Posttransplant Autoimmune Hemolytic Anemia and Other Autoimmune Cytopenias are Increased in Very Young Infants Undergoing Unrelated Donor Umbilical Cord Blood Transplantation

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Abstract

Autoimmune cytopenias are a recognized complication of hematopoietic stem cell transplant (HSCT), and are considered to be a feature of chronic graft-versus-host disease (cGVHD). We report on a cohort of very young infants (3 months of age) receiving HSCT from unrelated donor umbilical cord blood for genetic disorders who developed posttransplant autoimmune cytopenias at an increased rate compared to older aged controls. These infants received a conditioning regimen consisting of busulfan, cyclophosphamide, and antithymocyte globulin (ATG). All infants received HLA mismatched unrelated umbilical cord blood as graft source. GVHD prophylaxis was either cyclosporine + methylprednisolone (n = 16) or cyclosporine + mycophenolate mofetil (n =3). Engraftment, acute GVHD (aGVHD) and cGVHD, survival, treatment-related mortality (TRM), and deaths were evaluated. Ten patients developed cGVHD manifesting as autoimmune cytopenias at a median 247 days posttransplant with a cumulative incidence of 44% (95% confidence interval [CI] 21%–68%) and 56% (95% CI 32%–80%) at 1 and 2 years, respectively. In 6 of 10 patients developing autoimmune cytopenias, cGVHD presented as autoimmune cytopenia de novo. The cytopenias observed included anemia (n =4), thrombocytopenia (n =1), anemia with thrombocytopenia (n =3), and pancytopenia (n =2). No graft factors were identified as being significant to development of cGVHD. All patients responded to treatment with methylprednisolone, azithioprine ± rituximab. One patient required splenectomy. We hypothesize that posttransplant immunosuppression interferes with normal immune ontogeny creating immune dysregulation and graft directed cell destruction. Alternative strategies to prevent GVHD should be considered for this unique patient population.

Keywords

Autoimmune hemolytic anemia; Unrelated umbilical cord blood transplantation; Chronic graft versus host disease

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INTRODUCTION

Autoimmune cytopenias are a recognized complication after allogeneic hematopoietic stem cell transplantation (HSCT), and are considered to be a feature of chronic graft-versus-host disease (cGVHD) when another symptom of cGVHD is present [1,2]. In adults, the incidence of autoimmune hemolytic anemia (AIHA) is 3%–4% in patients receiving either conventional or T cell-depleted grafts [3–5]. A slightly higher rate of ~6% has been the reported in the pediatric literature [6,7]. There are limited reports about autoimmune cytopenias and cGVHD in very young babies undergoing HSCT. Over the past decade, we have transplanted a cohort of very young babies with early infantile lysosomal storage diseases and other genetic disorders. We have observed a higher than expected rate of autoimmune cytopenias in these infants and describe the cases in this report. We propose that autoimmune cytopenias can be a primary manifestation of cGVHD in young pediatric patients undergoing allogeneic HSCT.

MATERIALS AND METHODS

Patients

Between September 1998 and April 2007, 19 consecutive infants (3 months of age) diagnosed with early infantile lysosomal storage diseases (n = 18) or hemoglobinopathies (n = 1) who were referred to our center for transplantation and who lacked appropriate HLA matched related donors received cord blood transplants from unrelated donors. Fourteen were diagnosed prenatally and 5 were diagnosed at birth. All diagnoses were confirmed prior to transplant. Eight patients were enrolled in an institutional-based phase I/II study of cord blood transplantation for the treatment of nonmalignant diseases. Ten patients were enrolled on the Cord Blood Transplantation Study (COBLT) [8], and 1 patient was enrolled in a phase I trial using ex vivo expansion of ALDH cells [9]. All studies were approved by the institutional review board of Duke University Medical Center. Written informed consent was obtained from the infant's parents before enrollment.

Selection of Donors

Intermediate-resolution typing for HLA class I (A and B) and high resolution for HLA-DRB1 were used for donor selection. The cord blood unit with the highest number of nucleated cells (minimum, 3×10^7/kg of recipient body weight) and closest HLA match (minimum 4 of 6 HLA loci match) was selected. Units were tested to ensure that the donor was neither affected with nor a carrier of the condition for which the infant was being transplanted.

Transplantation Procedure

Cryopreserved units of cord blood were thawed and washed per the procedure of Rubinstein et al. [10]. For the single patient on the ALDH trial, the 80% fraction was transplanted using conventional methods, whereas the 20% fraction was sorted to isolate ALDH bright cells that were subsequently primed ex vivo for 5 days using cytokines before infusion [9]. All thawed cord blood units were tested for total number of nucleated cells (TNC), clonal hematopoietic progenitor cells, CD34$^+$and CD3$^+$cells, cell viability, ABO and Rh typing, and microbial cultures (bacteria and fungal).

Conditioning Regimen

The conditioning regimen for all patients included 16 doses of busulfan (Bu; 20 mg/m^2 orally) from days –9 through –6; 4 doses of cyclophosphamide (Cy; 50 mg/m^2 i.v.) from days –5 through –2; and 3 doses of equine antithymocyte globulin (ATG; 30 mg/kg i.v.) from days –3 through –1. Bu pharmacokinetics were monitored with the first dose to target

steady-state concentrations between 600–900 ng/mL. Patients received phenytoin during Bu as seizure prophylaxis and mesna during Cy for prophylaxis against hemorrhagic cystitis.

Posttransplant Regimen and Supportive Care

All children received prophylaxis against GVHD with cyclosporine (CSA) + methylprednisone (n = 16) or CSA + mycophenolate mofetil (MMF; n = 3). CSA was dosed to target levels between 200 and 300 ng/mL, and MMF was dosed at 15 mg/kg/dose i.v. every 8 hours. GVHD prophylaxis was continued at full dose for 6–9 months, then weaned as tolerated. Treatment options for GVHD were based on severity and included topical steroids, dose escalation of methylprednisone, change to tacrolimus, or addition of daclizumab (Hoffmann-LaRoche, Nutley, NJ).

All patients were nursed in reverse isolation rooms under positive pressure and HEPA filtration. Standard prophylaxis was used against pneumocystis carinii, acyclovir for viral, and voriconazole for fungal infections. Empiric antibiotic treatment was started with the first febrile episode and continued through engraftment. Intravenous immune globulin (500 mg/kg/dose) was administered weekly through day 100 then monthly through 1 year, then weaned as tolerated. As prophylaxis against veno-occlusive disease (VOD), a continuous infusion of low-dose heparin was used through day 28. Patients received transfusions of leukocyte-depleted, irradiated-packed red blood cells and platelets. Granulocytecolony stimulating factor (G-CSF) was administered from day 0 until hematopoietic recovery and then was weaned. Confirmation of engraftment was performed using fluorescein in situ hybridization (FISH) or restricted fragment length polymorphism (RFLP).

Study Subjects

Potential study subjects were identified by retrospective review of the transplant program research database using age at transplant as initial criteria. To be eligible for the study, patients had to be 3 months of age at transplant, recipients of unrelated UCBT with myeloablative conditioning, and have a diagnosis of a nonmalignant disorder. Data was collected via chart review for demographics, information regarding cord blood unit, complications following transplant including diagnosis, treatment, and response to treatment of an autoimmune cytopenia. Data were compared to 141 older aged historic and contemporary controls at our center.

Laboratory Evaluation

Autoimmune hemolytic anemia was defined as development of anemia in a patient with stable engraftment where there was a hemoglobin drop of >2 g/dL, transfusions needed in prior transfusion-independent patients, and evidence of hemolysis by positive direct antiglobulin test (DAT) and/or indirect Coomb's test, peripheral blood cell morphology, reticulocyte count, and bilirubin level. The DAT was performed to detect the presence of IgG or IgM antibodies and complement on the patient's red blood cells (Immucor, Norcross, GA). If initial testing was positive, further testing was conducted to determine if the reaction was the result of IgG and/or complement specifically C3. If IgG antibodies were present, an eluate was prepared and tested to determine antibody specificity. Thrombocytopenia was defined as platelet count <50,000 cells/μL. Neutropenia was present if the absolute neutrophil count was <500/mm^3. Direct and indirect antiplatelet antibody testing was performed if thrombocytopenia was present. Neutrophil cytoplasmic antibodies were enumerated when possible.

Acute and Chronic GVHD (aGVHD, cGHVD) Scoring

The severity of aGVHD was scored using standard criteria [11]. cGVHD was scored using National Institute of Health (NIH) consensus criteria [1]. However, patients with isolated immune cytopenias were also scored as having cGVHD.

Statistical Analysis

Neutrophil engraftment was defined as the first day of 3 consecutive days of an absolute neutrophil count (ANC) of 500 donor cells/mm^3; platelet engraftment was defined as the day of achieving an un-transfused count of 50,000 platelets/mm^3 for 7 days. aGVHD was scored as the maximum grade in all patients within 100 days [11] and the cGVHD at the highest level per consensus criteria [1]. The probabilities of neutrophil and platelet engraftment, aGVHD, and cGVHD and cytopenia were estimated using the cumulative-incidence-function method [12]. Neutrophil engraftment, platelet engraftment, aGVHD, and cGVHD and cytopenia were assessed in patients surviving past day 15 and treating death as a competing risk. Differences between subgroups were compared using Grays K-Sample Test [13]. The probability of overall survival (OS) was calculated with the use of the Kaplan-Meier estimator [14] and differences between groups were compared using the log-rank statistics [15]. Analyses were completed using the SAS system, version 8.2, and R, version 2.1.1.

RESULTS

Patients Characteristics

From September 1998 through April 2007, 19 very young infants (11 males, 8 females) received cord blood transplants from unrelated donors. The median weight and age at transplantation were 4.20 kg (range: 2.74 to 6.75) and 36 days (range: 18 to 117 days), respectively (Table 1). Diagnoses included Krabbe disease (n = 12), Hurler's syndrome (MPS I; n = 2), Hunter's syndrome (MPS II; n = 2), Metachromatic Leukodystrophy (n = 1), Tay Sachs disease (n = 1) and Beta-Thalassemia Major (n = 1).

Graft Characteristics

All infants were transplanted with a single unrelated cord blood unit selected by cell dose and HLA matching. The units contained a median TNC before cryopreservation of 18.78×10^7/kg of recipient body weight (range: 12.34×10^7/kg to 50.37×10^7/kg (Table 2). The median number of total nucleated infused cells were 15.70×10^7/kg (range: 8.42×10^7/kg to 32.40×10^7/kg). The median numbers of CD34$^+$ cells and CD3$^+$ infused were 3.18×10^5/kg (range: 1.27×10^5/kg to 11.02×10^5/kg) and 26.50×10^6/kg (range: 15.23×10^6/kg to 90.31×10^6/kg), respectively. Units were matched at 4 of 6 (n = 9), or 5 of 6 (n = 10) HLA loci using intermediate resolution DNA typing for Class I A + B and high-resolution DNA typing for DRB1.

Engraftment and Chimerism

Neutrophil (ANC >500/mm^3) and platelet engraftment (platelets >50,000 cells/μL) occurred in a median of 19 days (range: 10 to 40) and 60 days (range: 30 to 164), respectively (Table 3). The cumulative incidence of neutrophil engraftment at 42 days was 100% (95% confidence interval [CI] 89%–100%) and platelet engraftment at 180 days was 100% (95% CI 89%–100%). Red cell and platelet transfusions were no longer required at a median 59 days (range: 17 to 385) and 50 days (range: 25 to 164), respectively. Seventeen of the 18 evaluable infants obtained full donor chimerism (defined as >98% donor). One infant diagnosed with MPS II remained a mixed chimera throughout his life. An additional infant

with meta-chromatic leukodystrophy experienced secondary graft failure 2.5 years posttransplant.

aGVHD and cGVHD

In the 18 infants evaluable for aGVHD, 12 had either grade 0 (n = 1) or grade I (n = 11) (Table 3). Grades II–IV aGVHD occurred in 6 infants (grade II n = 5; grade III n = 1; grade IV n = 0). The median time to onset of among patients with grades II–IV was 12 days (range: 7–35) with a cumulative incidence of grades II–IV at 100 days of 33% (95% CI 11%–56%) (Figure 1). One patient experienced grade III aGVHD on day 35 posttransplant (Figure 1). Treatment included topical steroids (n = 10) and methyl-prednisolone pulse (n = 7), tacrolimus (n = 4), and daclizimab (n = 4).

The cumulative incidence of cGVHD defined according to conventional diagnostic criteria [1] was 17% (95% CI 0%–35%) and 23% (95% CI 2%–43%), at 1 and 2 years, respectively (Figure 2A). One patient had extensive cGVHD involving skin, gastrointestinal track, and the lung cGVHD, whereas 3 other infants had disease limited disease of the skin (Table 3). We examined possible factors contributing to GVHD, including TNC cell dose, HLA-matching, $CD34^+$ cell dose, $CD3^+$ cell dose, colony-forming units, ABO/Rh mismatch, and unit sex. None of these factors influenced the incidence or severity of aGVHD or cGVHD in this small patient cohort (data not shown).

Autoimmune Diseases

Ten infants were diagnosed with an autoimmune cytopenia (Table 4). The overall cumulative incidence of autoimmune cytopenias, with or without other manifestations of cGVHD, was 44% (95% CI 21%–68%) and 56% (95% CI 32%–80%) at 1 and 2 years, respectively (Figure 2B and C). The median onset was 247 days posttransplant (range: 92 to 687). The cytopenias observed included anemia (n = 4), thrombocytopenia (n = 1), anemia with thrombocytopenia (n = 3), and pancytopenia (n = 2).

Of the 9 patients with anemia, 8 had a positive Coombs with IgG polyspecific autoantibodies. Four patients also had complement fixation noted on red cells by direct antiglobulin testing. On eluate testing, the specificity of the antibody was identified as antiE in 2 patients with 1 patient having anti-A. Four of the 6 infants with thrombocytopenia had elevated levels of direct antiplatelet antibodies.

Nine of the 10 infants developed an autoimmune cytopenia while on CSA (n = 6) or tacrolimus (n = 3) with methylprednisolone for GVHD treatment or prophylaxis. One infant was on physiologic doses of hydrocortisone secondary to adrenal suppression.

Treatment of Autoimmune Cytopenias

All 10 infants received methylprednisolone for treatment of the cytopenias. Eight infants also received Rituximab therapy (2 infants were treated prior to widespread rituximab use). CSA was withdrawn in 5 infants. Other treatments included intravenous immu-noglobulins (n = 4), Azathioprine (n = 7), splenectomy (n = 1) (Table 4). Erythropoietin was administered to 1 infant without response. G-CSF was used in the 2 infants with neutropenia. The median treatment length was 21 months (range: 8–35).

As of April 2008, 3 of 10 infants are continuing to receive immunosuppressive therapies for autoimmune cytopenias. The DAT remains positive in 1 with active AIHA and 1 other infant who continues with intermittent hemolysis. One infant remains on immunosuppression for skin cGVHD.

Comparison with Older Children

We compared the incidence of cGVHD and auto-immune cytopenias with or without other manifestations of cGVHD in this group of very young infants to a cohort of 141 older children (median age = 1.50 years) with inherited metabolic disease who underwent cord blood transplantation between August 1995 and April 2007 at our center [16]. Although the overall incidence of cGVHD in the infants was equivalent to that of older children (Figure 2A), the cumulative incidence of autoimmune cytopenia with or without other manifestations of cGVHD for the infants was significantly higher at 1 and 2 years (44% [95% CI 21%–68%] and 56% [95% CI 32%–80%]) compared to the older children (1 year: 5% [95% CI 1%–8%], 2 years: 6% [95% CI 2%–10%], P < .01) (Figure 2B). The cumulative incidence of isolated autoimmune cytopenias was also significantly higher in the infants compared to the older children (28% [95% CI 6%–49%] versus 5% [95% CI 2%–9%], P < .01) (Figure 2C).

Survival

As of April 2008, 16 of 19 infants were alive (median: 1802 days; range: 353 to 3468 days) for an OS in this cohort of 95% (95% CI 85%–100%),89% (95% CI 75%–100%), and 80% (95% CI 59%–100%) at 1, 2, and 5 years, respectively (Table 3). One infant with Krabbe died on posttransplant day 15 from pulmonary hypertension. An infant with Hunter's (MPS II) died 653 days posttransplant from multisystem organ failure. An infant with Tay-Sachs died abruptly on day 1686 from unknown causes.

DISCUSSION

In this report, we describe a cohort of younger infants undergoing unrelated, single-unit UCBT for nonmalignant disorders after myeloablative chemotherapy-based conditioning who developed an unexpectedly high incidence of posttransplant immune mediated hematologic disorders. Rates of aGVHD and cGVHD were similar to those in previous reports. No patient had Epstein-Barr Virus lymphoproliferative disease and none of the immune diseases were triggered by a known viral illness.

Because of their small size, all infants received very high cell doses from their donor grafts. Engraftment and survival occurred at higher rates than those experienced by older children and adults. In this group, overall survival was 95%, 89%, and 80% at 1, 2, and 5 years, respectively. Neutrophil and platelet engraftment occurred (with a cumulative incidence of 100%) in a median of 19 and 60 days, respectively. Infants were treated with therapy first to remove T cell suppression and then to target B cells. We found that combination treatment with steroids, rituximab, and/ or azithioprine was most effective. In contrast to other reports [3–5,7], there was increase in mortality associated with the development of an autoimmune cytopenia. Rather, this cohort exhibited an excellent response to therapy and an excellent OS.

AIHA and other immune cytopenias have been reported in both adults and children undergoing allogeneic HSCT. In adults, Drobyski et al. [3] reported an overall incidence of AIHA of 3% in patients receiving T cell-depleted grafts with an incidence of 5% in patients who survived >6 months (3). Chen et al. [4] reviewed data from 293 patients and noted that the patients fell into 2 distinct groups: an early onset (2–8 months) with a cold antibody and a late onset (6–18 months) associated with a warm antibody. Sanz et al. [5] described 272 patients with an overall incidence of AIHA of 4.44%. Independent risk factors identified for AIHA included HSCT from an unrelated donor and the presence of extensive cGVHD. In contrast to the young patients in this report, AIHA in adults was associated with a poor overall prognosis, although often not directly because of the process [3–5,7]. O'Brien et al.

[7] described the largest series of pediatric patients who received unrelated donor HSCT with 19 (6%) of 303 patients developing AIHA. None of the patients who received a related donor transplant in their series developed AIHA (n = 136). In their series, children<10 years of age and those undergoing transplant for nonmalignant diagnosis (specifically metabolic diseases) were significantly more likely to develop AIHA as a posttransplant complication. Both stem cell source and graft manipulation to deplete T cells were not found to be risk factors, which are in contrast to other reports [3,6]. There was no difference in the overall rate of cGVHD in children who developed AIHA and those who did not. A high rate of mortality (53%) was reported in children developing AIHA in this series.

Horn et al. [6] described a high incidence (19.5%) of AIHA in a series of 41 children with SCID after T cell-depleted haploidentical bone marrow or PBSC transplantation. The incidence was 6% in patients who survived >6 months, which is comparable to other reports. All infants were found to have decreased CD4 and/or CD8 cells posttransplant. They hypothesized that the paucity of regulatory T cells allowed for B cell clonal expansion resulting in the autoimmune phenomena. Godder et al. [17] described 40 patients with hematologic malignancies or marrow failure who survived >120 days. Five of the 40 patients developed AIHA as the principal manifestation of cGVHD after receiving T cell-depleted partially mismatched related donor BMT. Immune function recovery was not different in the affected patients. aGVHD was also not noted to be a risk factor. It was proposed that HLA mismatch may have been a risk factor in addition to the T cell depletion process. Isolated case reports also exist, which describe AIHA and other cytopenias in children after all types of HSCT [18–26].

There are also rare case reports of patients developing autoimmune cytopenias from 6–9 years after cardiac transplantation (age: 7 months 4 years). Tubman et al. [27] described 3 pediatric patients with autoimmune cytopenias and hypothesized that the significant B cell immunosuppression by calcineurin inhibitions was a risk factor for development of the cytopenia. All were treated with and responded to rituximab therapy. Rawal et al. [28] described a pediatric patient transplanted at 15 months of age who developed at age 11 years multiple episodes of autoimmune cytopenias (anemia, thrombocytopenia, and neutropenia) and acquired glanzmann thrombasthenia several years after cardiac transplantation. This patient initially presented with a presumed viral myocarditis that may have recapitulated robust antibody formation.

The pathogenesis of autoimmune cytopenias in these young patients is not clear at this time. It may be because of an effect of treatment with calcineurin inhibitors or ATG on the developing thymus. All patients in this series received GVHD prophylaxis with cyclosporine, which is known to inhibit thymic-dependent clonal deletion, which disrupts the reconstitution of the immune system [29]. CSA also suppresses IL-2-dependent proliferation and function of regulatory T cells, which could then lead to the development of autoimmune disease [30]. However, this does not fully explain the increased incidence of autoimmune disease in these very young patients compared to our older patient cohort, who were also receiving cyclosporine for prophylaxis against GVHD. It is possible that the relatively higher T cell dose given to these patients from the cord blood unit graft may have accentuated the risk of both GVHD and immune dysregulation in the setting of CSA therapy. Future studies, characterizing the composition of the T cell compartment at the time of onset of autoimmune disease, may elucidate the mechanism underlying this problem.

In this report, we present the outcomes of a cohort of very young infants undergoing UCBT for genetic disorders in the first few months of life. We observed a markedly increased rate of posttransplant autoimmune cytopenias. In these patients, we suspect this was because of immune dysregulation associated with GVHD prophylaxis during the first year of life,

leading to aberrant immune ontogeny. We are planning a prospective study to characterize the early phases of immune reconstitution in this unique group of patients.
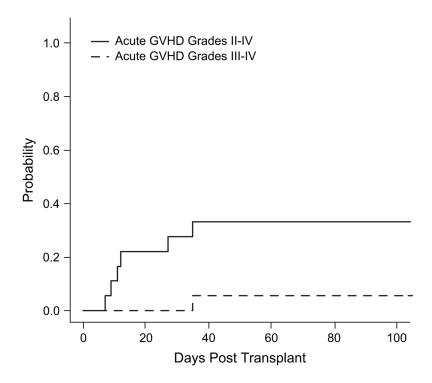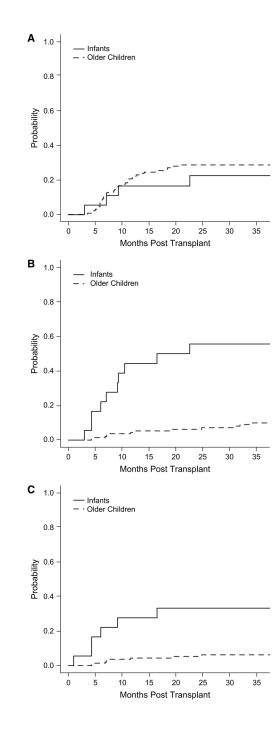
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Figure 1.
Cumulative incidence of aGVHD after unrelated cord blood transplantation in very young infants. Cumulative incidence of grades II–IV and grades III–IV aGVHD in the study patients is displayed.

Figure 2.
Cumulative incidence of cGVHD (A) and autoimmune cytopenias (B, C) in very young infants compared to older children undergoing UCBT. (A) The cumulative incidence of cGVHD in the infant population (solid line) compared to a historic control group of all other metabolic patients treated at Duke (dotted line). (B) The cumulative incidence of autoimmune cytopenias in the study patients (solid line) compared to the older control group of all other metabolic patients treated at Duke (dotted line). (C) The cumulative incidence of cytopenias without other manifestations of cGVHD between the infants (solid line) and older children (dotted line).

Table 1

Characteristics of the Study Population and the Unrelated Cord Blood Donors

Pt No.	Diagnosis	Days of Life at Time of Transplant	HLA Match (No./Total No.)	HLA Mismatch	Blood Type Pt	Blood Type Donor	Pt Sex	Unit Sex
Cytopenias without other GVHD manifestations								
2	Thalassemia	91	4/6	A31/30;Bbl/7	A+	A+	M	M
6	Krabbe	29	4/6	B57/8;DR0402/1302	B+	B+	M	M
12	Krabbe	117	5/6	A11/29	O+	A+	M	F
13	Krabbe	26	4/6	Abl/11;B39/18	O+	O+	F	F
15	Krabbe	22	5/6	DR9/8	O+	O+	F	F
19	Krabbe	33	5/6	A31/2	A+	A+	M	F
Cytopenias with cGVHD								
9	MPS II	44	5/6	DR11/7	O+	A−	M	F
10	MPS I	113	4/6	A33/68;DR16/13	B+	A+	M	M
16	MLD	62	5/6	A1/3	O+	O+	M	F
17	Krabbe	29	4/6	B55/51;DR15/4	A+	O+	F	M
No cytopenias or cGVHD								
1	MPS I	88	5/6	A28/29	A+	O+	F	F
3	Krabbe	55	4/6	A30/25;B44/53	O−	A+	M	M
4	Krabbe	29	4/6	A2/33;B39/bl	AB+	B+	F	M
5	Tay-Sachs	22	4/6	B50/41;DRB1 1302/1303	B+	A+	M	M
7	Krabbe	37	5/6	B40/58	O+	A−	F	M
8	MPS I	62	5/6	A68/2	O+	O+	F	F
11	Krabbe	26	5/6	B18/44	A−	A+	M	M
14	Krabbe	22	5/6	B44/14	A−	A+	M	M
18	Krabbe	18	4/6	A25/11;B18/44	B+	A+	F	M
Mean ± SD		48 ± 31						
Median		36						

GVHD indicates graft-versus-host disease; cGVHD, chronic graft-versus-host disease; Pt, patient; bl, blank.

Table 2

Characteristics of Transplanted Umbilical Cord Blood Units

Pt No.	Total Nucleated Cells Cryopreserved ($\times 10^7$/kg)	Total Nucleated Cells Reinfused ($\times 10^7$/kg)	Colony-Forming Units Infused ($\times 10^4$/kg)	CD34$^+$ Cells Infused ($\times 10^5$/kg)
Cytopenias without other GVHD manifestations				
2	31.82	19.10	7.64	6.11
6	39.20	25.00	23.75	5.65
12	14.74	19.90	15.92	3.18
13	13.48	9.92	4.46	1.69
15	18.78	13.20	10.56	8.45
19	42.75	31.60	0	2.84
Cytopenias with cGVHD				
9	14.85	10.00	4.50	1.48
10	24.63	20.73	14.51	4.68
16	16.42	12.60	3.15	1.51
17	38.23	27.40	20.55	5.48
No cytopenias or cGVHD				
1	18.63	15.70	51.81	8.01
3	21.93	14.20	6.39	1.80
4	19.79	11.50	10.93	1.27
5	18.49	12.41	13.03	1.81
7	17.36	15.84	11.09	3.72
8	12.34	8.42	8.00	1.94
11	17.76	15.40	23.10	2.60
14	50.37	32.40	105.30	11.02
18	33.03	18.60	13.02	7.25
Mean ± SD	24.45 ± 11.27	17.57 ± 7.14	18.30 ± 23.92	4.24 ± 2.86
Median	18.78	15.70	11.08	3.18

GVHD indicates graft-versus-host disease; cGVHD, chronic graft-versus-host disease; Pt, patient.

Table 3

Engraftment, Chimerism, Incidence of GVHD and Event-Free Survival

Pt No.	Diagnosis	Engraftment (Days)*		RBC Transfusion Independence (Days)†	Chimerism (% Donor)		Acute GVHD Grade‡	Chronic GVHD Grade	Event-Free Survival (Days)§	Cause of Death
		Neutrophil	Platelet		At 100 Days	At 1 Year				
Cytopenias without other GVHD manifestations										
2	Thalassemia	14	33	17	>99	>99	1	Cytopenia	3440	
6	Krabbe	20	101	115	>99	>99	1	Cytopenia	2684	
12	Krabbe	31	91	77	>98	>99	3	Cytopenia	1480	
13	Krabbe	14	48	55	>98	>98	1	Cytopenia	1368	
15	Krabbe	25	64	62	94	98	1	Cytopenia	1321	
19	Krabbe	31	141	385	97	NE	2	Cytopenia	353	
Cytopenias with cGVHD										
9	MPS II	20	56	52	50	22	1	Cytopenia, Extensive	Died	Multisystem organ failure (day 653)
10	MPS II	14	89	80	>99	>98	2	Cytopenia, Limited (Skin)	1879	
16	MLD	40	164	190	63	100	1	Cytopenia, Limited (Skin)	1293	
17	Krabbe	17	49	70	97	100	1	Cytopenia, Limited (Skin)	955	
No cytopenias or cGVHD										
1	MPS I	10	33	25	>99	>99	0	None	3468	
3	Krabbe	14	53	49	>99	>99	1	None	3391	
4	Krabbe	13	49	39	100	100	1	None	2977	
5	Tay-Sachs	20	90	103	>99	>99	2	None	Died	Probable infection (day 1686)
7	Krabbe	20	67	53	99	100	1	None	2264	
8	MPS I	19	47	47	>99	>99	2	None	1986	
11	Krabbe	19	74	70	>99	>98	2	None	1725	
14	Krabbe	11	30	51	>98	>98	1	None	1327	
18	Krabbe	NE	NE	NE	NE	NE	NE	NE	Died	Pulmonary Hypertension (day 15)
Mean ± SE		20 ± 2	71 ± 9	86 ± 20					1994 ± 238	
Median (range)		19 (10–40)	60 (30–164)	59 (17–385)					1802 (353–3468)	

MPS I indicates mucopolysaccharidosis type I (Hurler's Syndrome); Krabbe, globoid cell leukodystrophy, Krabbe disease; MPS II, mucopolysaccharidosis type II (Hunter's Syndrome); MLD, metachromatic leukodystrophy; EFS, event-free survival; Pt, patient.

*
Neutrophil engraftment was defined as an absolute neutrophil count of >500 per cubic millimeter on at least 3 consecutive days, and platelet engraftment was defined as a count >50,000 per cubic millimeter without any transfusion support for at least 7 days.

†
RBC transfusion independence was defined as 7 days from last transfusion with no further transfusion support needed.

‡The 15 infants with acute graft-versus-host disease (aGVHD) had skin manifestations.

§EFS as of April 1, 2008.

Table 4

Characteristics of Patients Transplanted in Neonatal Period that Later Developed an Autoimmune Cytopenia

Pt No.	Pretransplant Diagnosis	Initial GVHD Prophylaxis	Type of Cytopenia	Posttransplant Onset (Days)	RBC Antibody	Plt Antibody	Tacrolimus Exposure	Treatment	Outcome
Cytopenias without other GVHD manifestations									
2	Beta-thalassemia	CsA, Methypred	AIHA, thrombocytopenia	131	IgG+ panreactive		No	Methylpred, IVIG, Azathioprine	Alive. Resolved.
6	Krabbe	CsA, Methypred	Thrombocytopenia	279			No	Methylpred, IVIG, d/c CSA	Alive. Resolved.
12	Krabbe	CsA, Methypred	AIHA	503	IgG+, panreactive, C3+		No	Methylpred, IVIG, Azathioprine, Rituximab	Alive. On treatment with intermittent hemolysis.
13	Krabbe	CsA, Methypred	AIHA	183	IgG+ panreactive, C3+		Yes	Methylpred, Azathioprine, Rituximab, d/c CSA	Alive. Resolved.
15	Krabbe	CsA, Methypred	AIHA, thrombocytopenia, neutropenia	132	IgG+, panreactive, C3+	Yes	Yes	Methylpred, Azathioprine, Rituximab, EPO, GCSF, d/c CSA	Alive. Continues on azathioprine (weaning).
19	Krabbe	CsA, MMF	AIHA	320	IgG+, panreactive (Anti-e, C)		No	Methylpred, Azathioprine, Rituximab	Alive. On treatment.
Cytopenias with cGVHD									
9	MPS II	CsA, Methypred	AIHA, thrombocytopenia	92	IgG+ panreactive, C3+, (Anti-E)	Yes	Yes	Methylpred, IVIG, Azathioprine, Rituximab, Splenectomy, d/c CSA	Died of multisystem organ failure. Had secondary graft failure and underwent second transplant.
10	MPS II	CsA, Methypred	AIHA, thrombocytopenia	283	IgG+, C3+, (Anti-A)	Yes	Yes	Methylpred, Rituximab (daclizimab for skin GVHD)	Alive. Resolved.
16	MLD	CsA, MMF	AIHA, thrombocytopenia, neutropenia	687		Yes	No	Methylpred, Rituximab	Alive. Ongoing pancytopenia. Secondary graft failure requiring second transplant.
17	Krabbe	CsA, MMF	AIHA	215	IgG+ panreactive, (Anti-E)		Yes	Methylpred, Azathioprine, Rituximab, d/c CSA	Alive. On azathioprine and steroids for skin cGVHD.

Krabbe indicates globoid cell leukodystrophy (Krabbe disease); MPS II, mucopolysaccaridosis type II (Hunter's Syndrome); MLD, metachromatic leukodystrophy; DAT, direct antibody testing; Methylpred, methyl-prednisolone; CSA, cyclosporine; MMF, mycophenolate; EPO, erythropoietin; G-CSF, granulocyte-colony stimulating factor; GVHD, graft-versus-host disease; Pt, patient.