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Neurodevelopmental Outcomes of Umbilical Cord Blood Transplantation in Metachromatic Leukodystrophy

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A B S T R A C T

Metachromatic leukodystrophy (MLD) is an inherited demyelinating disease that causes progressive neurologic deterioration, leading to severe motor disability, developmental regression, seizures, blindness, deafness, and death. The disease presents as a late-infantile, juvenile, or adult form. Hematopoietic stem cell transplantation has been shown to slow disease progression. The purpose of this longitudinal study was to evaluate long-term treatment outcomes after unrelated donor umbilical cord blood (UCB) transplantation in pediatric patients according to disease burden and age at onset (ie, late-infantile versus juvenile). Engraftment, survival, treatment-related toxicity, graft-versus-host disease, neurophysiologic measures, and neurodevelopmental function were assessed. To evaluate whether signal intensity abnormalities on magnetic resonance imaging (ie, modified Loes scores) predict post-transplant cognitive and gross motor development, a general linear mixed model was fit to the data. Twenty-seven patients underwent transplantation after myeloablative chemotherapy; 24 patients engrafted after the initial transplantation. Seven patients died of infection, regimen-related toxicity, or disease progression. Twenty patients (6 with late-infantile onset and 14 with juvenile onset) were followed for a median of 5.1 years (range, 2.4 to 14.7). We found that patients with motor function symptoms at the time of transplant did not improve after transplantation. Brainstem auditory evoked responses, visual evoked potentials, electroencephalogram, and/or peripheral nerve conduction velocities stabilized or improved in juvenile patients but continued to worsen in most patients with the late-infantile presentation. Pretransplant modified Loes scores were highly correlated with developmental outcomes and predictive of cognitive and motor function. Children who were asymptomatic at the time of transplantation benefited most from the procedure. Children with juvenile onset and minimal symptoms showed stabilization or deterioration of motor skills but maintained cognitive skills. Overall, children with juvenile onset had better outcomes than those with late-infantile onset. As in other leukodystrophies, early intervention correlated with optimal outcomes. We conclude that UCB transplantation benefits children with presymptomatic late-infantile MLD or minimally symptomatic juvenile MLD.

INTRODUCTION

Metachromatic leukodystrophy (MLD) is an inherited lysosomal storage disorder caused by arylsulfatase A deficiency. This lysosomal enzyme is necessary for the breakdown of cerebroside 3-sulfatase, which accumulates within lysosomes of myelin-forming cells in the central and peripheral nervous system. Sulfatides also accumulate within lysosomes of cells in the liver, kidneys, and gallbladder but to a lesser extent than in the nervous system. MLD is characterized clinically by symptoms related to progressive demyelination and is classified according to age at onset as late infantile (6 months to 4 years), early juvenile (4 to 6 years), late juvenile (6 to 16 years), or adult (>16 years). Early signs in the late-infantile and early-juvenile forms include blindness, gait disturbance, loss of speech, loss of hearing, and quadriparesis. Older children and adults often present with gait disturbance, mental regression or frontal lobe syndrome, and behavioral abnormalities. Disease progression results in death within a few years (for the late-infantile form) to several decades (for the adult form) [1].

Although hematopoietic stem cell transplantation using bone marrow or umbilical cord blood (UCB) has been reported to provide some benefit for infants and children with MLD, its effectiveness in arresting disease progression is a matter of debate. Some case reports of children with juvenile MLD who underwent bone marrow transplantation indicate stabilization of clinical course, cognitive measures, and neurophysiologic measures [2,3]. However, these results could be attributed to limited follow-up rather than to arrested disease progression [4]. In other cases, motor deterioration continued after bone marrow transplantation even though cognitive decline appeared to be halted [5,6]. In general, patients with late-infantile or juvenile MLD who exhibited disease progression at the time of transplantation have experienced continued deterioration in neurologic status and/or neurophysiologic measures [7-9]. In the late-infantile form, deterioration has been reported when transplantation was performed less than 1 year before symptom onset [5,7,10-12].

UCB transplantation can improve neurologic outcomes in children with other lysosomal storage diseases, such as Hurler syndrome and Krabbe disease, as well as

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adrenoleukodystrophy, particularly when performed early in the course of the disease [13-16]. Thus, we hypothesized that patients with late-infantile or juvenile MLD who undergo UCB transplantation early in the disease course might stabilize or even gain neurodevelopmental function. In this study, we retrospectively evaluated clinical and functional outcomes of patients who underwent UCB transplantation at a single institution. We also evaluated whether pretransplant Loes scores (modified for MLD) were predictive of post-transplant cognitive and motor outcomes.

METHODS

Patient Characteristics

We reviewed the medical records of patients with MLD referred to the Program of Neurodevelopmental Function in Rare Disorders at the University of North Carolina at Chapel Hill between October 4, 1997 and March 1, 2011. The patients were referred for pre- and post-transplant evaluations by the Pediatric Blood and Marrow Transplantation Program at Duke University Medical Center. These patients were enrolled in different transplant protocols at Duke University, all of which were approved by the institutional review board for the time of study. This retrospective study was approved by the institutional review board of the University of North Carolina at Chapel Hill. For all patients, MLD diagnosis was confirmed by arylsulfatase A activity and presence of urinary sulfatides. Signed parental consent was obtained for both transplantation and neurodevelopmental follow-up.

Donor Characteristics and Procurement

Cord blood units were obtained from U.S. public banks. The cryopreserved units were tested, thawed, and washed before administration as previously described [14,17]. Cord blood units with the highest nucleated cell dose, matching at least four of six human leukocyte antigen (HLA) loci, were tested for arylsulfatase A activity. For each transplant, the unit with the closest HLA match, highest nucleated cell dose, and normal enzyme activity was selected. Nine patients were enrolled in the Cord Blood Transplantation Study (COBLT) (three under the expanded access protocol [COBLT-EAP]) and previously reported [18].

Conditioning Regimen, Transplantation Procedure, and Supportive Care

All but one patient underwent pretransplant conditioning therapy with myeloablative chemotherapy consisting of busulfan (16 doses of 20 to 40 mg/m^2 per dose to target a steady-state plasma concentration of 600 to 900 ng/mL), cyclophosphamide (200 mg/kg), and horse antithymocyte globulin (90 mg/kg) [16,18]. Cryopreserved units of cord blood were thawed, washed, and administered intravenously as previously described [16]. Prophylaxis against graft-versus-host disease (GVHD) was administered using cyclosporine for 9 months and methylprednisolone or mycophenolic acid for 2 to 3 months as long as GVHD was not active. One patient underwent reduced-intensity conditioning with alemtuzumab (3.2 mg/kg), fludarabine (150 mg/m^2), melphalan (140 mg/m^2), thiotepa (200 mg/m^2), and hydroxyurea. GVHD prophylaxis in this patient consisted of tacrolimus and mycophenolate. Supportive care was administered as previously described [16].

Myeloid engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count >500/mm^3. Platelet engraftment was defined as ≥50,000/mm^3 for 7 consecutive days without transfusion support. Donor chimerism was determined by restriction fragment length polymorphism analysis of total, +/−lymphoid, and myeloid cells in the peripheral blood at engraftment, 100 days, 6 months, and 12 months and annually thereafter. Arylsulfatase A levels were also evaluated at these time points.

Clinical Follow-Up

All patients underwent standard transplant follow-up studies at 100 days, 6 months, and 12 months and annually thereafter. Engraftment, chimerism, acute and chronic GVHD, and treatment-related morbidity were scored on these visits. In addition, patients underwent evaluations by multiple pediatric subspecialists at baseline and every 6 to 12 months thereafter, including in-depth neurodevelopmental assessment. Serial brain imaging, neurophysiologic examinations, and neurodevelopmental studies were performed within the same week. Details of these studies are presented below.

Neurodevelopmental Assessment

All patients underwent comprehensive neurodevelopmental examinations at the Program for the Study of Neurodevelopmental Function in Rare Disorders at the University of North Carolina at Chapel Hill. Follow-up data were available through December 3, 2012. Standardized and validated neurobehavioral tools were used to assess all children, and outcomes were compared with norms of typically developing children [19-25]. Age equivalents were used to allow comparisons across tests and to determine the acquisition of developmental skills. Gross and fine motor skills, cognitive development, receptive and expressive language, and adaptive behavior were longitudinally assessed.

Scoring of Magnetic Resonance Imaging Studies

Brain magnetic resonance imaging (MRI) was performed at baseline, 6 months, and 12 months and then annually for all surviving patients. A single neuroradiologist (blinded to clinical status but not diagnosis) graded the MRI scans using the Loes scoring system, which was developed for adrenoleukodystrophy [26]. Atrophy was uncommon in our patient population before transplantation but was an expected treatment outcome; therefore, atrophy scores were not included in the total score, hereafter referred to as a modified Loes score [26].

The reader was aware of patient age, which was needed to assess the degree of myelination, but no other clinical information. Images of all patients but one were reviewed on a PACS workstation (Duke University, Durham, NC), which allowed windowing of images to optimize signal intensity of structures for scoring and direct correlation of structures in different imaging planes.

Neurophysiologic Studies

Electroencephalogram (EEG), nerve conduction velocity (NCV), flash visual evoked potentials (VEPs), and brainstem auditory evoked responses (BAERs) were assessed before transplantation and at scheduled intervals thereafter. EEGs were considered abnormal if focal or generalized slowing, spikes, or sharp waves were present. VEPs were considered abnormal if the P100 wave was absent or delayed. BAERs were considered abnormal if wave I to V interpeak latency was prolonged or if any of the obligate wave forms (I, III, V) were absent. NCVs were considered abnormal if they showed prolonged distal latency, low amplitude, no evoked response, or prolonged F-wave latency.

Statistical Analysis

Cumulative incidences of engraftment and GVHD were calculated by standard methods [27]. The probability of overall survival (patients who are alive with durable engraftment) was calculated by the Kaplan-Meier method. The cut-off date for data analysis was June 30, 2012. Descriptive statistics were used to describe neurophysiologic outcomes. To evaluate whether the modified pretransplant Loes score is predictive of post-transplant cognitive and gross motor development, a general linear mixed model was fit to the data. Age at evaluation, modified Loes total score, and the age × Loes interaction term were regressed on both cognitive and gross motor age-equivalent scores.

RESULTS

Patient and Donor Characteristics

Twenty-seven children (.3 to 16.5 years old; boys, n = 18; girls, n = 9; white, n = 24; Asian Indian, n = 1; African American, n = 2) underwent transplantation with banked UCB from unrelated donors after myeloablative chemotherapy (Table 1). Ten children had late-infantile-onset MLD (4 were asymptomatic), and 17 had juvenile-onset MLD (4 were asymptomatic). Cord blood units were matched at 6/6 (n = 3), 5/6 (n = 8), or 4/6 (n = 16) HLA loci.

Six patients were enrolled in the COBLT, 3 in the COBLT-EAP, 11 in an ongoing single institution study, 1 in the reduced-intensity conditioning protocol, and the last 6 patients in an ongoing study of aldehyde dehydrogenase–bright cells. Median age at transplantation was 5.2 years. The median nucleated cell dose of units selected for transplantation was 6.54×10^7 cells/kg. After thawing, the median total nucleated cell dose administered was 5.19×10^7 cells/kg (range, 1.49 to 25.77) (Table 1), and the median infused CD34 cell dose was 1.53×10^5 cells/kg (range, 1.5 to 12.6).

Engraftment and GVHD

The cumulative incidence of neutrophil and platelet engraftment (≥50,000/mm^3) was 77.8% (95% confidence interval [CI], 57.0 to 98.6) at day 42 and 77.8% (95% CI, 55.6 to 100) at 1 year. The median time to neutrophil and platelet engraftment was 28 days (range, 14 to 76) and 120 days (range,

Table 1
Patient and Graft Characteristics and Outcomes

Patient No.	Age at Diagnosis (yr)	Symptoms	Age at Transplant (yr)	Loes Score	HLA Matches (No. Total)	Number of Nucleated Cells ($\times 10^7$/kg)					
						Cryopreserved	Reinfused	Time to Neutrophil Engraftment (Days)	Time to Platelet Engraftment (Days)	Chronic GVHD Grade	Overall Survival (Days)
Juvenile onset: asymptomatic at time of UCB transplantation											
7	2.2	A	2.3	3.0	4/6	11.37	8.97	37	NE		59*
8	4.7	A	5.7	2.0	5/6	4.67	4.32	38	109	Ext	766*
9	3.3	A	4.3	2.5	6/6	3.01	3.44	47	129		3193
26	.8	A	1.1	4.0	5/6	14.67	8.41	14	37	Ext	906
Juvenile onset: symptomatic at time of UCB transplantation											
10	8.5	S	8.6	15.5	5/6	6.68	5.86	20	113		3054
11	6.6	S	6.7	16.0	4/6	5.38	5.22	25	139	Ext	4075
12	7.7	S	7.9	18.5	4/6	5.08	4.99	62	199		2878
13	7.3	S	7.4	18.0	4/6	8.38	6.33	NE	NE		278*
14	16.1	S	16.5		4/6	2.28	1.49	25	146		5383
15	6.4	S	6.5	15.0	4/6	8.09	5.50	19	57		1934
18	13.9	S	14.0	13.0	4/6	6.44	5.21	24	53		1703
19	6.0	S	6.2	15.0	4/6	6.49	4.07	36	105		1708
20	6.9	S	7.0	15.0	4/6	4.69	3.52	38	99	Lim	1507
21	4.9	S	5.1	12.0	4/6	9.31	6.85	14	39		1352
22	6.3	S	6.5	14.0	5/6	4.39	3.19	28	77		1324
25	6.5	A	6.8		5/6	6.54	4.58	24	37		907
27	8.4	S	9.0		4/6	5.04	3.53	24	124		891
Median	6.8		6.6	15.0		5.91	4.79	25.0	105.0		1507
Late-infantile onset: asymptomatic at time of UCB transplantation											
1	.0	A	.1/2.8†	5.0	5/6	16.42	12.60	40	164	Lim	2852
2	.8	A	1.2	3.0	5/6		5.70	24	193	Ext	2647
23	.5	A	.7	.0	6/6	16.94	9.02	31	120		285*
24	.0	A	.3	1.0	5/6	25.77	12.70	28	88	Lim	1010
Late-infantile onset: symptomatic at time of UCB transplantation											
3	1.9	S	2.0	14.5	4/6	4.67	3.35	76	NE		173*
4	1.9	S	2.4	15.0	4/6	15.43	3.96	50	379		3818
5	3.9	S	5.3	10.0	4/6	5.22	3.84	51	NA		88*
6	3.2	S	3.6		4/6	6.04	5.14	33	NE		99*
16	2.0	S	2.2	6.0	6/6	7.15	6.34	24	48		1849
17	3.0	S	3.2	11.0	4/6	7.96	6.76	18	63		1858
Median	1.9		2.3	5.5		7.96	6.02	32.0	120.0		1429.5

A indicates asymptomatic at time of treatment; S, symptomatic at the time of treatment; Ext, extensive; Lim, limited to skin involvement; NA, not available; NE, no engraftment.
* Deceased.
† Transplanted twice.

37 to 379) after transplantation, respectively (Table 1). One patient experienced autologous recovery after his first transplantation and underwent a second transplantation with fludarabine, cyclophosphamide, and Campath conditioning, but again experienced autologous reconstitution. A second patient experienced late graft failure 31 months after transplantation and after a drug error in which cytosine arabinoside was administered instead of an antiviral medication; retransplantation with T cell–depleted haploidentical bone marrow resulted in full engraftment. A third patient experienced graft rejection and died shortly after transplantation.

The cumulative incidence of acute GVHD grades II to IV at day 100 was 40.7% (95% CI, 22.6 to 58.8), and the cumulative incidence of chronic GVHD at 24 months was 25.9% (95% CI, 9.3 to 42.5). Twenty children had acute GVHD (grade I, n = 8; grade II, n = 11; grade III, n = 1), and 3 patients died before evaluation for GVHD. Four of seven children with chronic GVHD had extensive GVHD and three had only skin involvement (Table 1). All patients who engrafted had enzyme within the normal range.

Morbidity and Survival

At the time of this writing, 20 of 27 patients are surviving, with a long-term (5-year) survival probability of 74.1% (95% CI, 53.2% to 86.7%); median follow-up for patients who survived was 5.1 years (range, 2.4 to 14.7) (Figure 1). Seven children died after transplantation (four late-infantile onset, three juvenile onset) (Table 2). The late-infantile group had a 5-year survival probability of 60.0% (95% CI, 25.3% to 82.7%), whereas the juvenile group had a 5-year survival probability of 82.4% (95% CI, 54.7% to 93.9%). Significant disease progression was noted in 10 patients (6 late-infantile onset, 4 juvenile onset). Other complications after transplantation included failure to engraft (n = 1) and mixed chimerism (n = 1) (Table 3).

Neurologic Outcomes

Brain MRI scans

MRI scans were available for 24 patients before transplantation and 21 patients after transplantation. The pretransplant modified Loes scores indicated baseline MRI abnormalities for all children except two asymptomatic patients diagnosed because of a family history. Median Loes scores were 14.5 for late-infantile MLD and 5.5 for juvenile MLD, indicating more severe central nervous system impairment for patients with late-infantile onset. Sixteen of 19 children who had both pre- and post-transplant modified Loes scores showed improvement after transplantation. Lower pretransplant Loes scores were associated with greater developmental gains in both cognitive skills (late infantile, r = –.72; $P = .10$; juvenile, r = –.68; $P = .022$) and

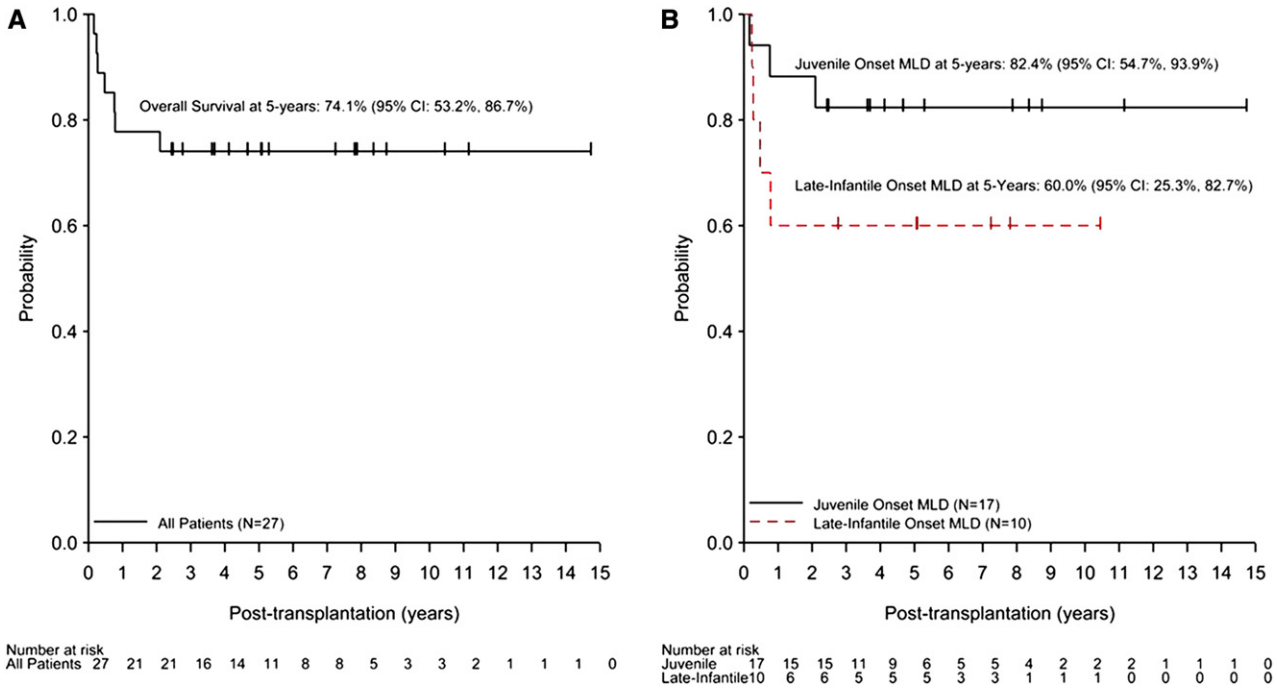

Figure 1. Overall survival of all patients (A) and according to age at onset (B) through the follow-up period.

gross motor skills (late infantile, r = –.84; $P = .037$; juvenile, r = –.88; $P = <.001$) (Figure 2).

Neurophysiology studies

In the late-infantile-onset group, VEP results were normal for 9 of 10 patients tested and remained normal after transplantation for all 5 patients tested during follow-up. In the juvenile-onset group, pretransplant VEP results were normal for 10 of 13 patients tested and remained normal for 8 patients after transplantation. In addition, two of three juvenile-onset patients with abnormal pre-transplant results had normal post-transplant results (Table 4).

In the late-infantile-onset group, pretransplant BAER results were abnormal for all 10 patients and remained abnormal after transplantation for the 6 patients tested during follow-up. In the juvenile-onset group, pretransplant results were abnormal for 14 of 15 patients tested and remained abnormal after transplantation. One asymptomatic patient had normal pretransplant results, which remained normal after transplantation. Interestingly, his sibling, who was symptomatic at transplantation and who was tested only after treatment, also had normal BAER results.

In the late-infantile-onset group, pretransplant NCV results were abnormal for eight of nine patients tested and remained abnormal for the six who were tested after transplantation (four worsened and two stabilized). One minimally symptomatic patient had normal pretransplant results but showed abnormal results 3 months after transplantation. In the juvenile-onset group, pretransplant results were abnormal for 12 of 14 patients tested. Of the 12 who were abnormal, 11 had available post-transplant results, and 10 of this 11 remained abnormal (including 3 who worsened). Of the two juvenile-onset patients who were normal at baseline, one became abnormal after transplantation.

In the late-infantile-onset group, 5 of 10 patients had normal pretransplant EEG results. Of the seven for whom post-transplant results were available, one was normal after transplantation, two showed seizure activity, and four showed diffuse generalized slowing. In the juvenile-onset group, 8 of the 14 tested had normal pretransplant EEG results. Five of the 16 tested after transplantation remained normal. Of the 11 who were abnormal, 7 showed only diffuse slowing and 4 showed diffuse slowing with voltage asymmetry or spikes (3 had seizure activity). Most seizures manifested as staring episodes. Although EEG recordings showed epileptiform activity for three patients, only one developed clinical seizures.

Neurodevelopmental function

Cognitive function. In the late-infantile-onset group, the patient treated as an asymptomatic 3-month-old infant

Table 2
Mortality of Patients with MLD Who Underwent Transplantation

Cause of Death (Infection, Toxicity, Disease Progression)	Number of Patients (n = 7)
Multiple organ failure	1
Respiratory failure (after chronic lung disease)	2
Viral infections/malignancy	3
Epstein-Barr PTLD	(1)
Disease progression and infection	1

PTLD indicates post-transplant lymphoproliferative disorder.

Table 3
Morbidity of Patients with MLD Who Underwent Transplantation

Complication	Number of Patients
Pericardial effusion	3
Pneumatosis intestinalis	3
Subdural effusion	2
Cholestatic disease requiring cholecystectomy	4
Neurologic disease progression	10
Aspiration	(4)
Seizures	(1)
Respiratory failure (tracheostomy)	1

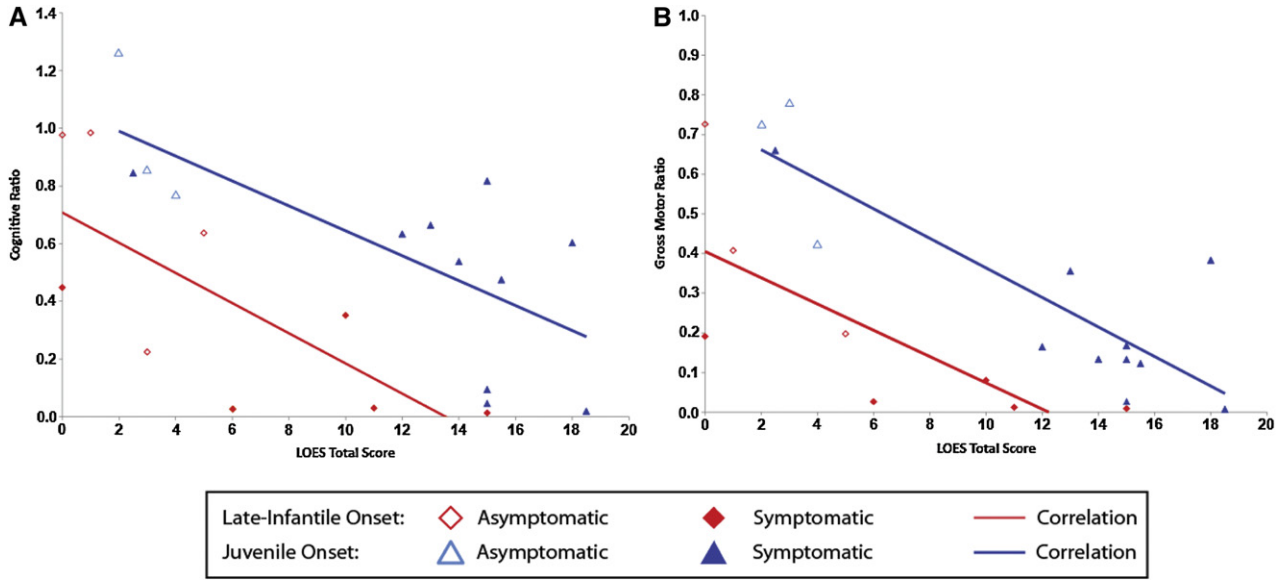

Figure 2. Gross motor and cognitive function versus Loes score. (A) Cognitive function and (B) gross motor at the last evaluation (ratio of functional age-to-chronologic age) are plotted against pretransplant modified Loes scores. Patients are represented by red markers (late-infantile-onset MLD) and blue markers (juvenile-onset MLD). Red and blue lines indicate the least-squares regression lines for each group. Symptom status at the time of transplantation is indicated by hollow markers (asymptomatic) versus solid markers (symptomatic).

continues to gain cognitive skills at a normal rate (Figure 3). The patient treated at 14 months, when he was cognitively normal but had mild motor impairment, maintained skills at a 14-month-old level through 6 years of age and did not have further deterioration. The patient treated as a newborn developed normally until the graft failed after a toxic insult at 15 months of age. After his second transplant, he continued to gain cognitive skills until 4 years of age when development reached a plateau. All patients who exhibited cognitive impairment at baseline experienced a rapid decline (n = 4). Three patients died before the post-transplant evaluations.

In the juvenile-onset group, five patients who were cognitively normal at baseline have continued to gain skills at a normal rate, except for one who died from transplantation-related complications. Five patients with borderline or delayed cognitive skills at baseline have continued to gain cognitive skills, but two have reached a plateau. Four patients declined rapidly, and three died before follow-up.

Gross motor function. In the late-infantile-onset group, the neonatal patient gained gross motor skills at an appropriate rate but experienced a loss of motor skills after the second transplant, followed by a plateau. The patient transplanted at 3 months showed a mild delay in motor development 14 months after transplantation. The patient transplanted at 14 months with minimal motor symptoms experienced a loss of motor skills and is not able to walk independently. The remaining patients are severely delayed and cannot ambulate.

In the juvenile-onset group, patients who were asymptomatic at transplantation have borderline motor function. One patient who showed mild motor impairment before transplantation is gaining skills at a normal rate and functions at the low-normal level. Two continue to gain skills and are in the borderline range, two exhibited mild deterioration but subsequently stabilized, seven lost most of their motor function (four of whom can sit up), three died, and two were lost to follow-up. Of the 12 patients with juvenile-onset disease, 4 patients can walk independently, 1 uses a walker, 3 are wheelchair-dependent for most of the day, and 4 are nonambulatory.

Fine motor function. Of the surviving patients with late-infantile onset who were asymptomatic at baseline, one is developing normally, but the other two reached a developmental plateau at approximately 2 years of age. Four of five symptomatic patients had extreme loss of function. The other patient stabilized at the pretransplant level.

Of the three asymptomatic juvenile-onset patients, two who were assessed post-transplant are developing normally. Of the six symptomatic patients who underwent post-transplant evaluations, four show significant deterioration in fine motor development, one stabilized, and one showed gains.

Adaptive behavior

Adaptive behavior is a standardized measure of independent and self-help skills based on parents' perceptions of their childs' abilities. All areas of development contribute to adaptive behavior. Because of the marked degree of motor involvement, many skills were affected in the study group. In the late-infantile-onset group, patients who were asymptomatic at the time of transplantation stabilized at the pretransplant level, but the symptomatic patients continued to decline. In symptomatic patients, adaptive behavior followed the same course as gross motor development.

In the juvenile-onset group, two of four asymptomatic patients made appropriate gains and two died. One died before the post-transplant evaluation could be completed. Of the 13 symptomatic children, 5 showed a rapid decline in adaptive behavior, 2 stabilized at their pretransplant level, and 3 showed initial decline with later improvement or stabilization. Two patients died before post-transplant evaluation, and no data are available for one patient.

Language

In the late-infantile-onset group, one patient who was asymptomatic at baseline shows normal language

Table 4
Neurophysiologic Measures at Baseline and Last Examination

Patient No.	Baseline BAERs	Last BAERs	Baseline EEG	Last EEG	Baseline NCV	Last NCV	Baseline VEP	Last VEP
Juvenile onset: asymptomatic at time of UCB transplantation								
7	A	A	N	A	N	N	A	N
8	A	A	N	N	A	A	N	N
9	A	A	N	N	A	A	N	N
26	N	N	N	N	A	N	N	N
Juvenile onset: symptomatic at time of UCB transplantation								
10	A	A	N	A	A	—	A	N
11	A	A	A	A	A	A	—	—
12	A	A	N	A	A	A	A	A
13	A	A	A	A	A	A	N	A
14	—	—	A	—	—	A	—	—
15	A	—		A	A	A	—	N
18	A	A	N	N	N	A	N	N
19	A	A		A	A	A	N	—
20	A	A	A	A	A	A	N	N
21	A	A	N	N	A	A	N	N
22	A	A	A	A	—	A	N	N
25	—	N		A	—	N	—	A
27	A	A	A	A	A	A	N	N
Late-infantile onset: Asymptomatic at time of UCB transplantation								
1	A	A	N	A	A	A	N	N
2	A	A	N	A	N	A	N	N
23	A	A	N	A	A	A	N	N
24	A	A	N	N	A	A	N	N
Late-infantile onset: symptomatic at time of UCB transplantation								
3	A	—	A	A	A	—	N	—
4	A	—	A	—	A	A	N	—
5	A	—	A	—	A	—	N	—
6	A	—	A		A	—	N	—
16	A	A	A	A	—	—	N	N
17	A	A	N	A	A	A	A	—

A indicates abnormal; N, normal.

development and the other has skills in the borderline normal range. Four of five symptomatic patients are severely impaired, and one gained skills but now functions at the level of a 12-month-old child.

In the 15 juvenile-onset patients assessed, 3 asymptomatic patients continued to gain skills. Three symptomatic patients show language development in the normal range, one stabilized at pretransplant levels, and five experienced rapid decline and are severely impaired. Three patients did not undergo post-transplant evaluation.

Comparative developmental trajectories

In the late-infantile group, the motor area was significantly more affected than any other area of development and showed no improvement over time. Cognitive and language developed similarly, showing gains but eventual plateauing. Adaptive and fine motor skills fall between language/cognitive and gross motor development (Figure 4).

For the juvenile group, all areas of development show improvement, with cognitive and language development being the strongest and gross motor development the weakest. Adaptive and fine motor development are similarly affected and fall between language/cognitive and gross motor development (Figure 4). This profile characterizes the phenotype of transplanted patients with MLD.

DISCUSSION

In this study we evaluated the overall survival, engraftment, and neurodevelopmental outcomes of 10 patients with late-infantile-onset MLD and 17 patients with juvenile-onset MLD who underwent UCB transplantation from unrelated donors after myeloablative chemotherapy. The feasibility, safety, and efficacy of UCB transplantation were similar to findings of previous studies [16,18]. Family history permitted the early diagnosis and treatment of eight children before the onset of clinical symptoms (four with juvenile onset, four with late-infantile onset). This is one of the few published comprehensive longitudinal studies of a large group of patients with various forms of MLD who underwent transplantation in a single institution. After serial neurophysiologic, neuroradiologic, and neurodevelopmental evaluations performed within a week of each other using standardized protocols, these patients were evaluated for a median follow-up of 5.1 years (range, 2.4 to 14.7).

UCB transplantation successfully replaced the missing enzyme and stabilized disease in patients who were asymptomatic at the time of transplantation, regardless of age at onset. However, children with moderate to severe symptoms did not benefit from transplantation, and seven patients in this group died. In the late-infantile-onset group, only asymptomatic patients with minimal disease burden benefited from transplantation. However, because peripheral neuropathy is severe even at 3 months of age, secondary muscle weakness affected motor skill development.

The modified Loes scores were useful in defining disease severity and showed that demyelination was greater in patients with late-infantile onset than in patients with juvenile onset. After transplant, Loes scores improved, showing fewer areas of demyelination and new areas of myelination. Although this was not compared with normal myelin development, the Loes scores improvement indicates fewer areas of increased intensity signal on T2 images. In our group of patients, those with Loes scores $\leq$5 derived greater benefit from transplantation. However, we do not recommend that Loes scores alone be used to guide treatment decisions. Other variables must also be considered, such as

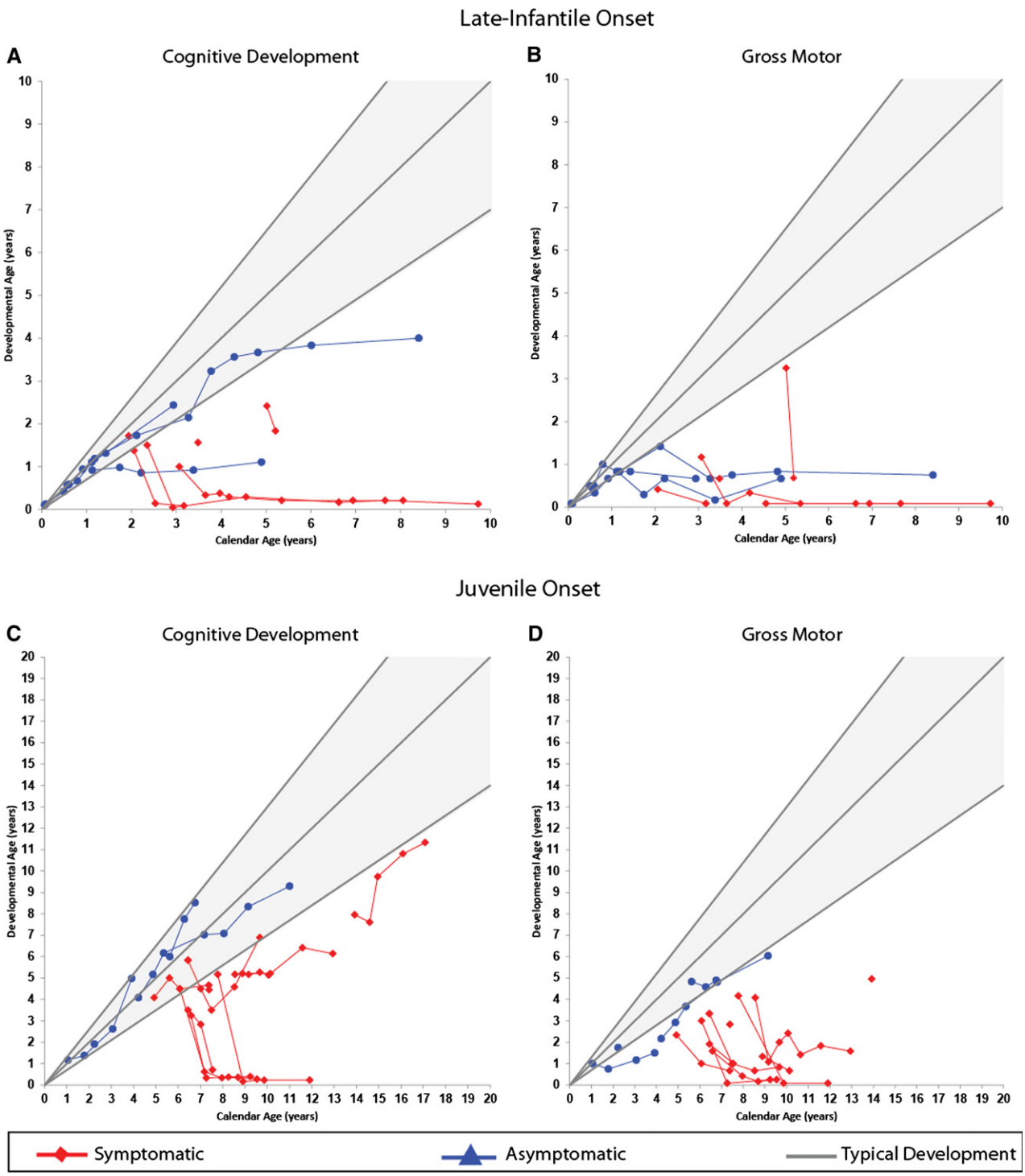

Figure 3. Developmental outcomes are shown by plotting age-equivalent score (developmental age) against actual age (calendar age) for late-infantile and juvenile patients. (A) Cognitive and (B) gross motor development for the late-infantile group. (C) Cognitive and (D) gross motor development for the juvenile group. Red and blue lines indicate individual values of children who were symptomatic (red) and asymptomatic (blue) at time of transplantation. Gray lines represent the mean and approximate variability (95% CI) observed in typically developing children.

the degree of peripheral neuropathy, which can be severe even in very young asymptomatic patients.

We found that gross motor abilities were greatly affected by peripheral neuropathy. Patients who had normal gross motor skills before transplantation also had normal NCV results. All patients with late-infantile onset and most patients with juvenile onset who underwent post-transplant evaluations had abnormal NCV results after treatment, and all but one patient exhibited a decline in gross motor function. Factors contributing to this decline may include disease progression during the period between transplantation and engraftment, muscle weakness due to corticosteroid use, prolonged inactivity, and neurotoxic effects of chemotherapy. It is also possible that transplantation fails to correct disease in

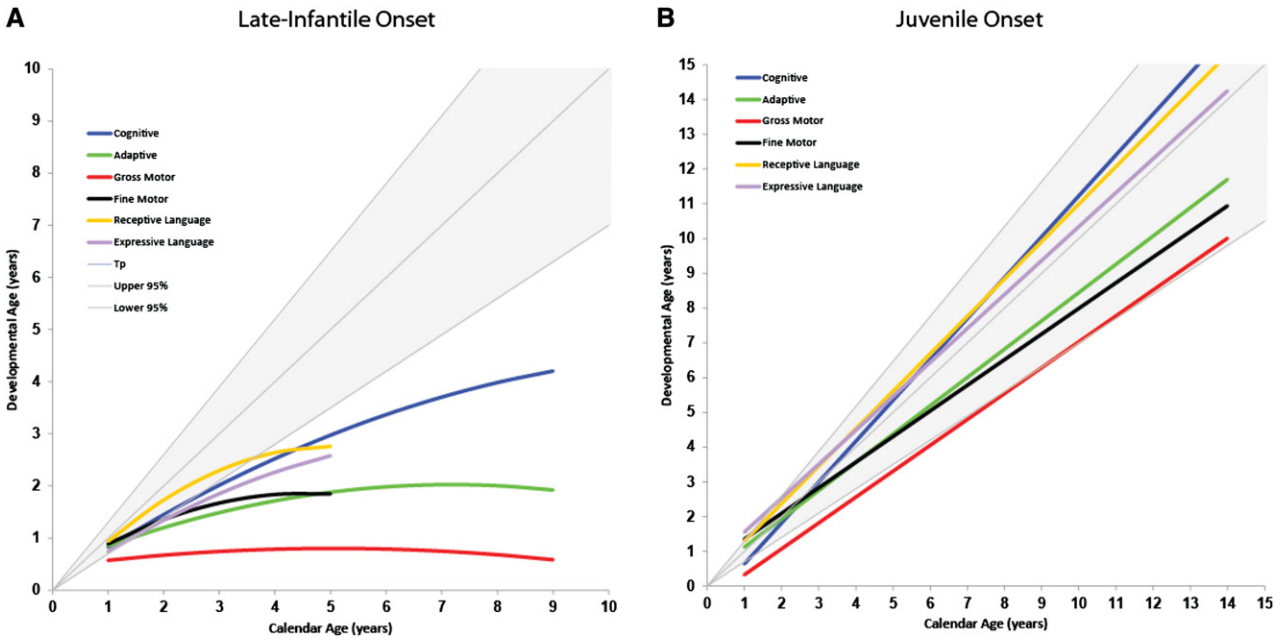

Figure 4. The average developmental trajectory for each developmental domain is presented for the patients who were asymptomatic at the time of transplantation. Developmental outcomes for each developmental domain are shown by plotting age-equivalent score (developmental age) against actual age (calendar age). (A) Developmental trajectories for late-infantile onset. (B) Developmental trajectories for juvenile onset. Gray lines represent the mean and approximate variability (95% CI) observed in typically developing children.

the peripheral nervous system, because enzyme is not delivered to the peripheral nerves.

In this study, the first physiologic test to detect abnormalities was the BAER test, followed by NCV studies. Changes on brain MRI scans and generalized slowing on EEG appeared later. Clinical seizures were evident in only one patient. VEP results were normal until late in the disease progression. In some patients with juvenile onset, VEP results normalized after transplantation.

In general, gross motor function was more severely affected and responded less well to treatment. Gross motor skills fell considerably below patient's cognitive abilities, adversely affecting adaptive behavior. Seven of 12 patients with juvenile onset who were followed long term showed normal language development. Unlike Krabbe disease, where cognitive and expressive language scores are lower than receptive language, in MLD cognitive skills and receptive and expressive language have similar trajectories. It is possible that receptive language is more affected in MLD because of the increased BAER abnormalities.

In our group of patients with late-infantile MLD we were able to evaluate transplantation outcomes for only three asymptomatic patients (one neonate, one 3-month-old infant, and one 14-month-old child). Unfortunately, the neonatal patient experienced toxic and an anoxic insult unrelated to his initial transplant, which limits the interpretation of his data. Despite this complication, his cognitive abilities continue to develop, but he is wheelchair-dependent. The patient who was treated at 3 months of age and was followed for 31 months has normal cognitive function but severe peripheral neuropathy and lower extremity weakness that interfere with her ability to walk independently. Nonetheless, rapid disease progression was expected at 13 months of age based on family history. The patient treated at 14 months who underwent transplantation when minimally symptomatic now has severe motor involvement and is wheelchair-dependent. His cognitive abilities at 6 years are delayed but are higher than his two older untreated siblings at the same age. The remaining patients with late-infantile-onset MLD were more severely affected at the time of transplantation and showed disease progression. Although outcomes of asymptomatic patients are significantly better than those of their siblings, the four asymptomatic patients with late-infantile onset showed early signs of peripheral neuropathy, which did not improve in the three who had follow-up studies. The effect of transplantation was better characterized in patients with juvenile onset because only 2 of 17 patients died before follow-up evaluations. However, additional follow-up is necessary because of the lack of natural history studies and the slower disease progression seen in patients with juvenile-onset MLD. Given the scarcity of patients treated while minimally symptomatic, collaboration among centers using standardized protocols is needed. To predict treatment benefits for an individual patient, the following may also be needed: MRI techniques that quantify brain damage, a closer look at the degree of peripheral neuropathy, a better understanding of the significance of certain mutations, and/or identification of biomarkers that reflect disease severity.

Only long-term follow-up of patients who are asymptomatic at transplantation will clarify whether neurologic disease progression can be halted or merely slowed by UCB transplantation. This study shows that early severe peripheral nerve damage occurs even before symptoms are apparent. Although improvements of the central nervous system were observed, peripheral neuropathy was either too advanced to be reversed or is not affected by transplantation. Newborn screening is needed to identify the patients likely to benefit most from transplantation and other future therapies.

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