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ARTICLE

Neurological outcomes after hematopoietic stem cell transplantation for cerebral X-linked adrenoleukodystrophy, late onset metachromatic leukodystrophy and Hurler syndrome

Desfechos neurológicos após transplante de células tronco hematopoiéticas na adrenoleucodistrofia ligada ao X, forma cerebral, na leucodistrofia metacromática de início tardio e na síndrome de Hurler

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ABSTRACT

Hematopoietic stem cell transplantation (HSCT) is the only available treatment for the neurological involvement of disorders such as late-onset metachromatic leukodystrophy (MLD), mucopolysaccharidosis type I-Hurler (MPS-IH), and X-linked cerebral adrenoleukodystrophy (CALD). **Objective:** To describe survival and neurological outcomes after HSCT for these disorders. **Methods:** Seven CALD, 2 MLD and 2 MPS-IH patients underwent HSCT between 2007 and 2014. Neurological examinations, magnetic resonance imaging, molecular and biochemical studies were obtained at baseline and repeated when appropriated. **Results:** Favorable outcomes were obtained with 4/5 related and 3/6 unrelated donors. Two patients died from procedure-related complications. Nine transplanted patients were alive after a median of 3.7 years: neurological stabilization was obtained in 5/6 CALD, 1/2 MLD, and one MPS-IH patient. Brain lesions of the MPS-IH patient were reduced four years after HSCT. **Conclusion:** Good outcomes were obtained when HSCT was performed before adulthood, early in the clinical course, and/or from a related donor.

Keywords: hematopoietic stem cell transplantation; leukodystrophy, metachromatic; mucopolysaccharidosis I; adrenoleukodystrophy.

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RESUMO
O transplante de células tronco hematopoiéticas (TCTH) é o único tratamento disponível para o envolvimento neurológico de doenças como a leucodistrofia metacromática (MLD), a mucopolissacaridose tipo I-Hurler (MPS-IH) e a adrenoleucodistrofia (CALD). **Objetivos:** Descrever a sobrevida e os desfechos neurológicos após o TCTH nessas doenças. **Métodos:** Sete pacientes CALD, 2 MLD e 2 MPS-IH realizaram TCTH entre 2007 e 2014. Avaliações neurológicas, ressonância nuclear magnética e estudos bioquímicos e moleculares foram feitos no baseline e repetidos quando apropriado. **Resultados:** Desfechos favoráveis foram obtidos em 4/5 TCTH de doadores relacionados e em 3/6 não relacionados. Dois pacientes faleceram de complicações do procedimento. Nove transplantados sobreviveram após uma mediana de 3,7 anos: estabilização neurológica foi obtida em 5/6 CALD, ½ MLD e em um caso MPS-IH. As lesões encefálicas de um caso MPS-IH reduziram-se quatro anos após o TCTH. **Conclusão:** Bons desfechos foram obtidos quando o TCTH foi feito antes da vida adulta, cedo no curso clínico e/ou a partir de um doador relacionado.

Palavras-chave: transplante de células-tronco hematopoiéticas; leucodistrofia metacromática; mucopolissacaridose I; adrenoleucodistrofia

Allogeneic hematopoietic stem cell transplantation (HSCT) was proposed as a treatment for inherited leukoencephalopathies in the eighties, based on the assumption that transplanted macrophages migrate through the blood-brain barrier, and differentiate into microglia that would replace or support native microglia with the functional enzyme through cross-correction[1]. Since then, HSCT has been the only available treatment for the neurological involvement of some lysosomal storage disorders that affect the central nervous system (CNS), such as juvenile and adult metachromatic leukodystrophy (MLD), mucopolysaccharidosis type I Hurler (MPS-IH), and others. In addition, HSCT has been mostly indicated for the cerebral form (CALD) of the peroxisomal disorder X-linked adrenoleukodystrophy (X-ALD)[2,3,4,5,6,7,8,9,10]. Considering that transplant-related mortality has declined to 10% and the rate of engraftment has substantially improved in recent years[11,12], risk of transplant is worthwhile in contrast to the certainty that the natural history of these disorders will lead to dementia, decorticate or vegetative states and death, some years after onset.

There is consensus about the efficacy of HSCT when performed early in life for MPS-IH[9,13,14] and at early stages in CALD[6,7,12,15]. Case reports suggested that HSCT might reduce demyelination in juvenile (onset between two and 14 years) and adult forms of MLD when performed as early as possible[3,4,5,16]. A recent case control study confirmed that HSCT, at a presymptomatic or early symptomatic stage of juvenile MLD, is associated with disease stabilization[10].

The aim of the present study was to describe a seven-year experience of a university hospital with HSCT for these disorders, focusing on survival and on neurological outcomes.

METHODS

Patients and study procedures

This is a retrospective study of a population consisting of seven CALD, one juvenile and one adult MLD and two MPS-IH patients, who underwent HSCT at Hospital de Clínicas de Porto Alegre between 2007 and 2014. All procedures were covered by the Brazilian Unified Health System, and received the same level of health insurance. Three CALD patients have been reported previously[17].

Biochemical diagnosis was obtained for all patients and was confirmed by molecular analysis of the appropriate gene. Neurological examinations, neuropsychological tests, magnetic resonance imaging (MRI), and specific biochemical markers were performed before HSCT, and six months to seven years after the procedure.

An HSCT was offered as early as possible when an HLA-matched donor or cord blood was available. Additional clinical criteria for offering HSCT were applied, according to each specific disorder. International recommendations were followed for CALD patients: the presence of white matter lesions in the CNS and a Loes score lower than 10 points[7]. An HSCT was also offered as early as possible following diagnosis of juvenile or adult forms of MLD, provided that some gross motor functions such as walking (with or without aid) were still present. Finally, HSCT was indicated for MPS-IH patients under 2.5 years of age if the clinical status, especially pulmonary function, allowed the procedure[9].

Biochemical and molecular analyses

Plasma docosanoic (C22:0), tetracosanoic (C24:0) and hexacosanoic (C26:0) acids were obtained from CALD patients, and analyzed as described previously[18]. Arylsulfatase A (ARSA) activity in leukocyte and a thin-layer chromatography of urinary sulfatides were studied in the MLD patients. In the MPS-IH patients, urinary glycosaminoglycans (GAGs), colorimetric assay (DMB test) and electrophoresis were performed, and α-L-iduronidase (IDUA) activity in leukocytes was measured. All biochemical studies were performed at the time of diagnosis: their values were subsequently compared to those obtained after HSCTs.

Molecular analyses of *IDUA*, *ABCD1* and *ARSA* were performed in DNA isolated from peripheral blood prior to the HSCT. In all patients, individual exons and flanking regions amplified by PCR were submitted for Sanger sequencing. For *ARSA* analysis we also evaluated regions associated with pseudodeficiency (PD) variants using a similar approach[19].

Brain MRI

Brain MRI data were obtained with a 1.0 or 1.5 T systems equipped with a standard circularly polarized head coil.

Axial fluid-attenuated inversion recovery (FLAIR), axial and coronal T2 and sagittal T1 weighted images were obtained in all patients. The MRI was performed at baseline and in the follow-up visits after HSCT. Disease-related MRI scores[20,21] were reviewed for each patient at the time of the present report. Images were analyzed by two independent neuroradiologists (LMV and LC) blind at the time of the given study. In case of discordant scores, both neuroradiologists conferred together to find a consensus. Any 0.5 T or low-quality MRI images were excluded from the analysis. An independent researcher (JAMS) rebuilt the time frame later.

HSCT procedures

The HSCT was performed according to institutional protocol recommendations, and all patients received the myeloablative conditioning regimen consisting of an combination of busulfan and cyclophosphamide, as previously published[22]. Details of the HSCT procedures are described in Table 1.

Ethics, consent and permissions. Consent to publish.

Consents for the transplant procedure and for follow up visits and ancillary tests were obtained from all individuals or their representatives at the time of the procedure, and according to ethical requirements of our institution. This report summarizes results from studies registered as GPPG 13-0390 (for X-ALD), 07-599 (for MLD) and 03-066 (for MPS-IH), all approved by the Ethics Committee (EC) of Hospital de Clínicas de Porto Alegre, following the World Medical Association International Code of Medical Ethics (Declaration of Helsinki).

RESULTS

The HSCT were performed in 11 patients (six with unrelated donors), in the last seven years. Patients and donor characteristics, conditioning, graft source and engraftment, are shown in Table 1.

Baseline results of the neurological findings, intelligence quotient (IQ), MRI and biochemical markers, as well as the follow-up findings after HSCT, are presented in Table 2. Two patients (one with CALD and one with MPS-IH) died from HSCT complications. Seven of the remaining nine individuals had stabilization of symptoms one year or more following HSCT.

Seven CALD patients underwent HSCT; preliminary data on three of them were reported previously[20]. Case reports are presented in the Supplemental Material. In summary, HSCTs were done in five CALD patient younger than 12 years, in one who was 19.4 years old and in one who was 28.2 years old. The oldest of them also presented with the highest Loes score at the time of his HSCT (8 points): he died nine months after the procedure. Five out of the six CALD patients who survived HSCT were clinically stable at the time of this report (in one patient, the Loes score had even improved). The progression of Loes scores of all seven individuals is shown in Figure 1.

Two MPS-IH underwent HSCT: their data are depicted in Tables 1 and 2, and also on Supplemental Material. Of note, a clear improvement of MRI findings of MPS IH-10 case appeared three years after her HSCT, with significant reduction of periventricular and deep WM lesions. The mild ventricular enlargement also improved (Figure 2).

Due to data scarcity on HSCT efficacy in MLD, individual case reports will be reported bellow.

Patient MLD-8b

Patient MLD-8b, female, eight years old, was the youngest sister of MLD-8a, the male index patient. A gait disturbance started when MLD-8a was nine years old. He was brought for evaluation at 11 years old: there was loss of cognitive abilities, spastic tetraparesis (wheelchair-bound) and dysarthria. MRIs showed diffuse leukodystrophy and brain atrophy (Eichler score of 21) (Figures 3 A, B, C). ARSA activity in leukocytes was 0.6 nmol/h/mg prot (normal range: 5-20) and thin-layer chromatography detected increased sulfatides in urine. ARSA sequencing revealed a homozygosis p.P426L mutation. The family and staff team agreed to not perform HSCT. At the time of the genetic counseling of this family, MLD-8b was eight years old, her school performance was normal and, on clinical examination, only an intermittent strabismus (exotropia) was noted. ARSA activity, urinary sulfatides and *ARSA* analysis confirmed the diagnosis of MLD. Her Eichler score on MRI was 6 (Figure 3D, Table 2). Patient MLD-8b was transplanted when she was nine years, two months old; her oldest sister did not carry p.P426L mutation at *ARSA* and was the donor. MRI lesions worsened at the time of HSCT (Eichler score of 10, Figure 3E), but she remained asymptomatic. She started with complex partial seizures at 12 years old, thee years after HSCT. An EEG showed left temporal paroxysmal spikes. Seizures were well controlled with valproic acid. The MRI lesions stabilized until the last observation, five years after HSCT (Eichler score of 12, Figures 3F-G). Her last follow-up was at 16 years of age: MLD-8b showed normal performance at school and normal neurological examination. Neurophysiological studies depicted subclinical abnormalities (Table 2).

Patient MLD-9

Patient MLD-9, male, presented with personality changes and cognitive decline at 19 years of age. An MRI scan revealed a diffuse leukodystrophy (Eichler score of 20). No ARSA activity was detectable in his leukocytes and was below normal values in his only brother. He was referred for evaluation almost a year after diagnosis, in order to search for unrelated donors. His family was then studied:

Table 1. Characteristics of the patients and donors, conditioning, composition of the graft, and engraftment.

Diagnosis; Case	CALD-1	CALD-2	CALD-3	CALD-4	CALD-5	CALD-6	CALD-7	MLD-8b	MLD-9	MPS IH-10	MPS IH-11
Patient sex	Male	Male	Male	Male	Male	Male	Male	Female	Male	Female,	Male
Disease	CALD	CALD+AMN	CALD	CALD	CALD	CALD	CALD	juvenile onset MLD	adult onset MLD	MPS IH	\ MPS IH-11
Age at HSCT indication (years)	8.8	18	6.5	9.5	7.5	6.2	27.5	8.5	19	0.9	0.9 (first HSCT); 2.6 (second HSCT
Age at HSCT (years)	9.4	19.4	8.4	10.8	8.4	6.5	28.2	9.2	20.6	2.2	1.9 (first HSCT); 2.8 (second HSCT)
Donor gender and age (years) at HSCT	Female, 5.2	Male, 31.9	Male, 48	Male, 35.4	Male, newborn	Female, 18.4	Male, 20.3	Female, 27y	Male, 24.4	Male, 34	Female, 33.5
Donor type	Sibling	Uncle	Unrelated	Unrelated	Unrelated	Sibling	Unrelated	Sibling	Sibling	Unrelated	Unrelated
Genotype of related donors	normal VLCFA;	non-carrier;				non-carrier		p.P426L -/- PD analysis -/-	p.P426L -/- PD analysis: c.1524+96A>G / -		
HLA donor/recipient match status	HLA identical	HLA identical	HLA identical	HLA identical	HLA mismatched (Cw allele)	HLA identical	HLA mismatched (DQB1 allele)	HLA identical	HLA identical	HLA identical	HLA identical
Conditioning Regimen	Bu and Cy	Bu and Cy	Bu and Cy	Bu and Cy	Bu and Cy	Bu and Cy	Bu and Cy	Bu and Cy	Bu and Cy	Bu and Cy	Bu and Cy
GVH Prophylaxis	MTX and Tacrolimus	CSA and MTX	ATG, CSA, and MTX	ATG, CSA, and MTX	ATG, CSA, and MMF	CSA, and MTX	ATG, CSA, and MTX	CSA and MTX	CSA and MTX	ATG, CSA. and MTX	ATG, CSA. and MTX
Graft source and NC dose (x 10^8/kg)	BM, 4.1	BM, 5.4	BM, 6.6	BM, 2.9	CB, 16.8	BM, 5.2	BM, 1.8	BM. 7.7	BM, 4.7	BM, 6.2	BM, 4.5
Days to ANC >0.5x10^9/L	15	19	17	28	15	21	15	22	15	21	19
Latest chimerism (recipient levels of the former cells) and time from HSCT (months)	100%, +62 m	NA	100%, +2 m	100%, +74m	100%, +49 m	100%, +22 m	100%, +7 m	100%, +57 m	100%, +17 m	34%, +46 m	7%, +11 m
Outcome	Favourable	Favourable	Unfavourable	Favourable	Favourable	Favourable	Death 9 months after HSCT (29yo)	Favourable	Unfavourable	Favourable	Death 23 after 2nd HSCT (2.8 yo)
Hb/WBC/PLT at last follow-up (x109/L)	150 / 6.2 / 325	96 / 2.5 / 142	132 / 4.6 / 110	120 / 3.2 / 151	135 / 5.4 / 179	122 / 7.2 / 177	\	127 / 5.0 / 151	115 / 4.7 / 270	129 / 4.5 / 191	

AMN: adrenomyeloneuropathy; ANC: absolute neutrophil count; ATG: anti thymocyte globulin; BM: bone marrow; Bu: busulfan; CALD: cerebral adrenoleukodystrophy; CB: cord blood; CSA: cyclosporin; Cy: cyclophosphamide; GVH: graft versus host disease; HLA: human leukocyte antigen; HSCT: hematopoietic stem cell transplantation; MLD: methachromatic leukodystrophy; MPS: mucopolysaccharidosis; MTX: methotrexate; NA: not available; NC: nucleated cells; NR: not reached; PMD: psychomotor development; VLCFA: very long-chain fatty acid.

Table 2. Baseline and follow-up results after HSCT: neurological and clinical findings, IQ, MRI, and biochemical markers.

Variable	CALD-1	CALD-2	CALD-3	CALD-4	CALD-5	CALD-6	CALD-7	MLD-8b	MLD-9	MPS IH-10	MPS IH-11
Genotype	p.L628Q	p.A232fsX64	p.A232fsX64	p.R518Q	p.S358X	p.A401W	p.P560L	p.P426L/p.P426L; PD negative at ARSA gene	p.P426L/ p.P426L; PD negative at ARSA gene	p.W402X/ p.W402X	p.W402X/ p.W402X
Age at onset of cerebral signs (years)	8.9 Addison-only at 7yo	18	6.5	9.5 Addison-only at 7yo	7.5	6.4	27.5	NA	19	0.8	0.9
Age at HSCT (years)	9.4	19.4	8	10.9	8.4	6.5	28.2	9.1	20.6	2.1	1.9 (first HSCT) and 2.8 (second HSCT)
Neurological and clinical examination findings at the time of HSCT	Asymptomatic	AMN without encephalic manifestations	Ataxia, ankle clonus and attention deficit to auditory stimuli	Asymptomatic	Asymptomatic	Brisk reflexes on the left hemibody	Hemiparesis, dysarthria and a conduction aphasia	Intermittent strabismus	Personality changes, seizures and cognitive loss. Babinski sign on right side	Recurrent upper air infections, hepatomegaly, failure to thrive, developmental delay, and coarse facies. Severe PMD retardation: unable to walk or talk.	Moderate PMD retardation: at the time of first HSCT, still starting to walk and talk.
Age and status at last follow-up (time elapsed)	16yo (7 years after HSCT): No complains Mild signs of neuropathy on neurological examination	27yo (8 years and 9 months after HSCT)): AMN without encephalic manifestations. Progressive worsening of gait since 18yo (needs canes for walking since 25yo).	9yo (12 months after HSCT) Severe GVHD. Marked worsening: aphasia, tetraparesis	16yo (5 years after HSCT): Stable (clinically normal). NCS disclosed peripheral neuropathy.	13y10m (5 years after HSCT): Stable (asymptomatic)	8y8m (2 years after HSCT): Stable (brisk reflexes on the left hemibody)	29yo (9 months after HSCT): Death due to Chronic GVH + opportunistic infection 9 months after HSCT	16yo (7 years and 6 months after HSCT) Stable. Normal neurological examination. Focal seizures controlled with valproic acid. Subclinical demyelinating polyneuropathy on nerve conduction studies, and slowed P40 latencies at somatosensory evoked responses. Normal visual evoked responses.	22 yo (18 months after HSCT) Akinetic mutism	5y11m (3 years and 8months after HSCT) Stable. No further cognitive losses, Communication was nonverbal, though enough to her daily needs. Coarse facies, weight and height on percentile 3, gibbosity, and joint restriction in hands, arms and legs still present. No hepatomegaly on ultrasound. Thickening of the leaflets of the aortic and mitral valves without changes in transvalvular flow on echocardiogram.	3 years: Death due opportunistic infection at 23 days after 2nd HSCT
Baseline IQ	Performance 103 Total 96	Performance 105 Total 91	NA	Performance 141 Total 140	Performance 99 Total 86	NA	NA	NA	NA	NA	NA
Last IQ (time elapsed)	Performance 106 Total 105 (5 years later)	NA	NA	Performance 136 Total 123 (2 years later)	Performance 108 Total 105 (2 years later)	NA	NA	NA	NA	NA	NA
Baseline MRI: (CALD-Loes score, MLD-Eichler score)	Loes of 2	Loes of 6.5	Loes of 4.5	Loes of 4.5	Loes of 4	Loes of 3	Loes of 8	Eichler score of 10	Eichler score of 20	WM lesions in the periventricular and deep WM associated with mild ventricular enlargement (Figure 4A-B)	-----

Continua

Last MRI: (CALD-Loes score, MLD-Eichler score)	Loes of 1 (7y after HSCT)	Loes of 4 (6 years and 9 months after HSCT)	Loes of 7 (12 months after HSCT)	Loes of 6.5 (5 years and 6 monts after HSCT)	Loes of 4 (5 years after HSCT)	Loes of 7 (2 years after HSCT)	Loes of 8 (2 months after HSCT)	Eichler score of 12 (5 years after HSCT)	NA	Significant reduction of periventricular and deep WM lesions. Mild ventricular enlargement also improved (Figure 4C and D) (3 years after HSCT)	NA
Baseline biochemical markers	C26: 2.2 µmol/L C26/22 ratio 0.04	C26:3.47 µmol/L C26/22 ratio 0.10	C26:2.90 µmol/L C26/22 ratio 0.15	C26: 1.94 µmol/L C26/22 ratio 0.06	C26: 4.33 µmol/L C26/22 ratio 0.049	C26:3.54 µmol/L C26/22 ratio 0.08	C26:4.53 µmol/L C26/22 ratio 0.07	ARSA activity in leukocytes:1 nmol/h/mg prot Urinary sulfatides ++	ARSA activity in leukocytes: undetectable Urinary sulfatides:++	α-L-iduronidase activity in leukocytes: 0.2 nmol/h/mg prot Urinary GAGs:532 ug/mg creatinine	α-L-iduronidase activity in leukocytes: 4.0 nmol/h/mg prot Urinary GAGs: 638 ug/mg creatinine
Last biochemical markers(time elapsed)	C26:2.9 µmol/L C26/22 ratio 0.05 (6 years)	NA	C26:5.7 µmol/L C26/22 ratio 0.16 (1 month)	C26:1.9 µmol/L C26/22 ratio 0.13 (6 years)	C26:1.8 µmol/L C26/22 ratio 0.17 (5 years)	C26: 0.7 µmol/L C26/22 ratio 0.12 (2 years)	NA	ARSA activity in leukocytes: 9.5 ml/mg of prot/h Urinary sulfatides ++ (6 years)	ARSA activity in leukocytes: 5.3 nmol/h/mg prot	α-L- iduronidase activity in leukocytes: 22 nmol/h/mg prot Urinary GAGs:185 ug/mg creatinine	α-L-iduronidase activity in leukocytes: 12 nmol/h/mg prot Urinary GAGs:289 ug/mg creatinine

AMN: adrenomyeloneuropathy; ARSA: arylsulfatase A; CALD: cerebral adrenoleukodystrophy; HSCT: hematopoietic stem cell transplantation; IQ: intelligence quotient; GAG: glycosaminoglycan; GVHD: graft-versus-host disease; MLD: metachromatic leukodystrophy; MPS: mucopolysaccharidosis; MRI: magnetic resonance imaging; NA: not available; NCS: nerve conduction studies; PMD: psychomotord; VLCFA: very long-chain fatty acid; WM: white matter. normal leukocyte ARSA activity = 5-20 nmol/h/mg prot, normal leukocytes α-L-iduronidase activity = 32-52 nmol/h/mg prot, normal urinary GAGs levels = 79-256 ug/mg creatinine.

he appears as individual V-14 in Figure 4. Born from consanguineous parents (IV-11 and IV-12, Figure 4), MLD-9 was genotyped as p.P426L/p.P426L; *PD* - / - at *ARSA* (pseudodeficiency variants not present). Mother was p.P426L/ - ; *PD* - / -, and presented with normal ARSA activity and absent urinary sulfatides. Father was p.P426L/-; *PD* c.1524+96A>G/ -: urinary sulfatides were absent, and ARSA activity was compatible with pseudodeficiency (Figures 3 and 4). The older brother of MLD-9, individual V-13 in Figure 4, was HLA identical to the patient, but was formerly excluded as a candidate donor due to low ARSA activity. He was genotyped as p.P426L - / -; *PD* - c.1524+96A > G / -: absent urinary sulfatides, and ARSA activity were compatible with pseudodeficiency. He was chosen to be the HSCT donor, performed when the patient was 20 years, seven months old. Patient MLD-9 was discharged 79 days after HSCT. He presented with acute graft-versus-host disease (GVHD) with skin and conjunctival involvement and no other major complications. At the follow-up six months after HSCT, MLD-9 presented with akinetic mutism, being able to walk when forced to, and daily vomiting associated with cyclosporine use. In the last follow-up, 18 months after HSCT (22 years of age), the clinical picture had not changed. His family did not agree to perform MRI and nerve conduction follow-ups.

DISCUSSION

In the present study, we reported the neurologic progression of Brazilian patients with CALD, MLD, and MPS-IH following HSCT.

These diseases are associated with untreatable and progressive neurological impairment, leading to vegetative states and death[11,17,23,24]. Pathophysiology varies according to gene defect, and notable variability in age at onset and progression rate occurs in all them. To date, HSCT is a unique therapy with potential to bring long-term survival and neurological stabilization for CALD and for some lysosomal storage disorders with brain involvement. Around 500 children with MPS-IH, 114 with MLD, and 465 with CALD underwent HSCT worldwide before 2012[11,12]. In the same period, South American HSCT procedures were described in seven CALD patients (the follow-ups of three of them were reported by Jardim et al.[17]), six MPS and one Gaucher disease type 1[25]. Our present report adds information about four new and three already-reported CALD patients, two new MLD, and two new MPS-IH patients, focusing on their neurological outcomes.

Nine out of our 11 patients (81%) were alive after a median of 3.7 years after procedure: 6/7 CALD, 2/2 MLD and 1/2 MPS-IH. According to the literature, survival rates improved from 55-65% in early series[6,8,25,26] to 85–95% in the most recent series[7,11]. Our rates were comparable to the latter. A 28-year-old CALD man (CALD-7), and a three-year-old MPS-IH boy (MPS-IH-11) died 23 and 270 days, respectively, after

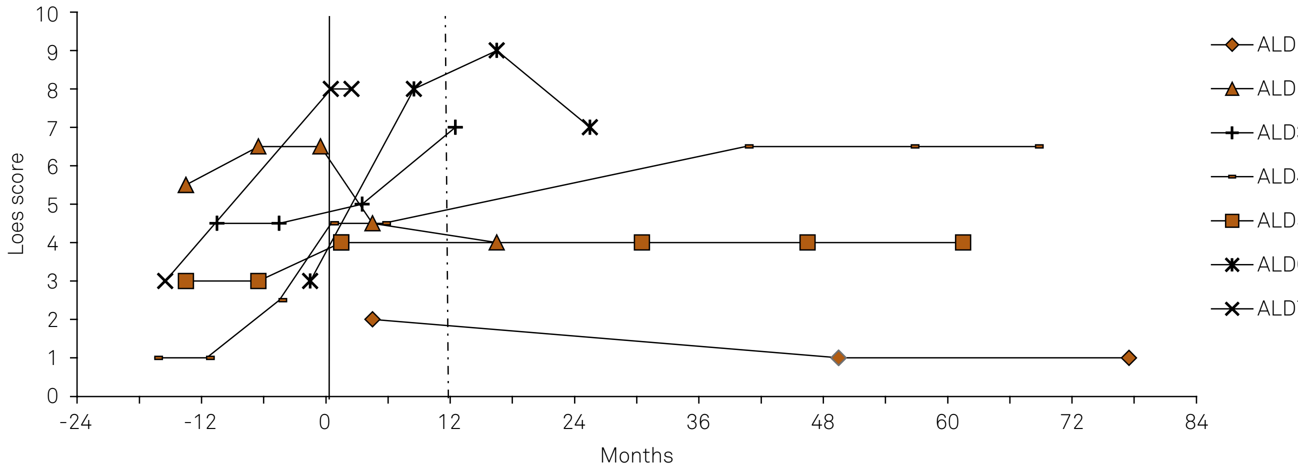

Figure 1 . Loes score progression of seven CALD patients before and after HSCT. Individual dots indicate when brain magnetic resonance imaging was performed. The first MRI was the image obtained at the time of HSCT indication. Time zero (continuous line) indicates when HSCT was performed. The 12-month interval (dotted line) indicates the time window for efficacy.

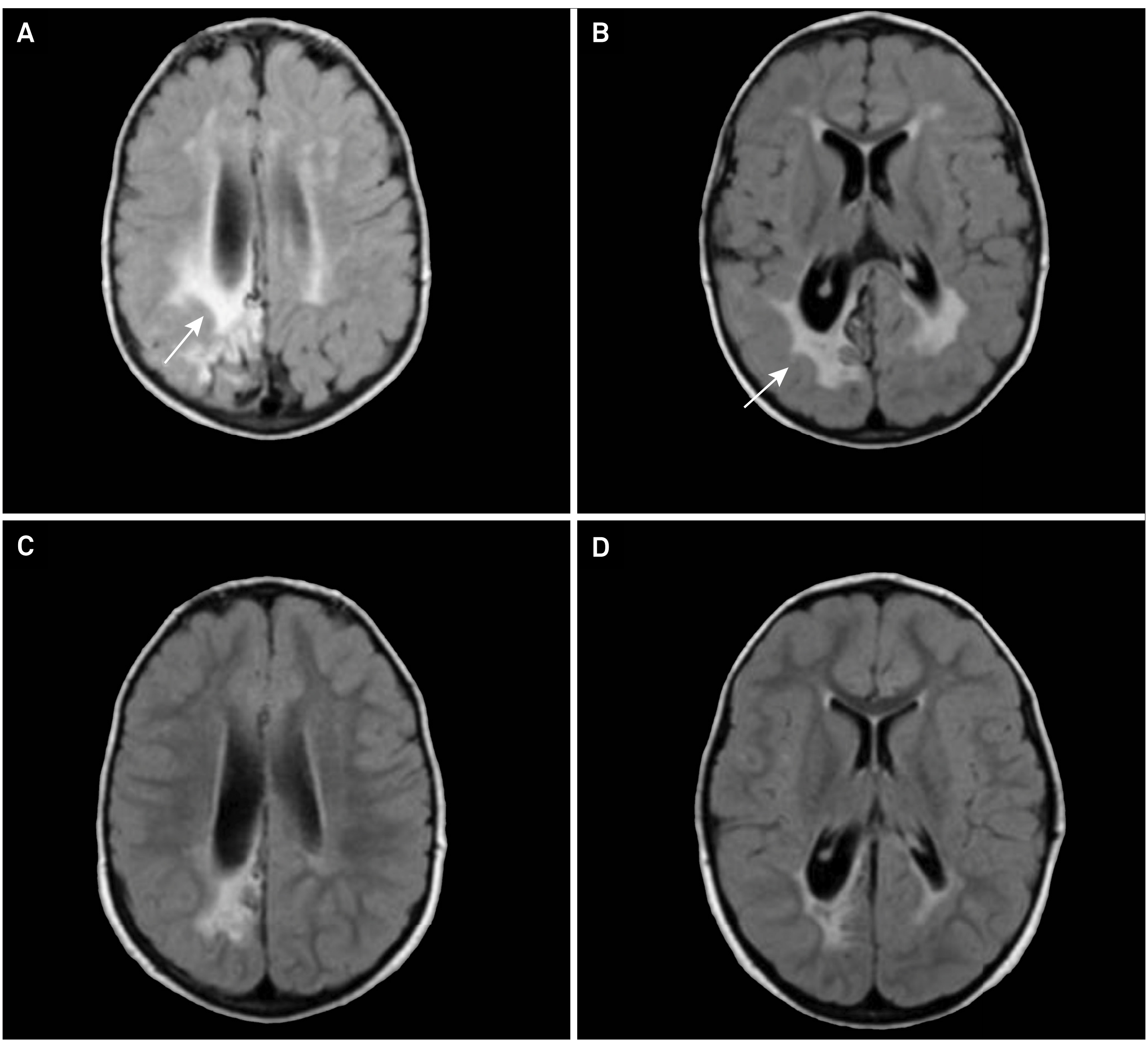

Figure 2. Brain FLAIR axial magnetic resonance images (MRI) of MPS-IH-10 patient. (A and B) MRI before HSCT. Hyperintensity lesions were mainly found in the periventricular and deep white matter (WM) (arrow). There was also mild ventricular enlargement. (C and D) MRI obtained three years after HSCT. Significant reduction of hyperintensities in the WM (arrow). The mild ventricular enlargement was smaller than before HSCT.

HSCT, from opportunistic infection, with or without GVHD. Multiple reasons probably contributed to their deaths. In common, both deceased patients received grafts from unrelated donors, an already-known factor associated with increased risk of mortality[26,27]. Moreover, the CALD-7 patient started with symptoms in adulthood, another factor associated with higher HSCT morbidity and mortality risks[27]. Although mortality rates after HSCT in CALD of less than 5% have been reported[7], none of the studies on HSCT outcomes published so far included adult forms[15]. A report on a 36-year-old CALD man showing cognitive deterioration in the previous year described the patient's death from GVHD three months after HSCT[28].

The remaining nine individuals of the present report had sufficient follow-up time to be described. They were six CALD, two MLD and one MPS-IH patients.

One out of the six CALD patients surviving HSCT presented with several complications from HSCT and worsening of Loes score up to 12 months after HSCT. In contrast, the other five patients showed neurological stabilization after transplantation: CALD-1, CALD-2, CALD-4, CALD-5 and CALD-6 (Table 2 and Figure 1). Mean age and Loes scores at the procedure were 10.9 years and 4 points. Mean Loes scores changed to 5.9 after a mean time of four years after their HSCTs; their neurological status was mostly stable. One adolescent patient (CALD-2) presented simultaneously with adrenomyeloneuropathy (AMN) and CALD at the time of HSCT. Nine years later, AMN findings worsened while CALD findings stabilized. All the other subjects showed normal functioning at school and in daily life activities. Signs of peripheral neuropathy appeared five and seven years after HSCT in two 16-year-old males (CALD-1 and CALD-4), probably related to AMN evolution. These results are in agreement with data showing that HSCT is effective in stabilizing CALD, but not AMN[7,15].

Therapeutic results in MLD transplanted patients were completely divergent. While HSCT in patient MLD-8b seemed to be a success, it did not halt the disease progression in the adult form of patient MLD-9. Both patients shared the same genotype: the main difference was the clinical picture at the time of HSCT. Patient MLD-8b was a completely asymptomatic girl (Eichler 10) transplanted at nine years old, and her neurological examination remained normal until the last follow-up at 16 years of age. Although other juvenile onset patients remained stable after HSCT[29,30], the lack of association of genotype p.P426L/p.P426L with age at onset complicates the evaluation of HSCT success. For instance, two untreated patients homozygous for p.P426L were still asymptomatic at

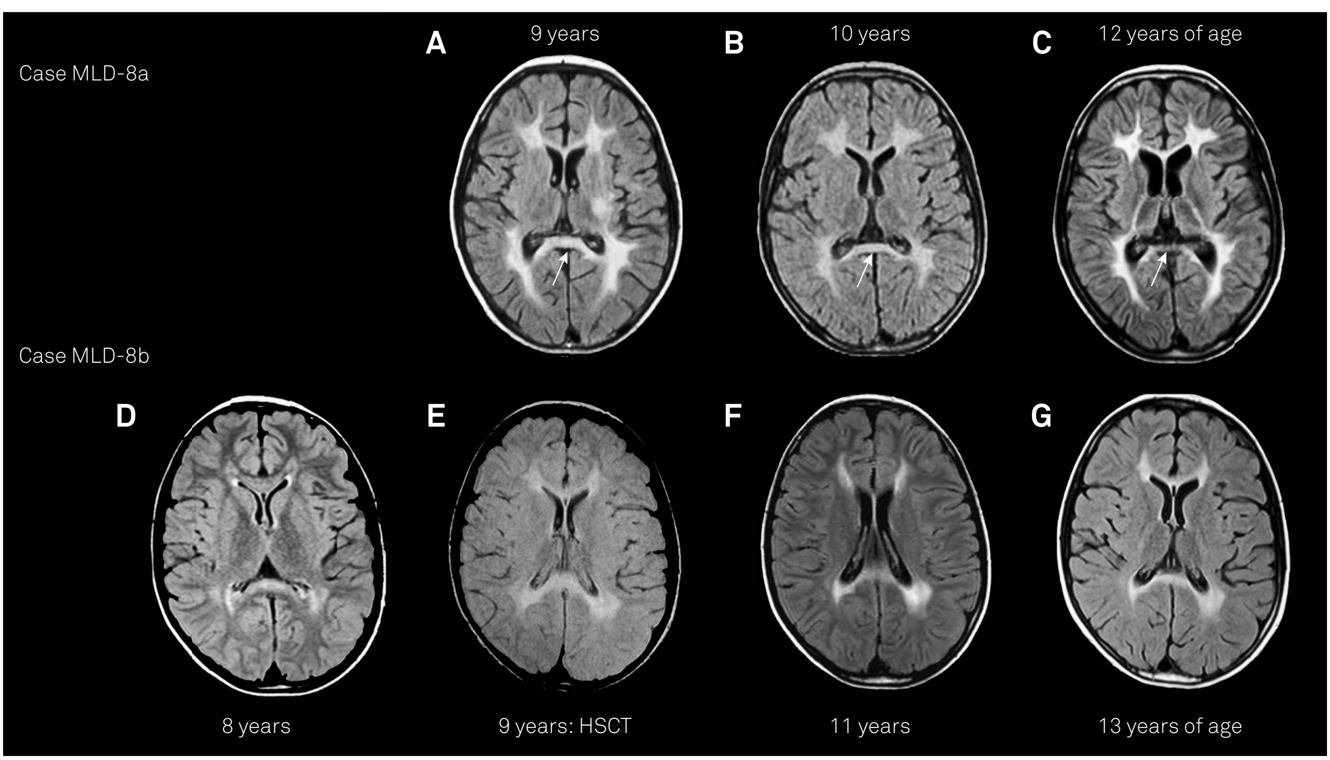

Figure 3. Brain FLAIR axial magnetic resonance images (MRI) of the transplanted patient MLD-8b and her non-transplanted brother MLD-8a. A, B and C. FLAIR axial MRIs from MLD-8a (non-tranplanted) patient. Images show hyperintense lesions diffusely distributed in the frontal, temporal, parietal and occipital white matter (WM) and in genu and splenium of the corpus callosum at 9 (A), 10 (B) and 12 years of age (C). There was a progressive worsening of brain atrophy during the follow up. White arrows highlight the progressive atrophy of the splenium of corpus callosum. D, E, F and G. FLAIR axial MRIs from MLD-8b patient show WM lesions distributed in the frontal, parietal and occipital lobes at 8 (D), 9 (E) (immediately before HSCT), 11 (F) and 13 years of age (G). Hyperintensities on the splenium of corpus callosum worsened until HSCT, with stabilization thereafter. The Eichler score was the same at 11 (F), 13 (G) and 14 years of age (image not shown).

ages 14 and 32[2]; our patient MLD-9 was also homozygous for p.P426L and had a disease onset at 19 years old. Late onset among p.P426L homozygotes points to longer follow-up observations to confirm the effectiveness of HSCT in these patients. It is important to note that evolution of patient MLD8a cannot be used as an untreated control for HSCT efficacy in patient MLD8b. His report emphasizes the importance of genetic counseling for the early, presymptomatic diagnosis of MLD. Patient MLD8a came to our attention too late, when an HSCT would not offer a favorable outcome. However, his diagnosis allowed the detection of patient MLD8b before disease onset. And indeed, two recent studies – a prospective cohort and case-control study – have raised evidence that HSCT is associated with a reasonable chance of disease stabilization if performed pre-symptomatically[10,16].

Patient MLD-9 came for HSCT evaluation one year after the beginning of cognitive losses. An HSCT was offered to this patient based on the assumption that adult forms progress slower than early-onset forms and that there was a chance that microglial cross correction could happen before the development of more severe neurological deterioration. However, follow-up revealed that this was not the case. Soon after HSCT, he developed an akinetic mutism. Since therapeutic effects are expected some months (usually 12) after HSCT, MLD-9's deterioration was probably related to a rapid disease progression in an individual with already-extensive leukodystrophy on MRI at presentation. Recently, HSCT performed in four adult MLD patients was associated with stabilization four to 18 years after the procedure[6]. Three of them showed high Eichler scores, from 18 to 26, before transplantation. Patient series have reported either stabilization[31] or failure after HSCT[32] in juvenile and adult MLD patients. Better evidence favoring HSCT in juvenile MLD was recently obtained, when the neurological follow-up of 24 transplanted patients was better than those of 41 non-transplanted patients[10]. Several factors were associated with a better prognosis after HSCT: gross motor function preserved, IQ of at least 85, and age at onset older than four years.

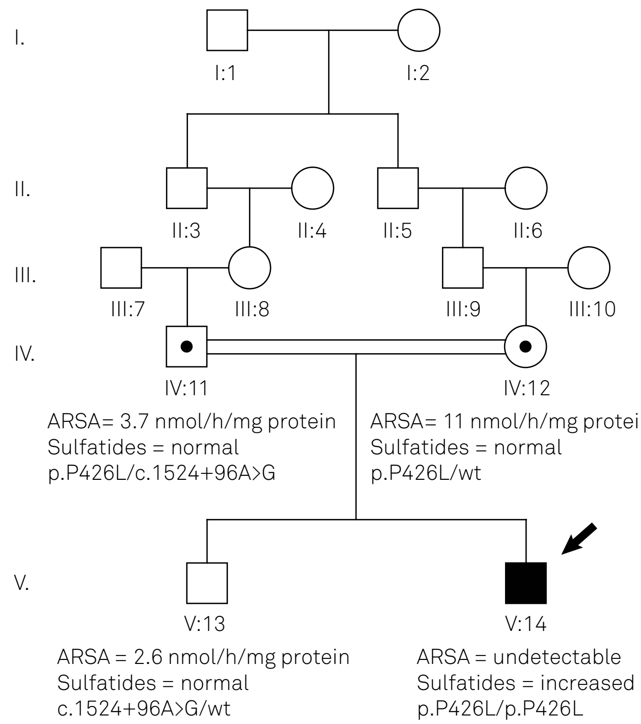

Figure 4. Pedigree of MLD-9 family. Proband is indicated with a black arrow. Squares indicate males and circle, females; dark fill indicates affected individuals. Arylsulfatase A (ARSA) activity in leukocytes (normal range: 5-20 nmol/hg/mg protein), urinary sulfatides, and *ARSA* genotypes are depicted for MLD-9 patient (V:14 individual), his parents IV:11 and IV:12, and his brother and bone marrow donor, V:13. Gene sequencing detected p.P426L as the pathogenic mutation in homozygosis in MLD-9 (V:14); both parents (IV:11 and IV:12) carried this mutation (were heterozygous), as expected. In addition, father (IV:11) and brother (V:13) were carriers of the variant associated with ARSA pseudodeficiency, c.1524+96A>G.

Table 3. Factors potentially associated with unfavorable outcomes in the present patient series.

Factors	All	Favorable outcome	Unfavorable outcome: Death due to HSCT or worsening of clinical picture
	11	7	4 (2 CALD, 1 MLD, 1 MPS-IH)
HSCT related donor	5	4	1
HSCT unrelated donor	6	3	3 (two deaths)
Adult-onset disease	2	0	2 (one MLD and one CALD)
Loes score at the time of HSCT, in CALD Mean (range)	7 (2 to 8)	4 (2 to 6.5)	6.25 (4.5 and 8)
Disease duration before HSCT *, in years Mean (range)	0.94 (0 to 1.6)	0.8 (0 to 1.4)	1.2 (0.7 to 1.6)

*Disease duration = the difference between age at onset of cerebral signs and age at HSCT (see Table 2). HSCT: Hematopoietic stem cell transplantation; CALD: Cerebral from of X-linked adrenoleukodystrophy ; MLD: Metechromatic Leukodystorphy ; MPS-IH: mucopolysaccharidosis type I-Hurler.

Early transplantation has been related to optimal long-term cognitive and language outcomes in MPS-IH[9,14]. Although our MPS-IH survivor (patient MPS-IH-10) was a late transplanted patient, her follow-up four years later was very encouraging. Clinical improvement, despite relatively low engraftment, should not be surprising. Studies in fibroblasts from MPS-I patients with different phenotypes and different genetic backgrounds confirmed that tiny differences in residual enzyme activity are related to important clinical differences[33]. Several studies on HSCT in MPS-IH reported improvements in cardiopulmonary function, hearing and vision, and preservation of neurocognitive function[9,14,34]. Some of them documented that subsequent cognitive deterioration might occur after HSCT, mostly if it is performed late[35]. None of these studies, however, reported a reduction of brain lesions on MRI after HSCT, such as happened in this patient (Figure 2). Since this patient was not receiving enzyme replacement therapy, the clear reduction of periventricular and deep white matter lesions as well as the reduction in ventricular dilatation should be related to an HSCT effect.

Our cohort is small and included three different diseases. Even so, we looked for differences between patients with favorable and unfavorable outcomes after HSCT - considering death due to HSCT or deterioration of the clinical picture as the unfavorable outcomes (Table 3). In summary, better outcomes were obtained in patients whose HSCT was performed before adulthood, early in the disease clinical course, and from a related donor.

In conclusion, the present study showed short- to long-term results of neurological functions after HSCT in CALD, MLD and MPS-IH, in Brazil. Our survival rate was of 81% after a median of 3.7 years. While the results for the two adult forms were negative – one procedure-related death in a 28-year-old CALD patient and a rapid deterioration in a 20-year-old MLD patient – the response to HSCT was very satisfactory for seven of the other nine individuals. Better outcomes were obtained in those patients whose HSCT was performed early, as were the cases of CALD patients with a mean baseline Loes of 4, and of the pre-symptomatic MLD individual. The outcome of the late-transplanted MPS-IH patient was also very satisfactory, being accompanied by reductions in the lesions seen in MRI.

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