with different mean glucose levels,[21] we divided the patients into two groups based on mean glucose level 80–140 or 140+ and then determined whether glycemic variability was associated with an increased incidence of infections. However, there was no significant association between glycemic variability and the incidence of infections in both groups.

CRP levels

Figure 4 shows serial changes in the CRP level. Even though there was no difference in the CRP level between the two groups at the beginning of the conditioning regimen, the CRP level was significantly elevated in the SGC group compared to that in the IGC group 15 days after the beginning of the conditioning regimen, and this trend continued up to 40 days ($P<0.05$). The maximal CRP level during the neutropenic period in the IGC group was significantly lower than that in the SGC group (median (range), 6.9 (0.9–16.3) vs 11.5 (1.6–37.3), $P=0.007$).

Other clinical outcomes

The probability of grades II–IV acute GVHD within 100 days was 28 and 37% in the IGC and SGC groups (HR 1.05, 95% CI 0.38–2.91, $P=0.93$). The incidences of grades III–IV acute GVHD and NRM within 100 days were low in both groups (one and two patients, and one and one patient, in the IGC and SGC groups, respectively).

Discussion

This is the first study to evaluate the outcomes in allogeneic HSCT patients who were treated with a glucose management protocol. A salient finding of this study is that the incidence of documented infections, especially the incidence of bacteremia, was significantly lower in the IGC group than in the SGC group, as in a previous report in the ICU setting.[1] Moreover, there tended to be fewer organ dysfunctions in the IGC group, albeit this difference was not statistically significant. Furthermore, the CRP level, which might be a surrogate marker for produced cytokines,[19] was significantly lower in the IGC group than in the SGC group, as shown in Figure 4. Even though this study did not have enough power to detect a decrease in acute GVHD and NRM, it could be anticipated that IGC could reduce the CRP level, which would lead to a reduced incidence of acute GVHD and NRM.

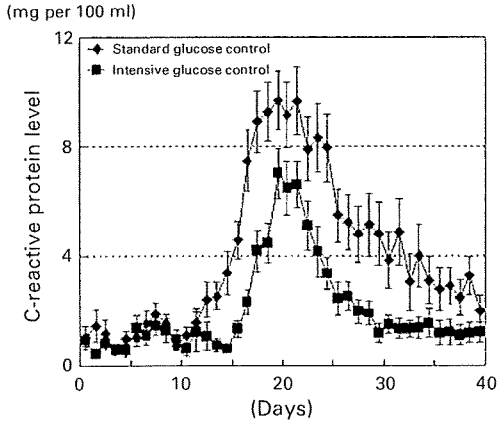

Figure 4 Serial change in the CRP level in the IGC and SGC groups. Values are mean + s.e.

This study has several limitations. One limitation is that only 64 patients were analyzed with no sufficient power to demonstrate any statistically significant changes in the incidences of organ dysfunctions, which was similar to the result in a previous report in the ICU.[1,2] An additional limitation was that the control of the glucose level could be suboptimal. This could be because of the glucose control protocol, which included monitoring of glucose level and the administration of insulin. With regard to the administration of insulin, we replaced the continuous infusion of insulin with the addition of Humulin R to the bottle of PN to control the glucose level within the target range because of the presence of fewer nursing staff in the HSCT unit than in the ICU. This could delay the normalization of hyperglycemia. Even though severe hyperglycemia (>180 mg per 100 ml) was reduced, a glucose value within the normal range (80–110 mg per 100 ml) could be achieved in only 49% of the IGC group as shown in Figure 1b. From a methodological point of view, it might be inappropriate to simply count the number of glucose value measurements, as patients with hyperglycemia were monitored more frequently, as defined in this protocol. Furthermore, as the mode of glucose monitoring was quite different between the IGC group and the SGC group, it could be inappropriate to compare the glucose values. A future protocol should include a more appropriate monitoring of glucose level and administration of insulin system that assures the fine tuning of glucose levels within the target range. Finally, there was a possible selection bias that may have affected the results, as this study was not a randomized-control study and there were many nonparticipants. However, the incidence of documented infections in nonparticipants within 100 days after allogeneic HSCT was 42%. Therefore, the reduction in the incidence of documented infections in the IGC group could not simply be explained by other causes such as the selection of antibiotics or catheter management.

With these limitations in mind, we took several steps to improve the quality of the study. First, we carefully matched patients and transplantation characteristics. Second, the IGC strategy was applied prospectively. Third, the low rate of patients who developed clinically significant hypoglycemia should be emphasized. As previously reported, the IGC procedure becomes very difficult in the medical ICU, especially in patients who have sepsis, a high APACHE score or mechanical ventilation.[1,2,22,23] The low rate of hypoglycemia could be because the medical acuity of our patients were relatively mild compared to those of patients in the medical ICU. Moreover, patients undergoing HSCT are younger and might have better β-cell function. The low rate of hypoglycemia could be important for maximizing the benefit of IGC because severe hypoglycemia could be associated with an increased risk of mortality.[23]

The biological plausibility of the intervention should be discussed. The reduction in infectious diseases by IGC may reflect the deleterious effects of hyperglycemia on macrophage or neutrophil function or insulin-induced protective effects on mucosal and skin barriers.[24–27] The improvement of innate immunity could be quite important, especially during the period of granulocytopenia after allogeneic HSCT. The protection of mucosal tissues could reduce bacterial translocation, which might lead to a reduced incidence of sepsis.

In conclusion, our results suggest that prospective IGC reduced the incidences of infectious diseases and organ dysfunction after allogeneic HSCT. To confirm these findings, a larger, prospective randomized-controlled trial is warranted.

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Positive impact of maintaining minimal caloric intake above 1.0 × basal energy expenditure on the nutritional status of patients undergoing allogeneic hematopoietic stem cell transplantation

To the Editor: Parenteral nutrition (PN) is frequently required for patients undergoing allogeneic hematopoietic stem cell transplantation (ASCT). However, the recommended dose of PN is associated with hyperglycemia [1,2], which leads to an inferior outcome [1,3]. Body weight (BW) and biochemical indices are used to assess the nutritional status, but these measures are affected by fluid status and inflammation [4]. Therefore, we retrospectively analyzed the values of nutritional variables in a cohort of 112 consecutive adult patients, who received myeloablative ASCT between January 2002 and June 2006. Sixteen patients who died before day 28, developed renal failure or liver failure, or received previous ASCT were excluded. Based on the mean caloric intake from the beginning of the conditioning regimen to day 28 or discharge, the remaining 96 patients were divided into low (n = 67) and high (n = 29) caloric groups [< or ≥ than 1.0 × basal energy expenditure (BEE)]. Patients' characteristics are summarized in Table I. During this period, nutritional support had been left entirely to the individual physicians. Six time periods were considered: (1) before the conditioning regimen, (2) from conditioning to day 0, (3) from days 1 to 7, (4) from days 8 to 14, (5) from days 15 to 21, (6) from days 22 to 28. Biochemical indices including total protein, albumin, cholinesterase, and prealbumin were monitored serially at least once a week.

TABLE I. Patients' Characteristics

Variable	N (%)/ median (range) Low caloric group <1.0 × BEE n = 67	High caloric group ≥1.0 × BEE n = 29
Age (year)	33 (18–57)	47 (20–55)
Body mass index (kg/m^2)	22.3 (15.2–38.1)	21.0 (15.1–27.2)
Sex		
Male	29 (43)	15 (52)
Female	38 (57)	14 (48)
Conditioning		
TBI-containing	34 (51)	15 (52)
Non-TBI-containing	33 (49)	14 (48)
Stem cell source		
Bone marrow	38 (57)	13 (45)
PBSC	28 (42)	12 (41)
Cord blood	1 (1)	4 (14)

Abbreviations: BEE, basal energy expenditure; TBI, total body irradiation; PBSC, peripheral blood stem cells.

Changes in BW are shown in Fig. 1A: a greater number of patients in the low caloric group lost more than 5% or 10% of their BW compared with the high caloric group (38 vs. 4, $P < 0.001$ and 8 vs 0, $P = 0.1$, respectively). No significant differences were seen for serum albumin, total proteins, cholinesterase, and prealbumin, whereas fasting glucose levels were significantly reduced from days 15 to 28 in the low caloric group (Fig. 1B). The significantly greater weight loss in the low caloric group could be associated with protein loss and organ dysfunction, although changes in fluid status and effects of chronic inflammation should also be considered. The absence of significant differences in biochemical indices between the two groups suggests that these parameters do not directly reflect malnutrition in ASCT patients [5]. Hyperglycemia was observed in patients receiving ≥1.0 × BEE caloric intake. We previously reported that hyperglycemia and neutropenia were associated with an inferior outcome [3]. The results suggest that a minimal caloric intake of >1.0 × BEE is necessary to maintain BW after ASCT, and that the assessment of nutritional status should not rely solely on biochemical indices. However, attention should be paid to the identification and prevention of hyperglycemia in these patients.

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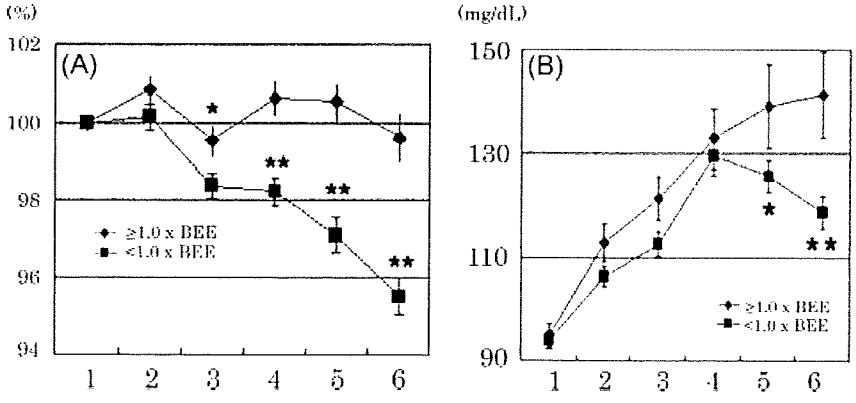

Figure 1. (A) Change in body weight in ASCT (*P 0.05, **P < 0.001). (B) Change in fasting serum glucose level in ASCT (*P 0.06, **P 0.003). The time course was divided into six periods: (1) before the conditioning regimen, (2) from conditioning to day 0, (3) from days 1 to 7, (4) from days 8 to 14, (5) from days 15 to 21, (6) from days 22 to 28.

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IGF-I treatment of patients with Laron syndrome suppresses serum thrombopoietin levels but does not affect serum erythropoietin

To the Editor: Growth hormone (GH) and insulin-like growth factor I (IGF-I) stimulate the proliferation and differentiation of many cell types including bone marrow cells. IGF-I was shown to stimulate erythropoiesis in in vitro studies [1]. In a previous study, we reported that children with Laron syndrome (LS, OMIM #262500) with congenital IGF-I deficiency responded to IGF-I treatment by an increase of hemoglobin (Hb) and red blood cells (RBC) and a decrease of a high platelet count (PLT) [2]. To investigate whether the effects induced by IGF-I are mediated by erythropoietin (Epo) and thrombopoietin (Tpo), we studied seven patients with LS: three untreated adults (ages: 43, 44, and 52) and four girls aged: 5, 9, 13, and 15 years receiving IGF-I replacement therapy (120–180 μg/kg/day s.c., Fujisawa, Osaka, Japan) for an average period of 9 ± 4 years. The mean age at initiation of therapy was 4.6 ± 3.5 years. Serum Tpo and Epo levels were measured using ELISA kits (Quantikine, R&D Systems, Minneapolis). In the children, before initiation of IGF-I treatment, Tpo levels were above normal for age, m ± SD: 285 ± 189 pg/ml (normal: 15–80 pg/ml). During IGF-I treatment Tpo levels dropped to 36 ± 19 pg/ml ($P = 0.04$). The mean PLT levels before treatment were 334 ± 53 × 10^9/l and decreased to 253 ± 30 × 10^9/l during therapy ($P = 0.04$). In the three untreated adult patients, Tpo serum levels were above normal but the PLT were within the normal limits (Table I). In the IGF-I treated-children, Epo levels did not correlate with the increase of RBC and Hb; and in the untreated adults, Epo levels varied within normal limits (1.0–21.5 mIU/ml). Experimental studies have indicated that the effects of GH on erythropoiesis are mediated by IGF-I of endocrine or paracrine origin [3]. We report for the first time that IGF-I administration reduces the high PLT count in young LS patients concomitantly with serum Tpo levels.

TABLE I. The Effect of IGF-I on Tpo and Platelets

	Before treatment	During treatment	*P* value
LS children			
Tpo (pg/ml)	285 ± 189	36 ± 19	0.04
Platelets (×10^9/l)	334 ± 53	253 ± 30	0.04
Untreated LS adults			
Tpo (pg/ml)	84 ± 60	–	
Platelets (×10^9/l)	240 ± 35	–	

Whether the reduction of Tpo during IGF-I treatment is due to a direct effect of IGF-I on the liver, or whether there exists a negative feedback mechanism between PLT and Tpo synthesis [4], remains to be clarified. The finding that Epo levels do not correlate with the IGF-I induced stimulation of erythropoiesis suggests that this effect is not Epo mediated as was also shown in rats [5] and in children [6]. Recently, it has been suggested that IGF-I secreted by macrophages may directly stimulate erythroblastic islands [7].

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LETTER TO THE EDITOR

Decreased insulin secretion in patients receiving tacrolimus as GVHD prophylaxis after allogeneic hematopoietic SCT

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As it has been reported that hyperglycemia is associated with a higher risk of non-relapse mortality after allogeneic hematopoietic SCT (HSCT), the efficient control of hyperglycemia has become an important consideration for safer HSCT.[1–3] A characteristic feature of this field is the use of calcineurin inhibitors, including tacrolimus (TAC), which may cause hyperglycemia as suggested in organ transplant settings, possibly by decreasing insulin secretion.[4] To evaluate this possibility, we serially monitored fasting glucose levels and serum immunoreactive insulin, and calculated homeostasis model assessment (HOMA)-IR and HOMA-β with the HOMA model[5] as recommended by Wallace *et al.*[6] HOMA-IR reflects insulin resistance and HOMA-β reflects the insulin secretion status.[5] If HOMA-IR increased after the administration of allogeneic HSCT, drugs that reduce insulin resistance, such as metformin or pioglitazone, might theoretically be effective. In contrast, if HOMA-β decreased, drugs that increase insulin secretion, such as glucagon-like peptide-1 analog or sulfonylureas, might be effective. The data from this study may help us to better understand how we should control glucose levels after HSCT.

Data obtained from 43 adult patients who received allogeneic HSCT from October 2006 to December 2007 were included in the analysis. The median age of the patients was 48 years (range: 19–66 years). When patients were not receiving s.c. long-acting insulin, systemic corticosteroid or parenteral nutrition, blood samples were obtained 1–2 months after HSCT. GVHD prophylaxis was started using CsA-based ($n=13$) or TAC-based regimens ($n=30$), with an additional short course of MTX in 35 patients. At the time of subsequent blood sampling, 15 patients were receiving CsA and 28 patients were receiving TAC, with no significant difference in various factors including age, gender, disease and intensity of the conditioning regimen (conventional vs reduced intensity), except that the TAC group included more HSCT with unrelated BM than the CsA group (89 vs 27%, respectively). The results regarding fasting glucose level, immunoreactive insulin and the HOMA model are summarized in Table 1.

We found that HOMA-β was significantly reduced in the TAC group compared with that in the CsA group, which was consistent with earlier studies in an organ transplant setting.[4] Clinically, it has been reported that GVHD prophylaxis with TAC is generally associated with a reduced incidence of acute GVHD compared with CsA. In contrast, hyperglycemia was associated with a higher risk of non-relapse mortality after allogeneic HSCT.[1,3] In our earlier study, patients with severe hyperglycemia had a significantly higher incidence of acute GVHD compared with normoglycemic patients.[3] Therefore, it is possible that hyperglycemia related to the use of TAC could offset the potential benefit of TAC, and drugs that increase insulin secretion, including the glucagon-like peptide-1 analog, may reverse the suppression of the insulin level.[7] Whether intensive glucose control could reduce the risk of acute

Table 1 Pretransplant and posttransplant glycemic status

Variable	N *(%)/ Median (range)*			
	Tacrolimus (n = *28*)		*CsA* (n = *15*)	
Fasting glucose level (mg per 100 ml)				
Pretransplant	87 (80–129)	$P=0.08$	89 (79–154)	$P=0.55$
Posttransplant	95 (79–129)		91 (80–116)	
Immunoreactive insulin level (μU/ml)				
Pretransplant	6.1 (1.6–17.3)	$P=0.60$	6.6 (2.9–13.5)	$P=0.25$
Posttransplant	6.5 (1.5–18.0)		5.3 (2.4–10.1)	
HOMA-IR				
Pretransplant	1.4 (0.3–4.6)	$P=0.75$	1.4 (0.6–5.13)	$P=0.40$
Posttransplant	1.5 (0.3–4.2)		1.3 (0.5–2.2)	
HOMA-β				
Pretransplant	90.9 (30.3–193.7)	$P=0.04$	65.4 (38.7–160.0)	$P=0.43$
Posttransplant	69.9 (15.8–202.5)		61.7 (28.5–180.0)	

Abbreviations: HOMA = homeostasis model assessment; IR = insulin resistance.

GVHD in patients using TAC should be evaluated by prospective randomized control trials.

In conclusion, this is the first study to assess the change in the glycemic status with the HOMA model in patients undergoing HSCT with CsA or TAC. We showed that GVHD prophylaxis with TAC was associated with decreased insulin secretion and a resultant tendency for hyperglycemia. It is possible that measures to keep insulin and glucose levels within their respective normal ranges are effective for reducing morbidity and mortality after HSCT.

Conflict of interest

The authors declare no conflict of interest.

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Possible graft-versus-host disease involving the central nervous system soon after cord blood transplantation

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The concept that central nervous system (CNS) could be a target of graft-versus-host disease (GVHD) is controversial. There are a few case reports which support the possibility of CNS-GVHD [1,2]. Here, we describe a patient who developed unique CNS symptoms soon after cord blood transplantation with reduced-intensity conditioning (RI-CBT). On Day 7 post-transplant, a high fever, slight skin eruption, moderate diarrhea, and liver damage suddenly developed. Three days later, her white blood cell (WBC) count rapidly increased to 1,700 μl^{-1} and consisted mostly of mature lymphocytes. Generalized convulsions developed on the same day. An analysis of the cerebrospinal fluid (CSF) revealed elevated proteins and pleocytosis comprising mostly mature lymphocytes. The lymphocytes found in the peripheral blood (PB) and CSF were phenotypically polyclonal T-cells that were donor derived. Extensive investigations did not detect any microorganisms or other causes for the T-cell proliferation and CNS symptoms. Considering the coexistence of CNS and systemic GVHD-like symptoms, proliferation of donor-derived polyclonal T-cells in the CSF and PB, and no microorganisms or other factors detected, CNS GVHD seems to be the most likely explanation for her clinical course.

Cord blood (CB) has been increasingly applied as a viable source of stem cells for allogeneic hematopoietic stem cell transplantation (allo-SCT) [3,4]. The incidence and severity of GVHD following cord blood transplantation (CBT) are lower than those after allo-SCT using bone marrow or peripheral blood stem cells from either matched siblings or unrelated donors [5–7]. On the other hand, unique immune-mediated complications, such as pre-engraftment immune reaction (PIR) and hemophagocytic syndrome (HPS), have been observed early after RI-CBT [8,9]. Thus, the spectrum of immune-mediated reactions after RI-CBT has not yet been fully clarified.

CNS complications have been described following allo-SCT [10]. Infections, drug toxicity, and metabolic and cerebrovascular disorders are the major causes, and there have been rare cases of apparent immune-mediated reaction to CNS [1,2].

Here, we present an interesting case of a patient who developed unique CNS symptoms soon after RI-CBT. A 40-year-old woman with follicular lymphoma that was refractory to chemotherapy was admitted to our hospital in September 2006. Her clinical stage was IV B at diagnosis in 2002. Six cycles of rituximab (R)-CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) resulted in complete remission, and rituximab therapy was maintained for 1 year. A relapse occurred in 2005 and was treated with R-ACES (high-dose Ara C, carboplatin, etoposide, and steroids), R-ICE (ifosfamide, carboplatin, etoposide), cladribine, and R-COP (cyclophosphamide, vincristine, and prednisone), which resulted in a partial response at each cycle. However, the disease gradually progressed thereafter, with the development of systemic lymphadenopathy, pleural effusion, and ascites. Since no suitable related or unrelated donors from the Japan Marrow Donor Program were available, unrelated CB was considered as an alternative graft, and she was referred to our hospital. The patient and graft were sex-mismatched and phenotypically two and genotypically three-loci mismatches in HLA-A, HLA-B, and DRB1 loci. The types of the HLA-A, HLA-B, and DRB1 loci were *A01* (*0101*)/A31 (3101), B35 (3501)/*B48* (*4801*), and DRB1*04

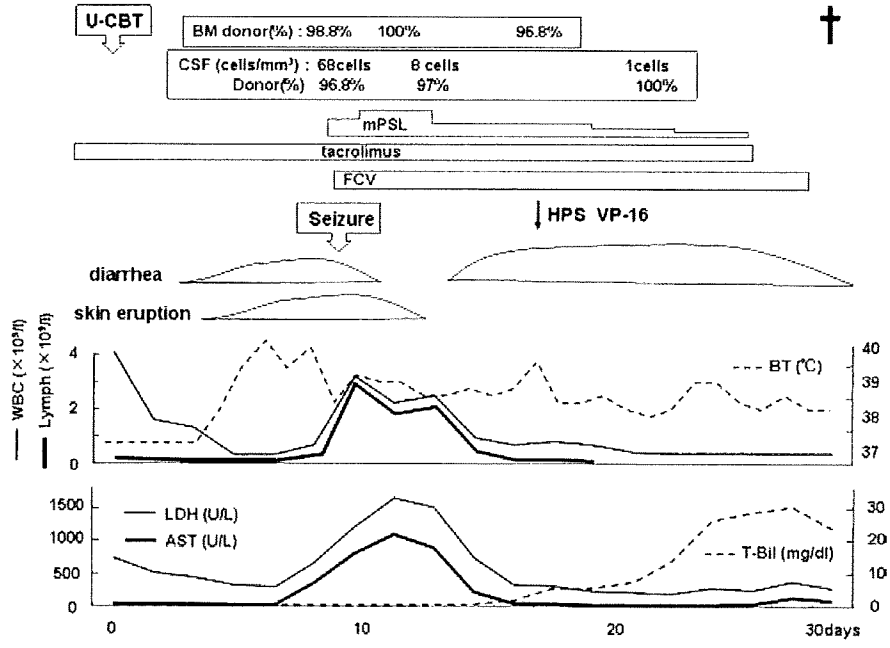

Figure 1. Clinical course of the patient. Abbreviations: U-CBT, unrelated cord blood transplantation; BM, bone marrow; CSF, cerebrospinal fluid; mPSL, methylprednisolone; FCV, foscarnet; HPS, hemophagocytic syndrome; VP-16, etoposide; WBC, white blood cell; BT, body temperature; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; T-bil, total bilirubin.

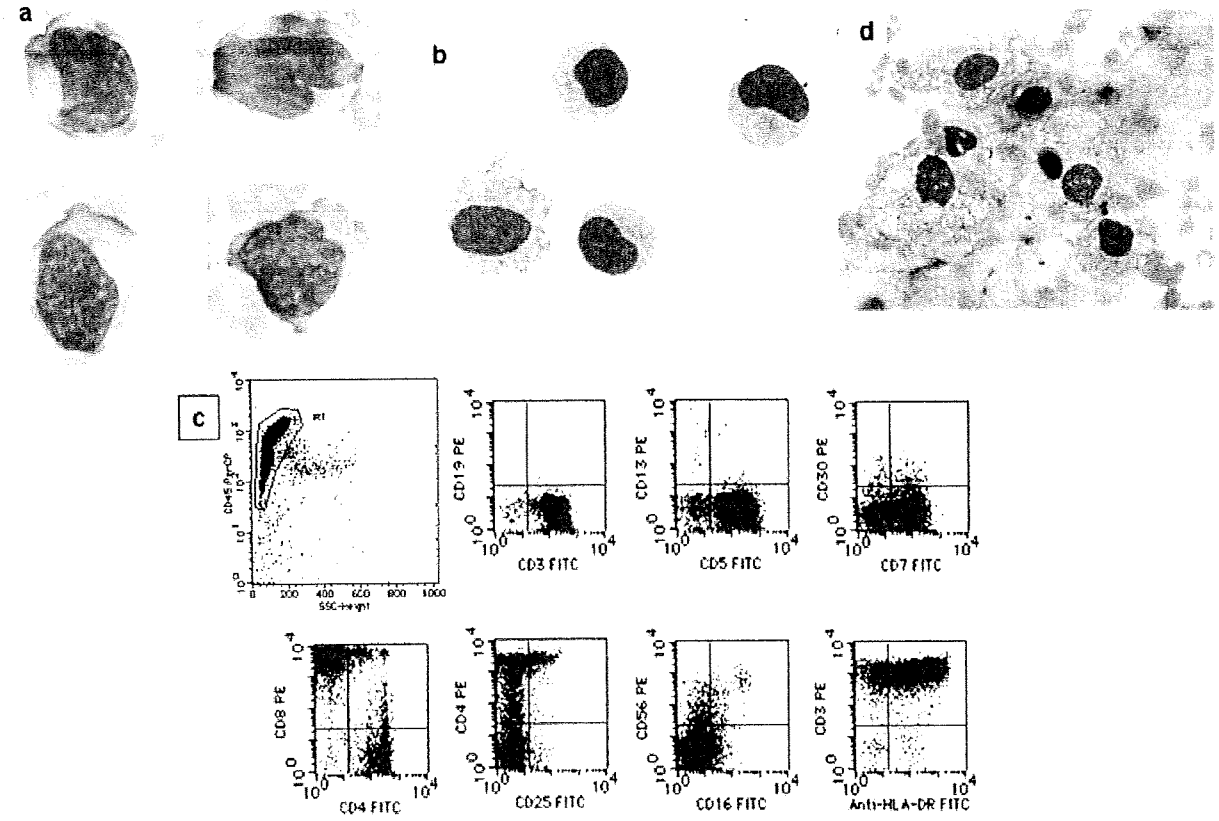

Figure 2. Activated lymphocytes in peripheral blood (a) and in cerebrospinal fluid (b) on Day 10 post-transplant. Flow cytometry of peripheral blood on Day 10 post-transplant (c). Activated macrophages in bone marrow on Day 17 post-transplant (d). May-Giemsa staining ×1000 (a, b) ×400 (c). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

(*0404*)/DRB1*09 (0901), respectively, in the recipient, and *A26* (*2601*)/A31 (3101), B35 (3501)/*B51* (*5101*), and DRB1*04 (*0401*)/DRB1*09 (0901), respectively, in the donor. The graft contained 2.4 × 10^7/kg total nucleated cells and 0.92 × 10^5/kg $CD34^+$ cells. The pretransplant conditioning regimen consisted of fludarabine (25 mg/m^2/day) for 5 days, melphalan (40 mg/m^2/day) for 2 days, and 4 Gy of total body irradiation. Tacrolimus alone was administered as GVHD prophylaxis. Granulocyte colony-stimulating factor was started from Day 1. Pretransplant viral serology was positive for HSV, HVZ, CMV, and EBV, and negative for HIV and HTLV-1. She received 600 mg/day of oral acyclovir, 400 mg/day of oral tosufloxacin, 200 mg/day of oral itraconazole, and trimethoprim-sulfamethoxazole (160 mg/day of the trimethoprim component) as for antimicrobial prophylaxis. Figure 1 shows her entire clinical course following RI-CBT. On Day 7 post-transplant, a high fever, slight skin eruption, and moderate diarrhea developed with a slightly increased WBC count (from 10 μl^{-1} on Day 6 to 30 μl^{-1} on Day 7). Her WBC count rapidly increased on Day 10 to 1,700 μl^{-1} and comprised 90% lymphocytes (Fig. 2a). Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels increased to 715 and 359 IU/l, respectively, and serum lactate dehydrogenase (LDH) levels increased to 1,101 IU/l. The patient suddenly lost consciousness along with generalized convulsions on the same day and required mechanical ventilation. Cerebrospinal fluid (CSF) analysis revealed an extremely elevated protein level of 675 mg/dl (normal range: 15–40 mg/dl) and pleocytosis (68 cells/μl), consisting mainly of lymphocytes (98%) (Fig. 2b). Magnetic resonance imaging scans of the brain revealed no specific abnormalities typically seen in cerebrovascular disorders, tacrolimus encephalopathy, thrombotic microangiopathy, or other CNS complications, and schistocytes were undetectable in the PB. Flow cytometry revealed that the excessive lymphocytes in both PB and CSF comprised polyclonal mature T-lymphocytes expressing CD3, CD4, CD5, CD8, and HLA-DR. The expression of CD4 and CD8 was variable, in which $CD4^-CD8^+$, $CD4^+CD8^-$, and $CD4^+CD8^+$ cells accounted for 65, 25, and 9%, respectively, of the cells in PB, and 38, 56, and 6%, respectively, of those in the CSF (Fig. 2c). Y chromosome-based fluorescence in situ hybridization analysis showed that most of these cells were derived from the donor (98.8% in PB and 96.8% in CSF). Furthermore, 98% of BM cells obtained on Day 10 were also donor derived. Routine cultures of PB and CSF for bacteria and fungi were negative. Analyses by real-time polymerase chain reactions were negative for HSV-1, HHV-6, VZV, CMV, and EBV in PB and CSF, and, for HSV-2, HSV-7, HSV-8, JCV, BKV, ADV, Parvovirus B19, HBV, and HCV in PB. Southern blotting of cells from the PB showed that the genes for both T-cell receptor Cβ1 and Jδ1 were in germ-line configuration, and EBV genome clonality was undetectable. Methylprednisolone (500 mg/day) was administered for 3 days, and acyclovir was switched to foscarnet, considering the possibility of acute GVHD and viral infection insensitive to acyclovir. After the initiation of these therapies, the numbers of lymphocytes in PB and CSF gradually decreased, and her clinical symptoms and laboratory data improved, so methylprednisolone was carefully tapered. However, high fever, diarrhea, and CNS symptoms recurred around Day 17, and then pancytopenia and cholestatic liver damage rapidly progressed. On Day 17, BM aspiration revealed an increase of activated macrophages (35%) with massive hemophagocytosis (Fig. 2d). The chimeric status of the BM cells revealed sustained donor cell dominance (96.8%), indicating that the hematopoietic cells and macrophages in the BM were both donor derived. Despite the administration of etoposide (50 mg/m^2) to control the hemophagocytosis, pancytopenia and cholestatic liver damage progressed and the patient died of bacterial sepsis 32 days after transplantation. An autopsy was not performed.

Polyclonal T-cell proliferation is the principal mechanism of the antigen-specific immune response that generally occurs upon infection and/or inflammation. GVHD is also primarily a T-cell-mediated event, and the subsequent expansion of donor T-cell clones-recognizing antigens causes tissue damage either directly through T-cells encountering recipient MHC-bearing cells in target tissues or indirectly through cytokine production [11].

We previously reported higher incidence of immune-mediated complications, such as PIR, characterized by high-grade fever, skin eruption, diarrhea, jaundice, and body weight gain developing before engraftment, and HPS early after RI-CBT [8,9]. Despite the known immunological naïvety of CB cells, the exceptionally high incidence of PIR and HPS suggests that the properties of CB cells are unique and distinctly different from adult donor cells.

The most striking features of our patient were the remarkable polyclonal T-cell proliferation both in PB and CSF, followed by sudden generalized convulsions and loss of consciousness. As the coexistent CNS and systemic GVHD-like symptoms, proliferating donor-derived polyclonal T-cells in the CSF and PB, and microorganisms or other factors that might be responsible for these symptoms or T-cell proliferation were undetectable. We therefore postulated that an alloimmune reaction of the CB graft against the CNS caused the CNS symptoms in our patient.

The concept that CNS could be a target of GVHD is controversial. Some case reports support the possibility of CNS-GVHD [1,2]. All of the patients in these reports were diagnosed with CNS-GVHD only when they responded to immunosuppressive therapy and had histologically and immunophenotypically documented perivascular T-cell infiltration without evidence of other CNS diseases with overlapping features. However, uniform diagnostic approaches or criteria have not been established. Most of the reported CNS-GVHD was diagnosed at the time of chronic GVHD development. Powles et al. [12] reported that convulsions, possibly due to cerebral edema, could develop as a manifestation of severe acute GVHD after haploidentical transplantation. This could explain the events in our patient, although information about the CSF, the presence or absence of T-cell proliferation, or detectable infectious organisms was not provided in the literature. We reported that early CNS complications are more frequent after RI-CBT than after transplantation with other stem cell sources and that hypercytokinemia associated with PIR could influence the development of CNS complications [13]. T-cell proliferation in CSF along with the severe systemic symptoms in our patient might have resulted from a type of hypercytokinemia that is unique to RI-CBT.

Moreover, severe HPS developed around 10 days after T-cell proliferation, and the activated macrophages in the BM were donor derived. Although HPS is a rare complication following allo-SCT, some investigators have suggested that a severe alloimmune response could result in HPS after PB transplantation [14,15]. Furthermore, we recently reported that the incidence of HPS following RI-CBT is higher than was previously reported and that HPS is a significant risk factor for engraftment failure [9]. Hypercytokinemia associated with engrafted T-cell proliferation may have played an important role in donor-derived macrophage activation and in the development of HPS in our patient.

In conclusion, we described a patient who developed sudden generalized convulsions and lost consciousness at the same time as polyclonal T-cell proliferation soon after RI-CBT. The findings of extensive investigations indicated that the CNS can be a target of GVHD. Further accumulation of clinical and laboratory data with the awareness of this devastating complication soon after RI-CBT is warranted to precisely understand the underlying basic mechanisms and to develop optimal intervention strategies.

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Treatment with hydroxyurea in a patient compound heterozygote for a high oxygen affinity hemoglobin and β-thalassemia minor

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Compound heterozygotes for β-thalassemia and high oxygen affinity hemoglobin (Hb) have been documented, but experience in the management of such rare cases is minimal. Although hydroxyurea (HU) has never been used in a heterozygote with high oxygen affinity Hb and β-thalassemia, we hypothesized that it would decrease erythrocytosis through a lowered production of abnormal cells and increase of P_{50} by induction of fetal hemoglobin (HbF). We present the case of a patient with compound high oxygen affinity Hb mutation with β-thalassemia. PCR analysis revealed combined Hb Regina and IVS1-110 G/A mutations. Treatment with HU caused a decrease in Ht (61.1% to 38.6%) and erythrocyte volume (74.87 mL/kg to 40.65 mL/kg), as well as an increase in P_{50} (6 mmHg to 10 mmHg) and HbF level (3.6% to

bjh research paper

High incidence of haemophagocytic syndrome following umbilical cord blood transplantation for adults

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Summary

Umbilical cord blood transplantation (CBT) is widely accepted, but one critical issue for adult patients is a low engraftment rate, of which one cause is haemophagocytic syndrome (HPS). We aimed to identify the contribution of HPS to engraftment failure after CBT, following preparative regimens containing fludarabine phosphate, in 119 patients (median age, 55 years; range; 17–69 years) with haematological diseases. Graft-*versus*-host disease prophylaxis comprised continuous infusion of a calcineurin inhibitor with or without mycophenolate mofetil. Of the 119 patients, 20 developed HPS within a median of 15 d (cumulative incidence; 16·8%) and 17 of them did so before engraftment. Donor-dominant chimaerism was confirmed in 16 of 18 evaluable patients with HPS. Despite aggressive interventions including corticosteroid, ciclosporin, high-dose immunoglobulin and/or etoposide, engraftment failed in 14 of 18 patients. Of these 14 patients, four received second rescue transplantation and all resulted in successful engraftment. Overall survival rates significantly differed between patients with and without HPS (15·0% vs. 35·4%; $P < 0·01$). 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). We concluded that engraftment failure closely correlated with HPS in our cohort, which negatively impacted overall survival after CBT.

Keywords: cord blood transplantation, reduced-intensity chemotherapy, haemophagocytic syndrome, engraftment failure.

Umbilical cord blood transplantation (CBT) is an alternative allogeneic haematopoietic stem cell transplantation (HSCT) strategy for patients with haematological diseases who do not have a matched related or unrelated donor and who need urgent transplantation. The value of CBT using myeloablative preparative regimens has already been confirmed among paediatric and adult patients (Laughlin *et al*, 2004; Rocha *et al*, 2004; Takahashi *et al*, 2004). However, conventional myeloablative preparative regimens are associated with significant morbidity and mortality, particularly in older patients or in those who have experienced extensive prior therapy or organ dysfunction associated with transplantation-related mortality. Various reduced-intensity preparative regimens that have been applied to such patients by several groups, including the authors of the present study, have proven feasible (Barker *et al*, 2003, 2005; Chao *et al*, 2004; Jacobsohn *et al*, 2004; Miyakoshi *et al*, 2004, 2007; Yuji *et al*, 2005; Misawa *et al*, 2006; Ballen *et al*, 2007; Brunstein *et al*, 2007; Komatsu *et al*, 2007; Uchida *et al*, 2008).

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Engraftment failure is a critical problem that can arise after CBT. The limited doses of infused total nucleated and $CD34^+$ cells contained in umbilical cord blood are thought to influence the rate and kinetics of haematopoietic recovery. In order to overcome engraftment failure, various kinds of strategies, such as multiple unit or *ex-vivo* expanded CBT, and co-infusion of peripheral blood stem cells, have been employed (Shpall *et al*, 2002; Fernandez *et al*, 2003; Barker *et al*, 2005).

Several recent reports have described HPS that arose after autologous and allogeneic HSCT followed by engraftment failure (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). In a reduced-intensity conditioned CBT (RI-CBT) setting, we experienced one patient who failed to achieve engraftment due to HPS following HSCT (HSCT–HPS). Following this case, several similar cases were observed in our institute. We postulated that HPS could play a critical role in engraftment failure after CBT. This report describes the characteristics of 20 patients with HSCT–HPS among 119 who underwent CBT.

Materials and methods

Patients

The study population consisted of 119 adult patients with haematological diseases, who underwent CBT as the first allogeneic HSCT at Toranomon Hospital, Tokyo, Japan between January 2004 and December 2006. All the patients were incurable using conventional approaches, lacked a human leucocyte antigen (HLA)-identical sibling or a suitable unrelated donor from Japan Marrow Donor Program. Most of the patients were considered inappropriate for conventional myeloablative allogeneic HSCT due to being >50 years and/or having organ dysfunction (cardiac ejection fraction <50%, forced expiratory volume 1·0 s % <80%, or serum creatinine > 1·5× upper limit of normal range). Written informed consent was provided by all patients in accordance with the Declaration of Hersinki. The Institutional Review Board of Toranomon Hospital approved the study.

Transplantation procedures

Cord blood units that were serologically matched for ≥4 of six HLA antigens and which contained at least $1{\cdot}8 \times 10^7$ nucleated cells/kg of recipient body weight before freezing were obtained from the cord blood bank at the Japan Cord Blood Bank Network (Nishihira *et al*, 2003). The units were not depleted of T lymphocytes. All patients received purine analogue-based preparative regimens comprising fludarabine phosphate (125–180 mg/m^2), melphalan (80–140 mg/m^2) or busulfan (BU; 8–16 mg/kg) and 0–8 Gy of total body irradiation (TBI), as decided by the treating physician. Graft-versus-host disease (GVHD) prophylaxis comprised a continuous intravenous infusion of either 0·03 mg/kg of tacrolimus (TAC) or 3 mg/kg of ciclosporin (CsA), starting on day-1, except eight patients who received 2 g/d of mycophenolate mofetil (MMF) starting on day-1 in addition to TAC.

Supportive cares

All the patients were treated in reverse isolation in laminar airflow-equipped rooms and received trimethoprim/sulfamethoxazole for *Pneumocystis jirovecii* prophylaxis. Fluoroquinolone and azole and acyclovir were administered to prevent bacterial, fungal and herpes virus infection, respectively. Neutropenic fever was managed according to the guidelines (Hughes *et al*, 2002). Cytomegalovirus pp65 antigenaemia was monitored weekly and preemptive therapy with foscarnet was initiated in the event of a positive result (Matsumura *et al*, 2007; Narimatsu *et al*, 2007a). Haemoglobin and platelet counts were maintained at >70 g/l and 10×10^9/l, respectively. Granulocyte colony-stimulating factor was administered intravenously from day 1 until neutrophil recovery became durable.

Assessment of engraftment, chimaerism, pre-engraftment immune reactions, disease risk and survival

Engraftment was defined as the first of three consecutive days in which white blood cell counts were $>1{\cdot}0 \times 10^9$/l or the absolute neutrophil counts were $>0{\cdot}5 \times 10^9$/l. When the above definition was not met by day 28 without subsequent neutrophil recovery, the patient was considered to have primary engraftment failure. Delayed engraftment was defined as neutrophil engraftment after day 29. Secondary engraftment failure was defined as a decrease in the neutrophil count to $<0{\cdot}5 \times 10^9$/l for three consecutive days after successful engraftment. The date of platelet recovery was defined as the first of seven consecutive days during which the non-transfused platelet count was at least 20×10^9/l.

Chimaerism was assessed using fluorescent *in situ* hybridization (FISH) in sex-mismatched donor–recipient pairs. In sex-matched pairs, chimaerism was assessed using the polymerase chain reaction for variable numbers of tandem repeats with donor cells detected at 10% sensitivity (Thiede *et al*, 1999).

Pre-engraftment immune reactions (PIR) were diagnosed when febrile patients (body temperature ≥38·0°C) developed skin eruption, diarrhoea, jaundice (serum total bilirubin >34·2 μmol/l) or body weight gain of >10% of baseline, with no direct evidence of infection or adverse effects of medication, developing ≥6 d before engraftment (Kishi *et al*, 2005b).

Patients with acute myeloid leukaemia in first or second complete remission (CR) at the time of transplant, with acute lymphoblastic leukaemia in first or second CR, with chronic myeloid leukaemia in the chronic phase, with refractory

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/m^2)/Mel (80–140 mg/m^2)/TBI (2–8 Gy)	91
Flu (125–180 mg/m^2)/Mel (80–140 mg/m^2)	7
Flu (125–180 mg/m^2)/BU (8–16 mg/kg)/TBI (4–8 Gy)	14
Flu (150–180 mg/m^2)/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 $2{\cdot}40 \times 10^7$ cells/kg (range, 1·98–5·13) and $0{\cdot}52 \times 10^5$ 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.

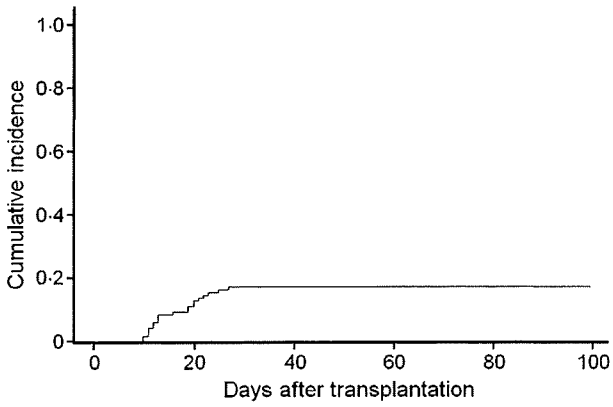

Fig 1. Cumulative incidence of HPS following CBT.

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.

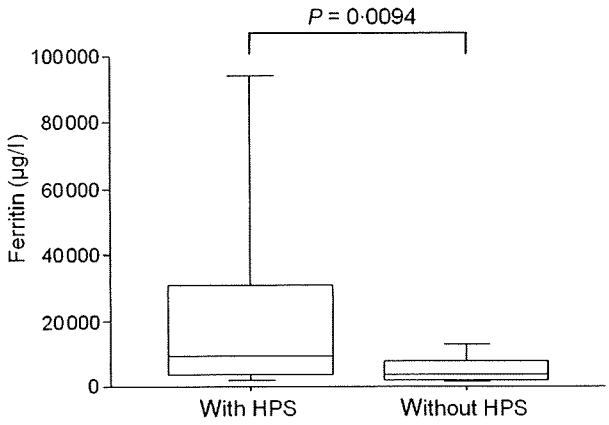

Fig 2. Comparison of highest value of serum ferritin by day 30 of CBT (with *versus* without HPS).

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

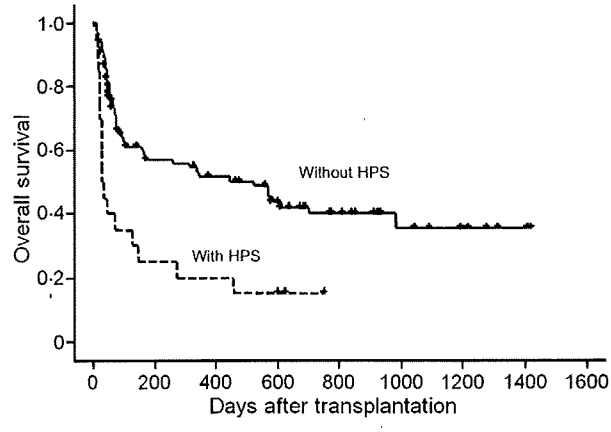

Fig 3. Comparison of overall survival (with *versus* without HPS).

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, tacrolimus; 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.

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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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