditioning regimens. Second, all of our patients had GVHD prophylaxis using single calcineurin inhibitors, whereas most of those in the Eurocord Registry received additional chemicals or anti-thymocyte globulin. Many institutes use methotrexate,[35,36] mycophenolate mofetil,[19,37] corticosteroids,[13] or anti-thymocyte globulin[38,39] in combination with a calcineurin inhibitor as GVHD prophylaxis in CBT. Narimatsu et al demonstrated that use of short-term methotrexate was associated with a lower rate of posttransplantation immune reactions without compromising engraftment.[36] Thus, more intensive immunosuppression may be beneficial for controlling early immune reactions and overcoming the issue of HLA mismatch. In addition, the unavoidable high incidence of gastrointestinal tract damage caused by TBI or melphalan in preparative regimens may have increased the chance of triggering GVH reactions.[40]

In the present study, HLA disparity had little association with the development of GVHD and survival, despite its obvious impact on engraftment. According to the Eurocord Registry data, better HLA match was not associated with better outcome in hematologic malignancies receiving CBT.[20] Further analyses are required to determine whether this is the result of the unique immunologic immaturity of CB or to the heterogeneous patient population with the majority being in the high-risk disease status.

In conclusion, HLA disparity in the GVH direction, especially class I disparity, was found to have a significant impact on engraftment. These results shed light on a novel mechanism responsible for graft failure in CBT and add a valuable clue for choosing a better CB unit to avoid graft failure.

Acknowledgments

The authors thank data coordinators Kaori Kobayashi and Naomi Yamada for their invaluable help in making this study possible; Dr Akinori Kimura, Department of Molecular Pathogenesis, Division of Pathophysiology, Medical Research Institute, Tokyo Medical and Dental University, for critical review of the manuscript; and the physicians, nurses, pharmacists, and support personnel for their care of patients in this study.

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This work was supported in part by a Research Grant for Tissue Engineering (H17-014) from the Japanese Ministry of Health, Labor, and Welfare.

Authorship

Contribution: N.M. and A.W. performed research and extracted data; A.Y. reviewed histopathologic methods; N.M. and Y.K. performed statistical analysis; N.U. and S. Taniguchi reviewed study design and methods; and K.I., H.A., S. Takagi, M.T., H.Y., D.K., Y.M., S.S., K.M., S. Miyakoshi, and S. Makino contributed to the writing of the paper.

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

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Scandinavian Journal of Infectious Diseases, 2010; 42:97–101

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ORIGINAL ARTICLE

Prediction of infectious events by high-sensitivity C-reactive protein level before undergoing chemotherapy for acute myeloid leukaemia

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Abstract

We retrospectively evaluated the serum high-sensitivity C-reactive protein (CRP) level before chemotherapy for the prediction of infectious events during neutropenia in patients with acute myeloid leukaemia. Thirty-eight patients who underwent first induction chemotherapy and 37 patients who underwent first consolidation chemotherapy were analyzed separately. A receiver-operating characteristic (ROC) curve revealed that the serum CRP level just before the first consolidation chemotherapy, but not just before the induction chemotherapy, had a significant predictive value for febrile neutropenia (FN) at a cut-off value of 0.19 mg/dl and documented infection (DI) at a cut-off value of 0.26 mg/dl. The high-sensitivity CRP measurement enabled the detection of slight increases in the serum CRP level, which might reflect a minute inflammation by occult infection, and discriminated high-risk patients for infectious events.

Introduction

Infection is the most common complication in neutropenic patients undergoing chemotherapy for haematological malignancies. Several measures, such as the use of high efficiency particulate air (HEPA) filters and the prophylactic administration of antibiotics, have been shown to be effective in preventing infectious events. However, considering the cost and the emergence of resistant bacteria, these interventions should not be applied to low-risk patients. Therefore, predictive factors before starting chemotherapy have been investigated to discriminate high-risk patients for infectious events during neutropenia.

C-reactive protein (CRP) is an acute phase reactant that is mainly produced in the liver. Serum CRP levels rapidly rise within 24 h in response to infection or tissue injuries [1]. Several reports have demonstrated that the prognoses of infectious events can be predicted by the serum CRP level measured at their onset in patients with neutropenia [2,3]. Other studies have demonstrated that changes in serum CRP levels reflect the response to antibiotic therapy [4,5]. However, it has been difficult to predict infectious events by the serial measurement of serum CRP levels [4]. High-sensitivity quantitation of serum CRP levels has recently become available; this can determine quantities of CRP of <0.3 mg/dl in sera, which is the detection limit in the conventional measurement of CRP, and enables the detection of slight inflammation. It has been reported that a slight increase in the serum CRP level in patients with atherosclerosis is associated with the risk of ischaemic heart disease, suggesting the clinical usefulness of the sensitive measurement of CRP [6]. Therefore, in this study, we examined the relationship between the high-sensitivity serum CRP level before chemotherapy and infectious events during neutropenia in patients with acute myeloid leukaemia (AML), to evaluate its predictive value for such events.

Patients and methods

High-sensitivity measurement of CRP became available in routine practice at our centre in October 2003. Therefore, we retrospectively analyzed consecutive patients

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(Received 16 July 2009; accepted 11 September 2009)

ISSN 0036-5548 print/ISSN 1651-1980 online © 2010 Informa UK Ltd. (Informa Healthcare, Taylor & Francis AS)
DOI: 10.3109/00365540903331019

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with AML treated at our institution from October 2003 to January 2008. Patients who underwent first induction chemotherapy or first consolidation chemotherapy for AML and who had neutropenia (neutrophil count <500/μl) for 7 days or longer were included in the study. We excluded patients who had had fever or definite infection and those who had received intravenous antibiotics at the start of chemotherapy. All the included patients received oral antibacterial and antifungal agents prophylactically. Antibacterial prophylaxis was performed with levofloxacin or polymyxin B. Oral fluconazole, itraconazole, or amphotericin B was given for antifungal prophylaxis. Antibacterial agents were changed to intravenous antibiotics when the neutrophil count became <500/μl, at the discretion of the attending physician (in 8 patients in the induction group and 12 patients in the consolidation group).

The serum high-sensitivity CRP level was measured at least twice a week as routine practice by latex immunoagglutination assay (Nanopia CRP, Sekisui Medical, Tokyo, Japan; minimum detection level 0.01 mg/dl). We collected data on serum CRP levels just before the start of chemotherapy. Infectious episodes were categorized into 2 groups: febrile neutropenia (FN) defined as fever during neutropenia with axillary temperature ≥37.5°C, and documented infection (DI), which included microbiologically documented infection and presumed infection based on clinical and/or radiological findings [7].

Patients who received induction chemotherapy and those who received consolidation chemotherapy were analyzed separately. The predictive value of the serum CRP level was evaluated using a receiver-operating characteristic (ROC) curve. ROC curves were drawn by plotting the sensitivity (y-axis) against (1 − specificity) (x-axis). The points on the ROC curves closest to the left upper corner were considered to be the best cut-off values, with which the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated. In addition, the predictive value of the following factors on the incidence of infectious events was also evaluated: sex, age, type of AML, performance status, history of recent infection, use of central venous catheter, prophylactic use of intravenous antibiotics after the start of chemotherapy, and duration of neutropenia. The incidence of FN was calculated using the Kaplan–Meier method. Univariate comparisons for dichotomous, continuous, and time-to-event variables between groups were performed with the Fisher's exact test, t-test, and the log-rank test, respectively. Factors associated with at least borderline significance ($p < 0.15$) in the univariate analysis were subjected to a multivariate analysis using proportional hazards modelling.

Results

Patient characteristics and infectious events during hneutropenia

Patient characteristics are summarized in Table I. Thirty-eight patients who underwent first induction chemotherapy and 37 patients who underwent first consolidation chemotherapy were analyzed separately. Seventeen patients were included in both analyses. In the induction group there were 26 males and 12 females with a median age of 54 y (range 16–78) and in the consolidation group there were 22 males and 15 females with a median age of 50 y (range 16–72). The median serum CRP level was 0.3 mg/dl (range 0.02–5.69) just before the first induction chemotherapy and 0.18 mg/dl (range 0.02–2.19) just before the first consolidation chemotherapy. The median durations of neutropenia <500/μl and <100/μl were 22 and 14 days, respectively, in the induction group and 18 and 10 days, respectively, in the consolidation group.

During the induction chemotherapy, 31 patients developed FN and 18 patients developed DI. During the consolidation chemotherapy, 26 patients developed FN and 11 patients developed DI. All patients who developed DI in both groups had experienced FN. Therefore, 18 patients in the induction group and 11 patients in the consolidation group had neither FN nor DI. The details of DI are summarized in Table II. In most of these patients, DI was documented by clinical or radiological evidence and the

Table I. Patient characteristics

	Induction (n = 38)	Consolidation (n = 37)
Age (range), y	54 (16–78)	50 (16–72)
Sex (M/F)	26/12	22/15
Performance status (0/1/2/3/4)	29/7/2/0/0	33/4/0/0/0
Antibiotic prophylaxis	38	37
HEPA filter	21	20
Central venous catheter	21	20
CRP level (range), mg/dl	0.3 (0.02–5.69)	0.18 (0.02–2.19)
Duration of neutropenia <500/μl (range), days	22 (7–69)	18 (9–51)
Duration of neutropenia <100/μl (range), days	14 (0–37)	10 (4–18)

HEPA filter, high efficiency particulate air filter; CRP, C-reactive protein.

Table II. Documented infections (DI)

	Induction chemotherapy	Consolidation chemotherapy
DI	18	11
Blood stream infection	5	4
Anorectal infection	4	2
Oral infection	3	0
Cutaneous infection	2	1
Upper respiratory tract infection	1	1
Lower respiratory tract infection	1	3
Peripheral venous catheter-related infection	1	2
Central venous catheter-related infection	0	1
Others	1	2

Some patients in the consolidation group developed 2 or more episodes of DI.

causative pathogen was not identified. However, in patients with blood stream infections, the most frequent causative pathogens were Staphylococcus epidermidis ($n = 4$), followed by Staphylococcus aureus, Staphylococcus capitis, Streptococcus mitis, and Enterococcus faecalis ($n = 1$ each).

Statistical analyses

The area-under-the ROC curve (AUC) plot, based on the serum CRP level just before induction chemotherapy was 0.48 for FN and 0.56 for DI, suggesting that the serum CRP level before induction therapy had poor predictive value for FN and DI. On the other hand, the serum CRP level just before consolidation chemotherapy had a better predictive value for FN and DI, with AUCs of 0.77 and 0.67, respectively (Figure 1). The best cut-off value of serum CRP level for FN was 0.19, which gave sensitivity, specificity, PPV, and NPV of 0.64, 0.82, 0.89, and 0.50, respectively. The best cut-off value of serum CRP level for DI was 0.26, which gave sensitivity, specificity, PPV, and NPV of 0.64, 0.80, 0.58, and 0.83, respectively. With these cut-off values, the likelihood ratio for a positive result of CRP for FN was 3.6 and for DI was 3.2. The likelihood ratio for a negative result of CRP for FN was 0.44 and for DI was 0.45.

In a univariate analysis, the prophylactic use of intravenous antibiotics after the start of chemotherapy and serum CRP level less than 0.2 mg/dl were associated with a lower incidence of FN during consolidation chemotherapy with at least borderline significance ($p = 0.12$ and $p = 0.07$, respectively; Table III). In a multivariate analysis, the adjusted p-value of the serum CRP level for the prediction of FN was 0.057. Cumulative incidences of FN calculated by the Kaplan–Meier method were 88% and 59% in patients with serum CRP levels of <0.2 mg/dl or in those with higher CRP levels ($p = 0.05$; Figure 2A). However, no difference in the incidence of FN was observed when the patients in the induction group were grouped according to the serum CRP level using the same cut-off value (Figure 2B).

Discussion

High-sensitivity measurement of the serum CRP level has enabled the detection of slight inflammatory change. We examined the relationship between the serum CRP level measured just before chemotherapy and the incidence of infectious events during neutropenia in patients undergoing first remission induction therapy or first consolidation therapy for AML. The serum CRP level before the first consolidation chemotherapy demonstrated a high predictive value for infectious events. High-risk patients for FN and DI could be discriminated by the serum CRP level, with cut-offs of 0.19 mg/dl and 0.26 mg/dl, respectively. This means that high-risk patients could not be discriminated by

Table III. Predictive factors for febrile neutropenia during consolidation chemotherapy

	Univariate analysis		Multivariate analysis	
	Incidence	p-Value	OR (95% CI)	p-Value
Sex (M/F)	68%/73%	>0.99		
Age (<50 y/≥50 y)	68%/72%	>0.99		
FAB (M3/other than M3)	63%/72%	0.67		
ECOG-PS (0/1)	68%/83%	0.65		
History of recent infection (+/−)	82%/60%	0.17		
Central venous catheter (+/−)	70%/71%	>0.99		
Prophylactic intravenous antibiotics (+/−)	50%/98%	0.12	0.26 (0.05–1.37)	0.11
Serum CRP level (<0.2 mg/dl/≥0.2 mg/dl)	57%/88%	0.07	5.70 (0.95–34.3)	0.057
Duration of neutropenia, <500/μl (<18 days/≥18 days)	63%/78%	0.48		
Duration of neutropenia, <100/μl (<11 days/≥11 days)	67%/75%	0.72		

OR, odds ratio; CI, confidence interval; FAB, French–American–British Cooperative Group criteria for the classification of acute myeloid leukaemia; ECOG-PS, performance status according to the Eastern Cooperative Oncology Group scale; CRP, C-reactive protein.