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Outcomes of Unrelated Umbilical Cord Blood Transplantation for X-Linked Adrenoleukodystrophy

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ABSTRACT

Adrenoleukodystrophy (ALD) is an X-linked disorder caused by a defect in the metabolism of long chain fatty acids leading to demyelination, neurodegeneration, and death. The disease typically presents in young boys and adolescent boys. Allogeneic bone marrow transplantation has been used to halt progression of the disease. However, many patients lack suitable HLA- matched related donors and must rely on unmatched donors for a source of stem cells. The purpose of this study was to evaluate outcomes of unrelated donor umbilical cord blood transplantation after chemotherapy-based myeloablative conditioning and retrospectively determine if baseline studies correlate and help predict outcome. Between November 22, 1996, and November 3, 2005, 12 boys with X-linked ALD who lacked HL- matched related donors were referred to Duke University Medical Center for transplantation. These children were conditioned with myeloablative therapy including busulfan, cyclophosphamide, and antithymocyte globulin before receiving umbilical cord-blood transplants from unrelated donors. Baseline studies of neurophysiologic, neuroimaging, and neurodevelopmental status were performed and patients were subsequently evaluated for survival, engraftment, graft-versus-host disease, and neurodevelopmental outcomes. A substudy evaluated whether baseline neuroimaging and neurophysiologic studies correlated with cognitive and motor function and if these studies were predictive of posttransplantation outcomes. The umbilical cord blood grafts had normal levels of very long chain fatty acids. They delivered a median of 6.98×10^7 nucleated cells per kilogram of recipient body weight and were discordant for up to 4 of 6 HLA markers. Neutrophil engraftment occurred at a median of 22.9 days after transplantation. Three patients had grade II-IV acute graft-versus-host disease; 2 had extensive chronic graft-versus-host disease. Cumulative incidence of overall survival of the group at 6 months is 66.7% (95% confidence interval 39.9-93.3%). Median follow-up was 3.3 years (range 12 days to 6.3 years). As previously reported with bone marrow transplantation, symptomatic patients faired poorly with lower survival and rapid deterioration of neurologic function. This study included 3 patients transplanted at a very young age (2.6-3.5 years) before the onset of clinical symptoms who continue to develop at a normal rate for 3-5 years posttransplant. Although baseline Loes scores correlated with cognitive and motor outcome, neurophysiologic studies failed to show statistically significant differences. Transplantation of boys with X-linked ALD using partial HLA-matched umbilical cord blood yields similar results to those previously reported after bone marrow transplantation. Superior outcomes were seen in neurologically asymptomatic boys less than 3.5 years of age at the time of transplantation. Baseline Loes scores were a strong predictor of cognitive and motor outcome.

KEY WORDS

Umbilical cord blood transplantation • Neurodevelopmental outcomes • Adrenoleukodystrophy

INTRODUCTION

Adrenoleukodystrophy is an X-linked disorder (X-ALD) caused by the deficiency of the ABCD1 gene that encodes for a peroxisomal protein membrane. It is associated with the accumulation of very long chain fatty acids (VLCFA) that can be measured in the plasma for diagnosis. X-ALD affects the testes, adrenal cortex, and the nervous system. The cerebral form leads to demyelination and typically affects young boys who present with behavioral lability, visual impairment, seizures, progressive loss of cognitive and motor function, and death by 10 years of age [1-3]. A milder adult form referred to as adrenomyeloneuropathy involves mainly the spinal cord. Hematopoietic stem cell transplantation (HSCT) arrests progression of cerebral X-ALD and other leukodystrophies in patients with early stages of disease [4-7]. However, many patients cannot identify an appropriately matched sibling or unrelated adult donor [4,6,7]. Unrelated donor umbilical cord blood (UCB) has been shown to successfully reconstitute marrow in children following allogeneic transplant for malignant and nonmalignant diseases [8-10]. We now report the outcomes after UCB transplantation in a series of 12 boys with X-ALD. We also correlate baseline neurodevelopmental, neurophysiologic, and brain imaging findings with functional outcomes.

METHODS

Between November 22, 1996, and November 3, 2005, 12 boys with adrenoleukodystrophy lacking HLA-matched related donors were referred to the Pediatric Bone Marrow Transplant Program at Duke University. All patients were evaluated with a baseline brain MRI, peripheral nerve conduction velocity (NCV), brainstem auditory evoked responses (BAER), visual evoked potentials (VEP), electroencephalogram (EEG), and neurodevelopmental evaluations. The diagnosis of ALD was confirmed by the presence of abnormally high levels of long chain fatty acids (LCFA) in the blood measured by capillary gas chromatography of pentafluorobenzyl bromide fatty acid esters [11]. After parental informed consent was obtained, 4 patients were enrolled in the Cord Blood Transplantation Study (COBLT), 4 patients enrolled on the COBLT-Extended Access Protocol (COBLT-EAP), and 4 patients were enrolled in an ongoing single-institution study. All were approved by the institutional review board at Duke University Hospital. The transplant related outcomes of 8 patients transplanted on the COBLT or the COBLT-EAP studies were previously reported as part of a larger cohort of patients with lysosomal storage diseases. These included survival, engraftment, and graft-versus-host-disease (GVHD) [10]. Neurophysiologic, neuroimaging, or neurodevelopmental outcomes were not previously reported in any of the patients.

Selection of Donors

Cord blood units from unrelated donors were selected from public cord blood banks listing units through the National Marrow Donor Program or the New York Blood Center after a search using intermediate resolution HLA typing for class I (A and B) and high-resolution typing of HLA-DRB1. The units with the highest number of nucleated cells (minimum, 3×10^7 per kilogram of body weight) matching at least 4 of 6 HLA loci were selected and tested for normal levels of LCFA [11]. Cell dosing was prioritized over HLA matching for unit selection for transplantation. Final unit selection was based on highest cell dose with closest HLA match and normal very long chain fatty acids (VLCFA) levels.

Conditioning Regimen

Patients underwent a myeloablative preparative regimen of busulfan, cyclophosphamide, and antithymocyte globulin as previously described [12-14]. First-dose pharmacokinetic studies were performed during busulfan therapy targeting a steady-state concentration (Css) of 600-900 ng/mL. Patients received phenytoin prophylaxis against seizures during therapy with busulfan; mesna was administered during cyclophosphamide therapy for prophylaxis against hemorrhagic cystitis.

Transplantation Procedure

Cryopreserved units of cord blood were thawed and processed as previously reported [15]. Thawed units were tested for total number of nucleated cells, clonal hematopoietic progenitor cells, $CD3^+$, $CD34^+$ cells, ABO, and Rh typing, cell viability, and sterility.

Prophylaxis against and Treatment of GVHD

The patients received cyclosporine for 9 months and methylprednisolone for 2 to 3 months as prophylaxis against GVHD. In the absence of chronic GVHD (cGVHD), immune suppression was discontinued approximately 1 year posttransplant [10,12,13]. The severity of acute GVHD (aGVHD) was scored using standard criteria [16]. Acute grade 1 GVHD of the skin was treated with topical creams, an escalation in methylprednisolone, or both. Patients with moderate to severe GVHD received pulsed doses of methylprednisolone 500 mg per square meter given intravenously every 12 hours for 4 total doses before GVHD treatment was changed from cyclosporine to tacrolimus, alone or in combination with daclizumab.

Supportive Care

All patients were hospitalized in reverse isolation with high-efficiency particulate air filtration. Prophylaxis for pneumocystis carinii, viral, and fungal infections were given in a standard fashion. The first episode of febrile neutropenia was treated with broad-spectrum antibiotics, and therapy continued through engraftment. Intravenous immunoglobulin was given weekly through day 100. Continuous low-dose heparin was infused intravenously as prophylaxis for veno-occlusive disease (VOD) of the liver through day 28 posttransplant. Patients received Neupogen (10 μg/kg/day intravenously) from day of transplant to engraftment.

Clinical Studies

Patients were evaluated at baseline and every 6-12 months with echocardiograms, pulmonary function tests, ophthalmology, serial brain imaging, and neurophysiologic and neurodevelopmental assessments (including vision, hearing, speech and language, motor, cognitive, and adaptive behavior).

Outcome Measures

The outcome measures included engraftment and survival, change in neurophysiologic measures and 4 domains of neurodevelopmental function: cognitive, adaptive-behavior, language, and motor skills.

Engraftment and survival. Engraftment was defined as the first of 3 consecutive days with absolute neutrophil count of at least 500 cells per cubic millimeter. The probability of overall survival (OS) was calculated according to the Kaplan-Meier method. The cutoff date for analysis was June 1, 2006.

Neurodevelopmental assessment. Standardized and validated neurobehavioral tools were used to assess all patients at baseline and follow up (Table 1) [17-24]. Standard scores and developmental quotients were converted to age-equivalent scores to allow comparisons of results among various tests and to identify development of new skills. Motor, cognitive, receptive, and expressive language and adaptive behavior were assessed. Results were compared to norms of typically developing children.

MRI analysis. Two board-certified neuroradiologists scored pretransplantation MR studies using the modified Loes scoring system in a consensus manner in a single scoring session [25]. Readers were aware of patient age (which was needed to assess appearance of white matter relative to the normal population) but were blinded to all other clinical information. Images were reviewed on a PACS workstation in all patients except 1, which allowed windowing of images to optimize signal intensity of structures for scoring and also direct correlation of structures in different imaging planes.

Neurophysiologic Studies. EEG, nerve conduction veolcity (NCV), visual evoked potential (VEP), and brain auditory evoked repsonses (BAERs) were performed prior to transplantation and at scheduled intervals posttransplant, and interpreted according to the guidelines established by the American Clinical Neurophysiology Society [26]. EEGs were considered abnormal if focal or generalized slowing, spikes, or sharp waves were present. The flash VEP was considered normal if the P100 wave was present and abnormal if it was absent. The BAERs were considered abnormal if either the wave I-V interpeak latency was prolonged or if any of the obligate wave forms (I, III, V) were absent. Nerve conduction studies were considered abnormal if they showed prolongation of the distal latency, low amplitude, absent evoked response, or prolonged F-wave latency. Study results were interpreted by expert physicians blinded to the status of the patient.

Statistical Analysis

The probability of event-free survival (patient durably engrafted with donor cells and alive) and the incidence of acute grade II-IV GVHD were calculated by Kaplan-Meier analysis. Five baseline measures (BAERs, VEP, NCV, EEG, and brain MRI) were evaluated as predictors of posttransplant cognitive and motor development. To evaluate these predictors a mixed effects regression model was fit for each baseline measure, with cognitive development as the outcome and age, baseline measure, and the interaction of age and baseline measure as the predictors. The BAERs, VEP, NCV, and EEG were entered as dichotomous variables coded as normal or abnormal as described above. MRI Loes scores were evaluated on

Table 1. *Neurodevelopmental Testing*

Domain	Before 5 years	After 5 years
Cognitive	The Capute Scales (CAT/CLAMS) Mullen Scales of Early Learning	The Differential Ability Scales
Language	Preschool Language Scale—Third and Fourth Edition	Clinical Evaluation of Language Fundamentals 3—Third Edition
Motor	Peabody Developmental Motor Scales, Second Edition (PDMS-2)	PDMS-2 (up to 70 months developmental age) then the Bruininks-Oserestsky Test of Motor Proficiency
Adaptive Behavior	Scales of Independent Behavior Revised (SIBR)	Scales of Independent Behavior Revised (SIBR)

both an ordinal scale (0-34) and dichotomous coded as ≤10 versus 11 or greater.

RESULTS

Patients Characteristics

From April 2000 through November 2005, 12 boys with a median age at diagnosis of 7 years (neonate to 9.75 years) were admitted to Duke University Medical Center for transplantation. One patient was Hispanic, 1 Asian, and 10 Caucasian. The median age at initiation of chemotherapy was 7.1 years (range: 2.36-11.7) and the median weight was 22 kg (range: 12.7-50.9), respectively (Table 2). One boy died while receiving chemotherapy but before receiving his transplant. Four boys received Lorenzo's Oil [27] prior to transplant, but none continued this therapy during or after transplantation.

Donor Characteristics

All donor/recipient pairs were mismatched at 1 (n = 4) or 2 (n = 8) HLA loci. Major ABO incompatibilities were present in 3 donor/recipient pairs. The median number of nucleated cells per unit was 6.98×10^7 cells per kilogram (range: 2.83×10^7 to 14.19×10^7) before cryopreservation. UCB units delivered a median 5.66×10^7 cells/ kg (range: 2.54-11.9) based on the postthaw count. The median number of $CD34^+$ infused was 2.44×10^5 per kilogram (range: 0.39-9.16) (Table 2).

Survival, Engraftment, and GVHD

Twelve patients received conditioning but 1 died on day −7 of shock and brain herniation likely secondary to adrenal crisis (Table 2). Eleven patients were successfully transplanted. The OS at 6.25 months was 66.7% (95% confidence interval 39.9-93.3%) (Figure 1a). There were no events after this time point. The median follow up was 3.3 years (range: 12 days-6.3 years). Posttransplant, 2 patients died of progressive ALD (patients 7 and 10) and 1 patient died of severe VOD (patient 11).

Ten of 11 patients engrafted neutrophils in a median of 22.9 days (range: 13-49) and platelets in a median of 86.8 days (range: 55-227). Two patients could not be evaluated for platelet engraftment because they died while still requiring transfusions because of hemorrhagic cystitis or secondary effects of viral reactivation therapy. One patient experienced primary graft failure, but engrafted after a second UCB transplant, 67 days after the first transplant with conditioning with cyclophosphamide and ATG (patient 9). Five of the 11 evaluable patients developed grade I GVHD, 2 developed grade II, and 2 developed grade IV GVHD (Figure 1b). cGVHD occurred in 2 patients (1 limited and 1 extensive)

Table 2. *Patient and Graft Characteristics, Engraftment, Incidence of GVHD, and Event-Free Survival after Transplantation*

Patient Number	Age at Diagnosis	Age at Transplant		HLA Matches		Number of Nucleated Cells ($\times 10^7$/kg)		Neutrophil Engraftment	Platelet Engraftment	Chimerism(%)		Chronic GVHD		Event- Free Survival
								Day		Day 100	1 Year	Grade		Day
	Years	Years	Loes Score	No. Total		Cryopreserved	Reinfused							
1	9.16	9.27	15	5/6		2.83	2.54	49	187	100	100	0		2307
2	0	3.52	5	5/6		6.84	5.67	32	60	100	100	0		1586
3	0.6	2.36	7	5/6		8.48	7.83	15	25	>99	>99	0		1565
4	6.88	8.24	5.5	5/6		4.00	2.81	14	138	100	100	Skin, * brain		1538
5	0	11.71	8	4/6		4.27	3.12	22	58	>99	>99	0		1406
6	7.5	7.70	18	4/6		9.47	7.40	20	55	100	>99	0		1216
7	6.41	6.58	13	4/6		14.19	9.37	13	No engraftment	>99	NE	NE		died on day 190
8	9.33	9.53	21	4/6		7.12	5.77	14	37	>99	>99	AHA		1110
9	0	2.59	5.5	4/6		12.83	11.90	NE	NE	NE	NE	0		graft failure on day 42
9*	0	2.75	5.5	4/6		8.30	5.64	25	62	87	67	0		851
10	9.75	10.05	23	4/6		3.77	3.48	25	No engraftment	>98	NE	0		died on day 185
11	5.91	7.12	3	4/6		5.97	3.94	NE	NE	NE	NE	0		died on day 12
12	4.16	NE	NA	NE		NE	NE	NE	NE	NE	NE	NE		died on day −7
Range	0-9.75	2.36-11.71	3-23	NA		2.83-14.19	2.54-11.9	13-49	25-187	87-100	67-100	NA		−7-2307
Median	6.16	7.41	7.5	NA		6.98	5.66	21	59	NA	NA	NA		1110

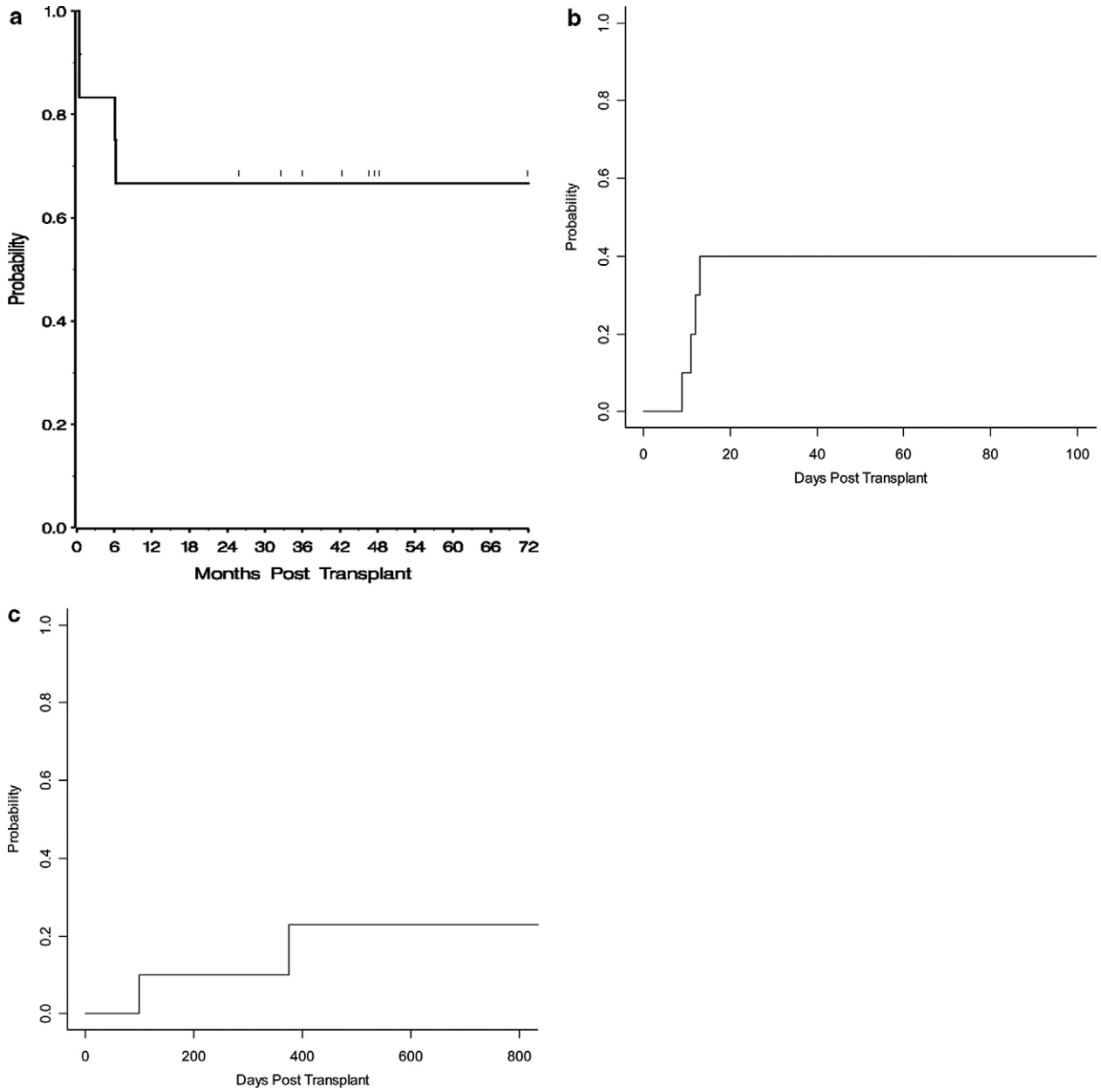

Figure 1. (a) Overall survival after transplantation. Kaplan-Meier estimates of the probability of overall survival. Cumulative incidence of overall survival at 6.25 months was 66.7% (95% confidence interval 39.9%-93.3%). (b) Cumulative incidence of acute GVHD grades II-IV (n = 10, 2 events). Cumulative incidence at day 100: 40.0% (95% confidence interval 7.5%-72.5%). (c) Cumulative incidence of chronic GVHD (n = 10, 2 events). Cumulative incidence at day 365: 10.0% (95% confidence interval 0.0%-29.6%) CINC at day 730: 22.9% (95% confidence interval 0.0%-53.2%).

(Table 2, Figure 1c). The patient with extensive cGVHD experienced severe neurologic deterioration 2 years posttransplant. The patient developed new white matter changes on MRI and visual motor and cognitive deficits, which temporarily improved with high-dose steroids and later stabilized on Etanercept. A brain biopsy showed diffusely increased microglia and reactive astrocytosis in the cortex and white matter, preservation of myelination, and no evidence of lymphoproliferative disorder or active ALD. A presumptive diagnosis of brain GVHD was made and the patient stabilized with immunosuppressive therapy.

Myeloablative doses of oral Busulfan were used in the conditioning regimen. Busulfan was administered at 20-40 mg/m^2/dose orally (based on age) $\times$ 16 doses on days -9 through -6, busulfan pharmacokinetics were measured after the first dose, and subsequent doses were adjusted to target a steady state concentration of 600-900 ng/mL. Only 1 patient developed signs of grade III-IV hepatic toxicity and VOD 9 days posttransplant. He died of progressive VOD on day +12.

Neurophysiologic Studies

VEP. VEP were available in 9 patients pretransplant and 8 patients posttransplant. Pretransplant, 2 of the patients had abnormal findings. Patient 1 had no reproducible waveforms and lost all vision in the peritransplant period. Patient 5 had prolonged absolute latencies in the P100 waveform but has normal vision posttransplant. Of the 7 remaining patients, the 3 younger ones were normal pretransplant and remained normal posttransplant. The other 4 patients, who were normal pretransplant, had no reproducible waveforms or lost the P100 wave posttransplant. Two of these patients (7 and 10) lost vision in the peritransplant period. Patient 4 developed anisotropia but preserved functional vision.

BAERs. BAERs were available in 10 patients pretransplant and 7 posttransplant. Pretransplant, 8 patients had normal responses as well as normal hearing as measured by serial behavioral audiometry (patients 1-5, 10, 11, and 12). Abnormal results were seen in 2 patients (7 and 9) who demonstrated prolongation of I-V latency. Posttransplant, these 2 patients normalized and 4 additional patients remained normal. One patient who initially had normal BAERs and behavioral audiometry, developed prolongation of I-V latency, a focal brainstem lesion on BAERs, and right peripheral hearing loss (patient 4).

NCS. NCSs were available in 10 patients pretransplant with normal findings in 9 of these patients (patients 1, 3-5, and 7-11). Posttransplant 4 of the 6 patients studied remain normal (patients 3, 4, 8, 9). Of the other 2, 1 (patient 6) who was unable to walk, showed pre- and posttransplant peripheral motor neuropathy and left peroneal nerve damage and the other (patient 10) developed a severe decrease in amplitude and latency posttransplant and died of progressive disease.

EEGs. EEGs were available in 11 patients pretransplant, of which 5 were normal (patients 2, 3, 4, 9, and 11). Of the remaining 6 patients, 1 showed diffuse background slowing (patient 7), 1 had polymorphic delta slowing in the right posterior quadrant (patient 10), 1 had intermittent right temporal slowing (patient 5), 1 had frontal occipital slowing (patient 6), and the additional 2 patients had focal seizure spikes (patients 8 and 12). Posttransplantation, 8 patients were studied, 2 patients remained normal (patients 3 and 9), 1 showed temporal slowing (patient 4), and 5 patients (patients 1, 6-8, 10) with abnormal pretransplant EEG progressed to have focal seizure activity.

Neurodevelopmental Function

Cognitive function. Of the 11 patients who were transplanted, 9 had normal to strong cognitive skills (patients 1-7, 9, and 11) and 2 were delayed pretransplant (patients 8 and 10). Ten patients had follow-up assessments posttransplant. Five patients (2-5, and 9) continued to show average cognitive gains. Patient 8 who had comorbid mental retardation at baseline remained stable but has not gained new skills after the 2 years of follow-up. Four patients who experienced rapid neurologic deterioration had scores in the spatial area 1-5 standard deviations lower than their verbal scores (patients 1, 6, 7, and 10) (Figure 2a).

Adaptive behavior. This domain, unlike the others, is measured by parent's responses to a standardized measure of the child's independent and self-help abilities. Nine patients were assessed pre and posttransplant. Two other patients had a single visit: 1 pretransplant and 1 posttransplant. Pretransplant, 10 patients had average skills. Of these, 2 were in the low average range and deteriorated during the peritransplant period. Posttransplant 5 patients (1, 6, 7, 8, and 10) deteriorated rapidly and 1 patient (patient 4) lost most adaptive skills 2 years after transplant but regained some function after treatment of presumed GVHD of the brain. Patients 2, 3, 5, and 9 remained normal (Figure 2b).

Language. Four patients had pretransplant and posttransplant testing (patients 2, 3, 6, and 9), 2 patients had only pretransplant data (4 and 7), and 2 had only posttransplant data (5 and 8). Receptive and expressive language were normal to strong in all the patients tested pretransplant. Of the 6 patients tested posttransplant, 4 remain with average language skills (2, 3, 5, and 9), 1 (patient 8) deteriorated slightly after transplant, and 1 (patient 6) deteriorated rapidly after transplant and is not verbal (Figure 3c and d). One patient (patient 4) was not formally tested but had no difficulties communicating. All patients were noted to have some degree of articulation difficulties pretransplant that continued after transplantation.

Motor function. Eight patients were tested before transplant and 10 after transplant in the gross motor area (Figure 2e). Pretransplant, 4 patients had average skills (2, 3, 9, and 11) and 4 were delayed (4, 6, 7, and 10). Posttransplant, 2 patients continue to have average gross motor skills (2 and 9), and 1 not previously tested has average skills 6 years posttransplant (patient 5).

Pretransplant, patient 3 had average skills. However, he had hypotonia, mild ataxia, and posturing of 1 arm secondary to resection of low-grade astrocytoma discovered on initial MRI after diagnosis of ALD. After transplant, his motor function improved, and now, 4 years posttransplant, he only has a mild motor delay. Of the remaining 6 patients followed posttransplant, 1 was weak but stable for 2 years and then lost his ability to ambulate because of presumed GVHD of the brain. After treatment with immunosuppression, he stabilized and regained ability to walk short distances with a walker (patient 4). Another patient is regaining strength and is able to sit, stand, and walk with full

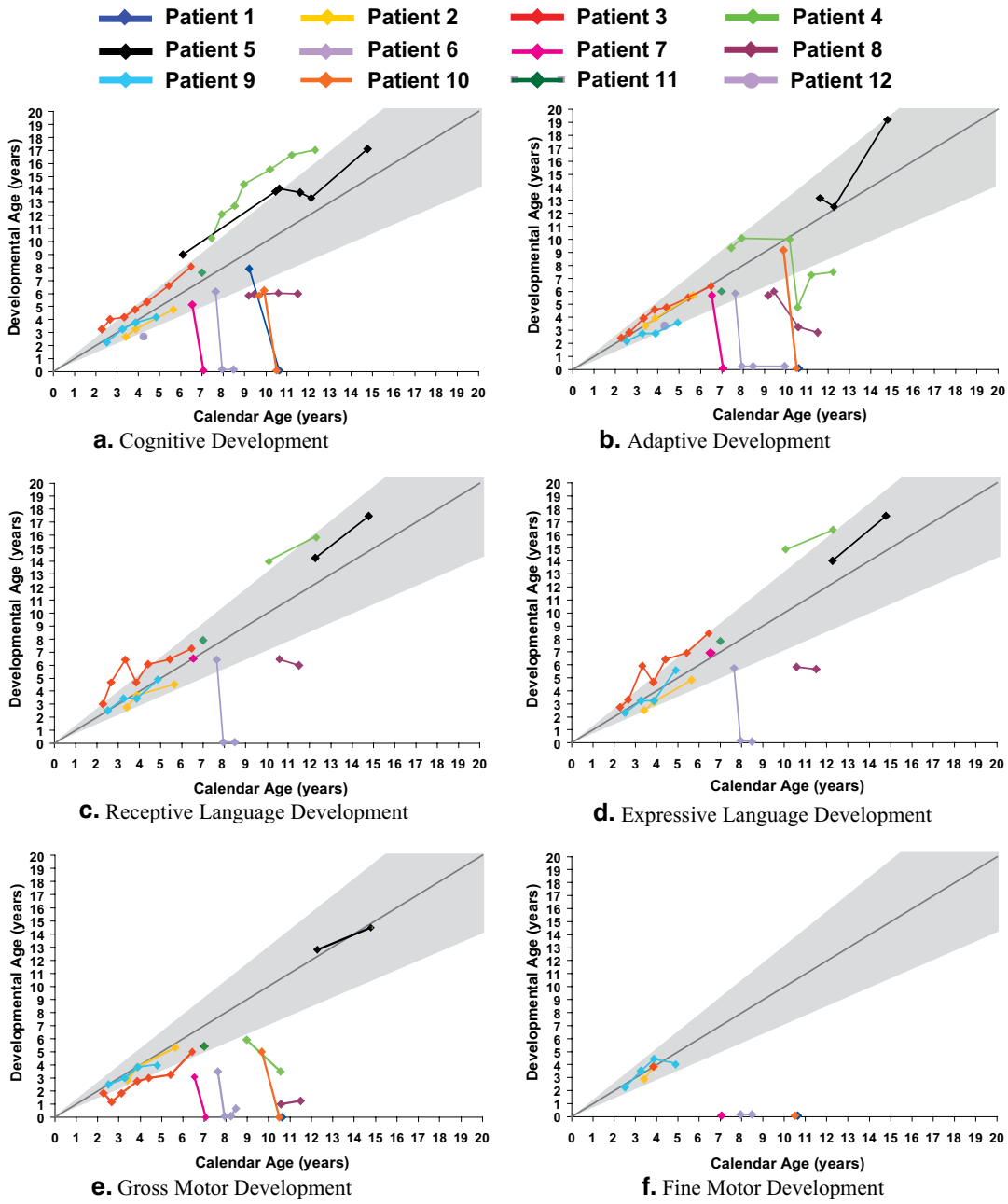

Figure 2. (a-f) Developmental function by domain. Each patient's development is represented by a unique color line. The green diagonal line represents typical population development. The gray area represents the approximate normal 95% range in the general population.

assistance and adaptive equipment (patient 8). One patient is able to hold his head and sit up with assistance (patient 6). The other patient is unable to perform any skills (patient 1). The 2 remaining patients died after rapid motor deterioration, spasticity, and inability to perform any spontaneous movement (patients 7 and 10).

In the fine motor area 7 children were tested. Two patients had severe disease progression posttransplant and developed clasped thumbs (1 and 6), and the other 2 patients died (Figure 3f). The 3 youngest patients continue to have normal fine motor skills posttransplant (patients 2, 3, and 9). The 3 older patients (4, 5, and 8) not formally tested, are able to write and use their hands without difficulty.

Neuroimaging

Brain MRI scans (MRI) were available in 11 patients pretransplant. The Loes scores ranged from 3-23 with a median of 8. Of the 5 patients with Loes scores >10, 2 died from progressive ALD (patients 7 and 10), 2 deteriorated neurologically and are severely affected (patients 1 and 6), and 1 who had mental retardation pretransplant, stabilized in the cognitive

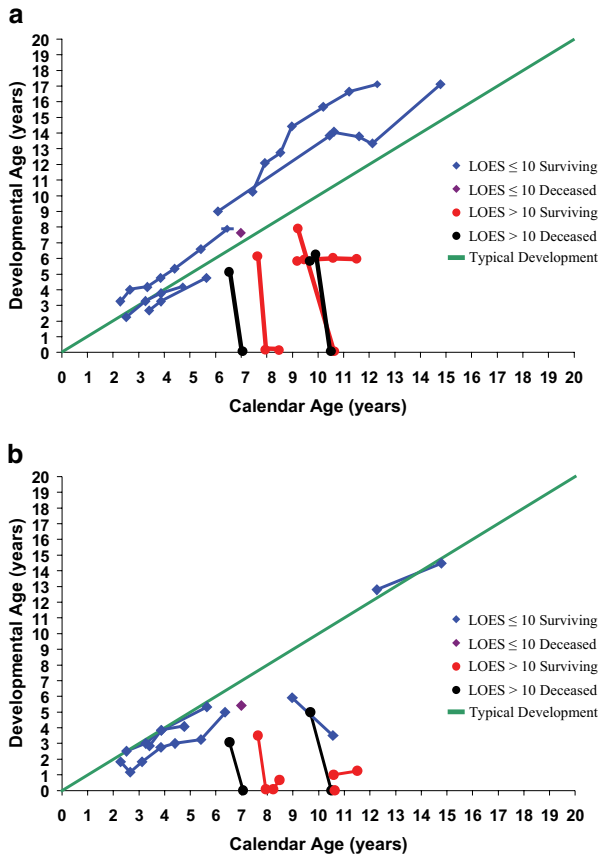

Figure 3. (a) Cognitive development and pretransplant MRI Loes scores. The green line represents typical development. Each blue line represents the longitudinal cognitive course of a surviving patient with baseline Loes Scores <10. The red lines represent the longitudinal cognitive course of surviving patients with Loes Scores >10. The black and purple represent deceased patients. (b) Gross motor development and pretransplant MRI Loes scores. The green line represents typical development. Each blue line represents the longitudinal motor course of a surviving patient with baseline Loes scores <10. The red lines represent the longitudinal motor course of surviving patients with Loes scores >10. The black and purple represent deceased patients. Note that motor scores are in general lower than cognitive scores in (a).

area but had motor deterioration posttransplant (patient 8) (Figure 3a and b). Five of the 6 patients with scores <10 are long-term survivors with favorable neurodevelopmental outcomes, and 1 (patient 11) died of transplant-related VOD. Four of the 5 patients have continued to learn and develop normally (patients 2, 3, 5, and 9), and 1 who has above average cognitive abilities is having mild difficulties in speed of processing and has deteriorated in the motor area presumably from GVHD of the brain (patient 4).

Predictors of Posttransplant Outcome

Five baseline measures (BAERs, VEP, NCV, EEG, and MRI) were evaluated as predictors of posttransplant cognitive and motor outcome. As previously described, pretransplant MRI Loes scores have been found to be associated with poor prognosis [28,29]. Pretransplant Loes scores significantly correlated with longitudinal cognitive ($P = .006$) (Figure 3a) and motor development ($p = .014$) (Figure 3b). Of the 6 children with Loes scores <10, the 5 that survived continue to show normal cognitive development. Of the 5 children with Loes scores >10, 4 showed rapid cognitive and neurologic deterioration and 2 of these 4 died. The fifth patient (patient 9) who had comorbid mental retardation stabilized but has low function. Baseline neurophysiologic results failed to show statistically significant differences in predicting cognitive or motor outcomes.

Interestingly, 4 of the 5 children with Loes scores >10 demonstrated significant differences between the verbal and nonverbal/spatial IQ at baseline. Conversely, all 5 patients with scores <10 had no significant differences between the verbal and nonverbal/spatial IQ and continued to have normal cognitive function posttransplant. All patients with normal motor function and Loes scores <10 continue to develop normal motor function.

DISCUSSION

We report the results of unrelated donor UCB transplantation in a group of 12 boys with cerebral X-ALD. Despite HLA mismatching, the transplant procedure yielded similar outcomes to those previously reported after HLA-matched bone marrow transplantation [4-7]. The probability of OS was 71.9% with the 3 younger children, demonstrating a survival of 100%.

There are several parameters that can be used to predict outcome of patients with ALD after HSCT. The pretransplant Loes score has been shown to correlate with outcomes after bone marrow transplantation [28]. Age and pattern of demyelination in brain MRI have been reported to aid in predicting progression [29]. Previous studies have also reported that patients with performance IQ <80 (also referred as nonverbal IQ) at baseline are significantly more impaired posttransplant and those with parietal-occipital pattern demonstrate greater mean loss in their performance IQ [7]. One of the objectives of this study was to determine whether baseline Loes score was a strong predictor of posttransplant cognitive and motor outcomes. Ten patients were available for this analysis. Loes scores were found to be strong predictors of outcome. Baseline nonverbal/spatial scores in combination with overall cognitive scores and Loes scores were as a group strongly associated with outcome. However, because our sample was not large, we did not report a definitive index combining these measures. Future studies will be needed to assess if the

combination of these measures will aid the clinician in counseling families regarding treatment outcomes when they are considering transplant.

A second objective was to ask if baseline neurophysiologic studies, BAERs, VEP, EEG, and NCV, correlated with cognitive and motor outcomes. These results were mixed. The BAERs and NCV were not always associated with progressive neurologic deterioration and became abnormal only later in the disease process. The VEP and EEG became abnormal earlier in the disease process. Most patients who had abnormalities in pretransplant EEGs developed clinical seizures posttransplant and had poor outcome. However, this finding was not statistically significant. Most of the children in our sample were <10 years. As many as 80% of children this age have the parietal-occipital white matter pattern of involvement [29]. This may explain the early abnormalities seen in VEP.

In this study, myeloablative doses of busulfan were used in the conditioning regimen for transplant. In previous studies, busulfan has been reported to cause adrenal insufficiency in healthy patients [30]. Therefore, careful consideration should be given to the use of busulfan in conditioning regimens in patients with ALD. All patients should be supplemented with stress doses of hydrocortisone during the preparative regimen. Even after dilantin prophylaxis, busulfan appeared to lower the seizure threshold and accelerate neurologic deterioration. Myeloablative agents with low neurotoxicity need to be studied in the future.

This is the first report that includes a cohort of young children transplanted before development of ALD symptomatology or progressive MRI changes. These children were referred for transplant because of strong family history of cerebral X-ALD and MRI changes consistent with this disorder. Two patients had adrenal insufficiency and 1 had an astrocytoma as comorbid conditions. Although these children have not reached the age at which cerebral X-ALD typically progresses, they tolerated the procedure well; continue to have normal neurologic exams, typical development, and no additional changes in MRI scans at 4, 6, and 8 years of age. In contrast, children who followed the present recommendation of waiting until progressive MRI changes or abnormalities in neurobehavioral testing are evident had increased risk of rapid neurologic deterioration, transplant complications, and dismal outcome. This study supports the use of unrelated UCB transplantation in young asymptomatic patients to maximize survival and improve neurodevelopmental outcomes. The window of opportunity to treat can be easily missed if there is no family history, and therefore it also provides support for inclusion of ALD in neonatal screening programs.

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