Published in final edited form as:

Ann Neurol. 2008 November ; 64(5): 583–587. doi:10.1002/ana.21522.

Umbilical cord blood transplantation for juvenile metachromatic leukodystrophy

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Abstract

Three siblings with metachromatic leukodystrophy underwent umbilical cord blood transplantation at different stages of disease. Neuroimaging, nerve conduction studies, neurological and neuropsychological exams were used to measure outcome over two years. Post-transplant, the oldest sibling experienced disease progression. His two siblings had near or total resolution of signal abnormalities on neuroimaging. Their neuropsychological testing remained stable and nerve conduction studies have shown improvement. These results indicate pre-transplantation neurological exams may be the most significant predictor of outcome after transplant. To our knowledge, this report is the first to document neurological outcome of metachromatic leukodystrophy treated by umbilical cord blood transplantation.

Introduction

Metachromatic leukodystrophy (MLD) is an autosomal recessive lysosomal storage disease (LSD) due to a deficiency of arylsulfatase A (ASA). Absence of ASA results in accumulation of toxic sulfatides causing demyelination. Neurological findings may include spasticity, neuropathy, dementia, and seizures. Importantly, time of onset and severity correlate with residual ASA activity^{1, 2}.

Many ASA gene mutations have been identified. Null mutations result in complete loss of enzyme activity, while residual mutations encode proteins with reduced enzyme activity¹. Infantile MLD occurs in individuals with two null mutations. Adult MLD occurs in individuals with residual mutations. Juvenile MLD patients often possess a null and a residual mutation, resulting in intermediate enzyme activity. Pseudodeficiency (Pd) alleles

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Statement of Conflict of Interest

None of the authors of this article have any potential conflict of interest or and potential conflict of interest regarding the work presented here. Tyler Mark Pierson was supported by NIH grant T32-HD043021-04 during the course of the study.

exist that encode functional proteins with low activity when assayed *in vitro*³. Individuals who are compound heterozygotes for the Pd and null mutations have normal ASA activity *in vivo*, but low enzyme activity *in vitro*. Because this may lead to false positive diagnoses, the diagnosis of MLD requires the presence of urine sulfatides¹.

Therapy for MLD was limited to supportive measures; however, recent interventions based upon unique features of lysosomal enzyme trafficking have shown promise. Lysosomal enzymes possess mannose-6-phosphate (M6P) residues, which bind M6P receptors (M6PR) located on endosomal or plasma membranes. Most of these enzymes are trafficked to the lysosome⁴, but some are released into the extracellular space where they can bind to adjacent cells, undergo endocytosis, and be sorted to lysosomes^{5, 6}. This feature allows a normal cell to cross-correct nearby deficient cells. Intravenous enzyme replacement therapy (ERT) uses this characteristic to treat some LSDs⁷. Unfortunately the blood-brain barrier is believed to hinder the transport of large amounts of enzyme into the CNS⁷; nonetheless, recent studies in ASA-deficient mice have shown that ERT has been modestly effective in reducing sulphatide storage in the brain⁸. This data has led to the recent initiation of human clinical trials using human recombinant ASA. An interesting alternative to ERT uses transplanted hematopoietic cells to deliver enzyme to the CNS. These cells, once transplanted, can migrate into the CNS and subsequently rescue nearby cells⁹. Reports have shown bone marrow and umbilical cord blood transplantation (BMT and UCBT) are able to stabilize the neurodegeneration associated with some LSDs^{2, 9, 10}.

This report documents our experience with three siblings diagnosed with juvenile MLD who underwent UCBT at different stages of disease. In this family, neurological exam at the time of UCBT was the best predictor of outcome, despite concomitant abnormal neuroimaging and NCS. Our results also include the stabilization or reversal of disease progression in two of these siblings two years after transplant. To our knowledge, this report is the first to document the neurological outcome of MLD patients treated with UCBT.

Results

The oldest brother was eight years-old at presentation with complaints of inattentiveness and episodic urinary incontinence for six months. His parents noted he was able to continue with many activities of daily living, but required occasional assistance. He had two healthy siblings, a six year-old brother and four year-old sister.

During examination, the child answered simple questions correctly (but slowly), had brisk patellar reflexes, and an left extensor plantar response. Subsequent brain MRI showed diffuse white matter signal abnormalities (WMSAs) consistent with MLD (Fig 1A). Positive urine sulfatide analysis confirmed this diagnosis. His siblings also had positive urine sulfatides, while their parents had negative urine sulfatides.

ASA analysis revealed the mother and all three children had low ASA activity (<5.6 nmol/hr/mg protein; normal range: 49–82). The father had normal ASA activity. Genetic analysis revealed that the mother was a compound heterozygote for the 635C>T (A212V) null mutation and one copy of a common Pd allele (resulting in her low *in vitro* ASA activity). The father was identified as a heterozygote carrier for the 1277C>T (P426L) residual mutation. All three children possessed A212V and P426L mutations.

Unrelated cord blood units were located through national registries. Cord blood typing was high resolution for DRB1 and intermediate resolution for class I antigens (A, B). Clinical details regarding matching, conditioning, engraftment and outcome are shown in Table 1.

Neurological exams and brain MRI were performed before UCBT and approximately every six months afterwards. Nerve conduction studies (NCS) were similarly scheduled, but could not be obtained before transplantation. Neuropsychological analysis (NPA) was performed before UCBT and approximately two years later.

White blood cell ASA (WBC-ASA) activity was tested every six months after UCBT with all of the children having persistently normal activity after UCBT. Every child had initial NCS with mild diffuse sensorimotor polyneuropathy typical of demyelinating/dysmyelinating disorders (Table 2).

Pre-transplant NPA testing of the oldest child noted extensive CNS dysfunction with a full-scale IQ score of 59 (2nd percentile). Two months after UCBT, he became non-verbal, did not follow commands, and developed spasticity. Two years after transplant, he developed migrating simple partial epilepsy that has been unresponsive to medication. His brain MRI showed late-stage leukodystrophy (see Fig 1B).

Before UCBT, the six year-old brother had a normal neurological exam. Brain MRI showed moderate diffuse WMSAs (Figure 1C). NPA showed mild inattentiveness and cognitive disturbance. He performed at a low-average level with a full-scale IQ score of 88 (21st percentile). After UCBT, his neurological exam remained relatively stable, although six months to a year afterwards he developed heel-cord tightness and decreased ankle deep tendon reflexes. Treatment with physical therapy and ankle braces has led to improvement of the contractures. Brain MRIs showed improvement with near resolution of WMSAs, associated with enlarged lateral ventricles (Figure 1D). Follow-up NPA remained at a low average to borderline level with a general-ability index in the eighth percentile (full scale IQ was not used due to a substantial variability among subtests). NCS revealed improvement of the median nerve sensory velocity from abnormal to normal over two years (Table 2). Median motor velocities remained stable and near 80% of the lower limit of normal (Table 2), as did sural sensory and peroneal motor nerve conduction (data not shown)^{11, 12}. Median nerve F-wave values remained stable (and may be trending toward improvement).

The younger sister had a normal neurological exam and brain MRI (Fig 1E) before UCBT. NPA revealed high average-superior ability with a full-scale IQ score of 120 (91st percentile). After UCBT, her neurological exam remained normal. Six months after UCBT, her brain MRI developed mild periventricular WMSAs with a posterior prominence (data not shown), which resolved within the next six months and has remained stable (see Fig 1F). After UCBT, her NPA was interpreted in the high average-superior range even though her follow-up full scale IQ score was 98 (21st percentile; the score reduction was an artifact of the patient's initially shy behavior during initial testing, later tests were scored in the high average to superior range). NCS revealed that median nerve sensory velocities improved from abnormal to normal for age over two years, while F-wave latency-to-height ratios remained stable or trended towards improvement (Table 2)^{11, 12}. Median nerve motor conduction remained near 80% of the lower limit of normal (Table 2), as did sural sensory and peroneal motor nerve conduction (data not shown).

Discussion

Reports of hematopoietic cell transplantation (with or without mesenchymal stromal cells) for MLD have yielded a wide range of results^{13–20}. Even with full engraftment, BMT often failed to alter the neurodegeneration⁹; however, numerous reports showed some stabilization of disease after transplantation^{9, 18–20}. UCBT may be a better option than BMT because stored and cataloged UCB can be rapidly identified and transplanted, producing a shorter period between diagnosis and transplantation, an important

characteristic to consider in neurodegenerative diseases. This feature was especially evident with the youngest sibling's development of WMSAs six months after UCBT and their subsequent resolution. Unrelated UCB donors are also more likely to have normal enzyme activity than family members (often used in BMT) who could be heterozygote carriers with reduced enzyme activity and therefore reduce the efficacy of the transplant²¹.

Previous recommendations for the use of BMT to treat MLD required patients to have adequate neuropsychological function and independence in activities of daily living; however, no firm guidelines exist²². Some reports have advocated that only non-symptomatic patients undergo HCT⁹; however, in practice this categorization may be too vague to implement.

In this family a normal neurological exam was the best indicator of prognosis. The oldest child had a mildly abnormal neurological exam at the time of transplant and continued to deteriorate neurologically. Whether the transplant or medical interventions associated with the transplant accelerated this progression cannot be determined. In contrast, the middle sibling of this family had a normal neurological exam on presentation, but could also have been viewed as being symptomatic with regards to his initial testing, and so a poor candidate for transplant. Importantly, after UCBT, he and his sister have had their disease stabilize or improve. Unfortunately, his neuropsychological status may have deteriorated slightly, although it is difficult to determine whether this deterioration occurred during the period directly after transplant while the transplanted cells were migrating into the brain or has been an ongoing process prior to and after transplantation.

Of note, the younger patient's trend in improving NCS may serve as a biomarker of improved peripheral myelination after UCBT. The median nerve sensory conduction velocities have changed from abnormal to normal over two years, while motor values have been stable or may be improving (given the improved f-wave latency-to-height ratios). These findings may be the result of the reversal of pathology or may represent disease stabilization associated with normal developmental changes that were previously unable to occur in the setting of active disease. Sensory fibers may be the earliest type to respond following UCBT and perhaps are the most sensitive indicator of change; further observation with these patients and others is warranted. and caution should be used so that these values are not overemphasized when discussing treatment response with patients and their families. In addition, it is very interesting that the youngest child's MRI data suggest a period of six months to a year for the transplant to start having a therapeutic effect.

Previous reports have shown there can be phenotypic variation in the onset and course of compound heterozygote patients possessing the P426L residual mutation and a null mutation²³. It may be that the course of each of these children's disease reflects this variation and is not a solely based on their UCBT; however, one can estimate from the MRI and NCS data that all three children started developing pathology (e.g. WMSAs and polyneuropathy) at approximately the same age (4–5 years old). Behavioral patterns for age were also similar by parental report. We believe these similarities allow for a realistic comparison of these children's responses after UCBT. Altogether, whether these are temporary improvements or permanent effects will require long-term monitoring. For these two younger siblings, UCBT has been beneficial in stopping progression of disease.

Acknowledgments

The authors would like to thank Kenneth H. Fischbeck and Barrington Burnett for critical review of the manuscript. They would also like to thank Joanne Molinari and Tracy Hayden for administrative assistance, as well as Thomas Flynn, Robert Zimmerman, and Larissa Bilaniuk for technical expertise and advice. T.M.P. was funded by NIH grant T32-HD043021-04

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Table 1

Transplantation Data

Patient age/Gender at UCBT	8 years/Male	6 years/Male	4 years/Female
UCB-HLA match	5/6 Mismatch at B	4/6 Mismatch at A&B	4/6 Mismatch at A&B
CD34+/kg	5.1 x 10 ⁵ /kg	6.0 x 10 ⁵ /kg	1.5 x 10 ⁶ /kg
Conditioning regimen	IV Busulfan with pharmacokinetics, Cyclophosphamide, alemtuzumab	IV Busulfan with pharmacokinetics, Cyclophosphamide, thymoglobulin	IV Busulfan with pharmacokinetics, Cyclophosphamide, thymoglobulin
GVHD prophylaxis	Tacrolimus, methylprednisolone	Tacrolimus, methylprednisolone	Tacrolimus, methylprednisolone
Time until engraftment Neutrophil=N Platelet=P	N:Day +24 P:Day +30	N:Day +18 P:Day +43	N:Day +5 P:Day +41
GVHD (overall)	Grade III (skin, gut)	None	None
Complications	CMV colitis Chronic sinusitis	BK viral infection Hemorrhagic cystitis	Right ankle infection Chronic sinusitis Hypothyroidism

Table 2

Nerve Conduction Studies

	Median Nerve Sensory Velocity (m/sec)	Median Nerve Motor Velocity (m/sec)	Median Nerve F-Wave Latency (msec)	F-Wave Latency / Ht (msec/cm)
Normal Ranges 9-10 (Age Range)	39.6–58.710 (4–8 years)	47.1–65.59 (4–6 years) 50.6–64.0 (6–14 years)	16.4–22.59 (4–6 years) 18.1–28.4 (6–14 years)	
Age (years) /Months Post-Transplant	Middle Sibling			
6/6	33	38	25.2	0.219
7/14	38	34	27.5	0.233
7.5/20	38	39	28.1	0.236
8/24	42	41	26.8	0.217
Age (years) /Months Post-Transplant	Youngest Sibling			
4.5/6	39	39	22.1	0.216
5.5/14	46	45	NR	
6/20	46	45	21.6	0.199
6.5/24	49	45	21.9	0.197