Metachromatic leukodystrophy:

Natural evolution and treatment effects

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Aan mijn ouders

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Metachromatic leukodystrophy: natural evolution and treatment effects

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Chapter 1

General introduction

Introduction

Metachromatic leukodystrophy (MLD; OMIM 250100) is an inherited lysosomal disorder, caused by recessive mutations in *ARSA,* located on chromosome 22q13.33 and encoding arylsulfatase A (ASA). The estimated birth prevalence of MLD is 1.4-1.8 per 100.000.[1] ASA is essential for sulfatide metabolism through the hydrolysis of the 3-O ester bond of galactosyl and lactosyl sulfatides. Deficiency of ASA results in accumulation of undegraded sulfatides in the lysosomes and membranes of all cell types, especially in myelinating cells, oligodendrocytes and Schwann cells.[2] This results in progressive demyelination of the central and peripheral nervous system (CNS and PNS). The disease is classified into a late-infantile, juvenile and adult onset type, based on the age of onset of presenting symptoms.

Genetics

More than 150 *ARSA* mutations have been described to date.[3] The characterized mutations are divided into two groups: 0 alleles, which are associated with extreme low enzyme activity, and R alleles which have detectable residual activity.[1] The following mutations are the most common ones in Europe: c.465+1G>A (p.?) (0 allele and commonly found in the late-infantile type), c.542T>G (p.Ile181Ser) and c.1283C>T (p.Pro428Leu) (R allele, common adult variant).[2] The c.827C>T (p.Thr276Met) variant is frequently associated with the late-infantile type. Pseudodeficiency alleles are the result of the c.1055A>G, p.(Asn352Ser) (traditionally named c.1049A>G)) and the c.*96A>G variant and result in 10-15% of normal enzyme activity, which is sufficient to hydrolyze sulfatides and does not cause disease symptoms, even in a homozygous state.[4]

The prosaposin gene (*PSAP*) is an activator protein of ASA, and mutations in this gene, though rare, also lead to MLD.[5] Multiple sulfatase deficiency (MSD) is characterized by deficiency of all sulfatase activities, caused by a mutation in the sulfatase modifying factor 1 (*SUMF1*) gene, that encodes a protein involved in the post translational modification of

sulfatases.[6] The phenotype of MSD is a combination of the different sulfatase defects. The neurodegenerative course of the disease is similar to MLD. In addition, hepatosplenomegaly, short stature, corneal clouding, ichthyosis and skeletal changes are common.[7]

Even between patients sharing the same genotype, there is considerable clinical variability. Still, the amount of residual ASA does correlate with the subtype; homozygosity for 0 alleles is mainly prevalent in the late-infantile type, whereas compound heterozygosity for 0 and R alleles usually causes the juvenile type, and patients with the adult type are mostly carrying two R alleles.[1] The involvement of the peripheral nervous system (PNS) varies depending on the genotype. In patients carrying at least one 0 allele (coinciding with rapid disease progression), the PNS is likely to be involved already at diagnosis, whereas in the adult type PNS involvement occurs late and is mild.[1] Cesani et al described the genotype-phenotype correlation in 432 patients that were published in the international literature up to 2015. They found the late-infantile form to be the most common disease variant (around 48%), followed by the juvenile (23%) and the adult (22%).[2] In our Dutch patient cohort however, the juvenile form is most prevalent (accounting for 61% of diagnosed cases between 2008 and 2017), followed by the late infantile (23%) and adult form (16%).

Pathophysiology of MLD

Sulfatide (3-O-sulfogalactosylceramide) is the most abundant sulfoglycolipid (Figure 1).[5] Sulfoglycolipids form a considerable fraction of glycolipids. These glycosphingolipids form an abundant component of cellular membranes in all eukaryotic cells.[5] Sulfatide is degraded in lysosomes, after a sphingolipid activator protein, saposin B, has extracted it from membranes to make it accessible for arylsulfatase A, which then hydrolyzes the sulfate group.[5] In the nervous system, sulfatides are mostly present in oligodendrocytes and Schwann cells.[5]

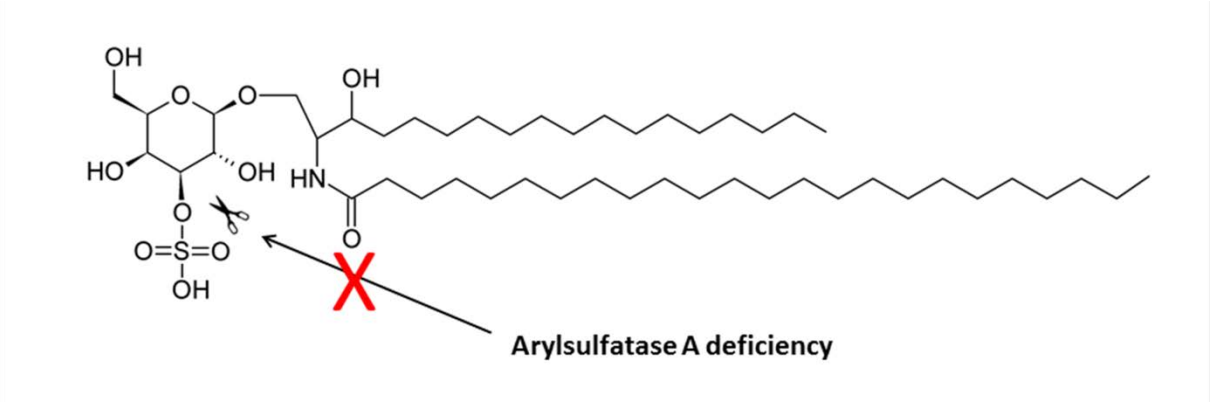

Figure 1. Structure of sulfatide.

Galactosylceramide, the precursor of sulfatide, and sulfatide are the two major glycosphingolipids of the myelin sheath and contribute to the stability, flexibility and compaction of myelin.[3] Sulfatide negatively regulates oligodendrocyte differentiation, has a function in the initiation of myelination and inhibits axonal outgrowth.[5,8] In MLD, the metabolic defect is the failure to catabolize sulfatide.[9] There is no, or insufficient ASA activity, and accumulation of sulfatides takes place in oligodendrocytes, Schwann cells, phagocytes, astrocytes, neurons and macrophages.[5] Why this accumulation leads to a loss of myelin and neuronal degeneration is not completely understood. A possibility is that lysosomes stop functioning because of the massive sulfatide accumulation, ensuing cell death.[10] Also, sulfatide loading triggers inflammatory cytokines,[11] thought to be involved in apoptosis.[12] Microglial activation, invasion of peripheral macrophages and astrogliosis, all indicating inflammation, are found in the CNS of MLD patients. This Ca^{2+} increase leads to activation of intracellular proteases and subsequent injury.[8] The precise trigger for the inflammatory response is not yet understood, but of great importance to fully understand the pathomechanism of the disease. In **chapter 6**, we study the inflammatory response in brain tissue of transplanted and non-transplanted MLD patients.

Pathology of the central nervous system

MLD owes its name to the pathological feature of metachromasia;[13] describing the accumulated sulfatides which are periodic acid-schiff (PAS)-positive (staining blue-violet) and pink or dull red/brown with the toluidine blue stain (Figure 2), in place of the orthochromatic blue staining for the latter.[13] This is caused by the shift of the absorption spectrum of anionic groups in sulfuric acid radicals when present in high concentrations.

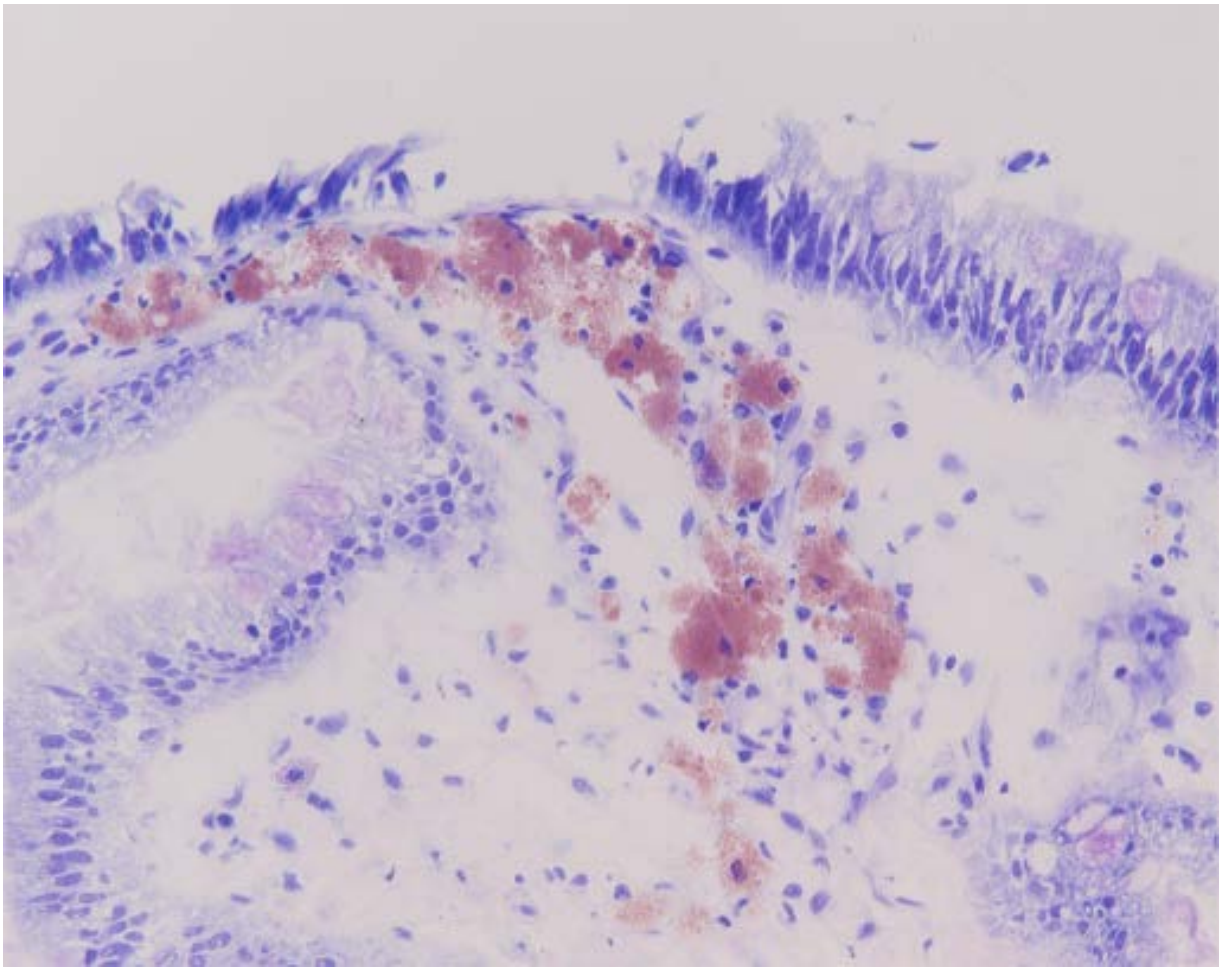

Figure 2. Macrophages are red with the toluidine blue stain indicating that they contain sulfatides; metachromasia.

Macroscopically, the brain of MLD patients appears initially normal, but with progression of the disease cerebral and cerebellar atrophy occurs. There is thinning of the corpus callosum and diffuse sclerosis of the frontal, parietal, temporal and occipital white matter.[14] The size of the thalamus can be markedly reduced. The brain stem and basal ganglia seem macroscopically unaffected.

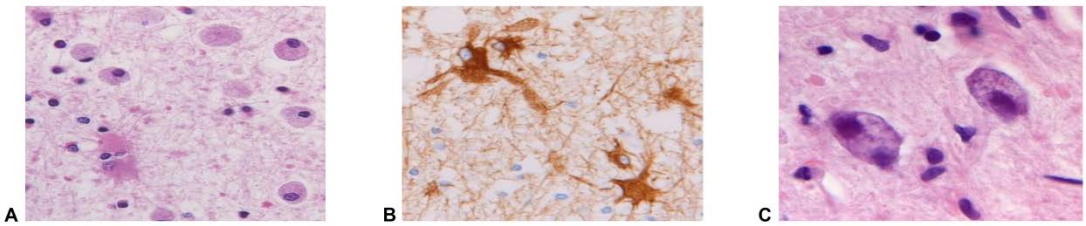

Figure 3. (A) Large, foamy macrophages containing metachromatic granules. (B) shows the storage of sulfatides in astrocytes. In (C) the sulfatide storage in neurons is visible.

Histologically, MLD is characterized by reactive macrophages and astrocytes containing metachromatic granules (Figure 3A).[13] These macrophages are present throughout and at the edges of the lesions. Analytical histopathological studies of the brain of MLD patients showed that the white matter is most severely affected by metachromatic deposits, with a sulfatide content up to 8 times higher than normal, with relatively few gross chemical changes in gray matter.[9] Isolated myelin sheaths with granules as a result of demyelination are found in the corpus callosum, the internal capsule, centrum semiovale and frontal, parietal and temporal white matter. Oligodendroglia are practically absent in demyelinated areas.[14] The U-fibers tend to be spared. The pyramidal tracts are usually early affected, containing numerous metachromatic granules. The sulfatide storage also affects axon density and neurons (Figure 3B,C).[13] Fibrous gliosis arises in the extracellular spaces of the demyelinated cerebral white matter as a result of astrocyte proliferation.[14] These astrocytes contain metachromatic granules. The cortex is usually spared from demyelination and oligodendrocyte loss. However, thinning of myelin, loss of neurons, reactive astrocytes and sulfatide containing granules have occasionally been observed.[15]

MLD has always been seen as a primarily white matter disease. However, since sulfatide is a constituent of membrane lipid rafts, it is logical that its accumulation also affects neurons. The cerebral cortical gray matter (GM) and thalamic volume are already reduced at diagnosis, regardless of age at onset, in MLD patients compared to controls.[16] The neurons of the cerebral and cerebellar cortex hardly ever contain metachromatic materials with exception of the large pyramidal neurons.[17] At the end stage of the disease, especially in the adult onset type, patients suffer from dementia. Interestingly, in these patients the cortical neurons and axons are usually relatively spared, suggesting that the white matter degeneration is mainly contributing to the dementia.

Pathology of the peripheral nervous system

The accumulation of sulfatides in the PNS results in peripheral demyelinating neuropathy; characterized by severe slowing of motor and sensory conduction. Histopathology shows reduced density of large myelinated fibers and accumulation of sulfatides in macrophages and Schwann cells.[8] Disease mechanisms in the CNS and PNS have been hypothesized to be separable processes. The degree of damage to the PNS (as measured by nerve conduction velocity and sural nerve sulfatide levels) does not always correlate with CNS disease manifestations (degree of affected motor function or change in metabolite concentrations such as N-acetylaspartate (NAA) in the white matter measured by quantitative magnetic spectroscopy (MRS)).[18] Interestingly, the level of sulfatides in the cerebral spinal fluid (CSF) does not reflect the extent of central white matter injury, but is proportional to PNS damage.[19] The peripheral nerves are enlarged (and not thinned as a result of atrophy as would be expected in a neurodegenerative disease) on ultrasound in MLD,[20] probably as a result of the accumulation of metachromatic inclusions.

Storage in other organs

The accumulation of sulfatides is not limited to the CNS and PNS, but also occurs in visceral organs, such as the gallbladder [21-23] which is further described in **chapter 9** of this thesis. Other organs affected by the disease are intestines, adrenal glands, lymph nodes and ovaries.[24] The accumulation of sulfatides in the kidney leads to an increased sulfatide excretion in urine in patients, which is further described in the discussion of this thesis.

Clinical spectrum

The three clinical subtypes vary in disease progression and prevailing symptoms. The late-infantile form starts before 30 months of age. First symptoms are usually psychomotor regression resulting in ataxia, weakness and areflexia.[1] The juvenile form starts between 30 months and 16 years and usually presents with a combination of motor regression (due to ataxia, a pyramidal syndrome and peripheral neuropathy), behavioral abnormalities and deterioration in school performance. The adult form starts after the age of 16 years with behavioral and intellectual changes.[11,25] Peripheral neuropathy follows as the disease progresses but remains mild in most cases. Beyond infancy, peripheral neuropathy as presenting sign is uncommon. In general, the earlier the disease onset, the faster it progresses.

In the late-infantile form, language regression usually occurs one year after onset, and complete loss of speech before the age of 3 years.[26] Kehrer and colleagues observed loss of any communication in half of the late-infantile patients 3 years after onset. Complete regression of gross motor function takes on average 15 months after the first signs of motor deterioration.[27]

In juvenile patients, a complete loss of language occurs around 6 years after disease onset in most patients, and complete loss of any communication nine years after onset in a quarter of patients. The deterioration of gross motor function has a similar pace; the loss of complete

motor function takes approximately 6 years after first signs of regression of motor function.[27] Eventually, all acquired skills are lost and patients die.

In the juvenile and adult form, psychiatric symptoms can precede overt neurological signs, sometimes by years. This makes obtaining a correct diagnosis challenging, which we further describe in **chapter 3.**

Diagnosis

Diagnosis of MLD is made through clinical presentation, brain MRI, measurement of ASA activity in leukocytes and sulfatides levels in urine, and *ARSA* mutation analysis. Brain abnormalities seen on MRI are bilateral symmetric abnormal hyperintense T2 signal changes starting in corpus callosum and subsequently involving the periventricular white matter.[28] Depending on the age of onset, the white matter abnormalities either start in the splenium of the corpus callosum and the parieto-occipital white matter (late-infantile form) or in the genu and the frontal white matter (adult form) (Figure 4A,C).

A pattern of radially oriented stripes of low signal intensity throughout the diffuse high signal intensity on T2-weighted images is typical for MLD and represents a combination of storage material and better preserved myelin (Figure 4B,D).[29] As the disease progresses, the subcortical white matter (U-fibers) become involved as well. With disease progression, the entire white matter becomes affected, and cerebral atrophy occurs with enlargement of the ventricles, eventually also cerebellar atrophy and demyelination.[30] The basal ganglia, especially the pallidum, and thalami usually have a decreased T2 signal intensity in this late disease stage, probably as a result of sulfatide accumulation.[31] Gröschel and colleagues correlated regression of cognitive function to more pronounced involvement of frontal WM areas in juvenile MLD patients, sparing the central motor parts of the WM. In certain patients, they found frontal demyelination to appear independently without impairment of the central region, accompanied by a relatively preserved motor function.[32]

Eichler et al developed an MRI scoring system specifically for MLD.[31] Brain abnormalities on MRI can be scored based on their extent and intensity of abnormal white matter signal. Cerebral and cerebellar atrophy is also taken into account. The total amount of points gives an estimation of disease severity; categorized as either mild, moderate or severe.

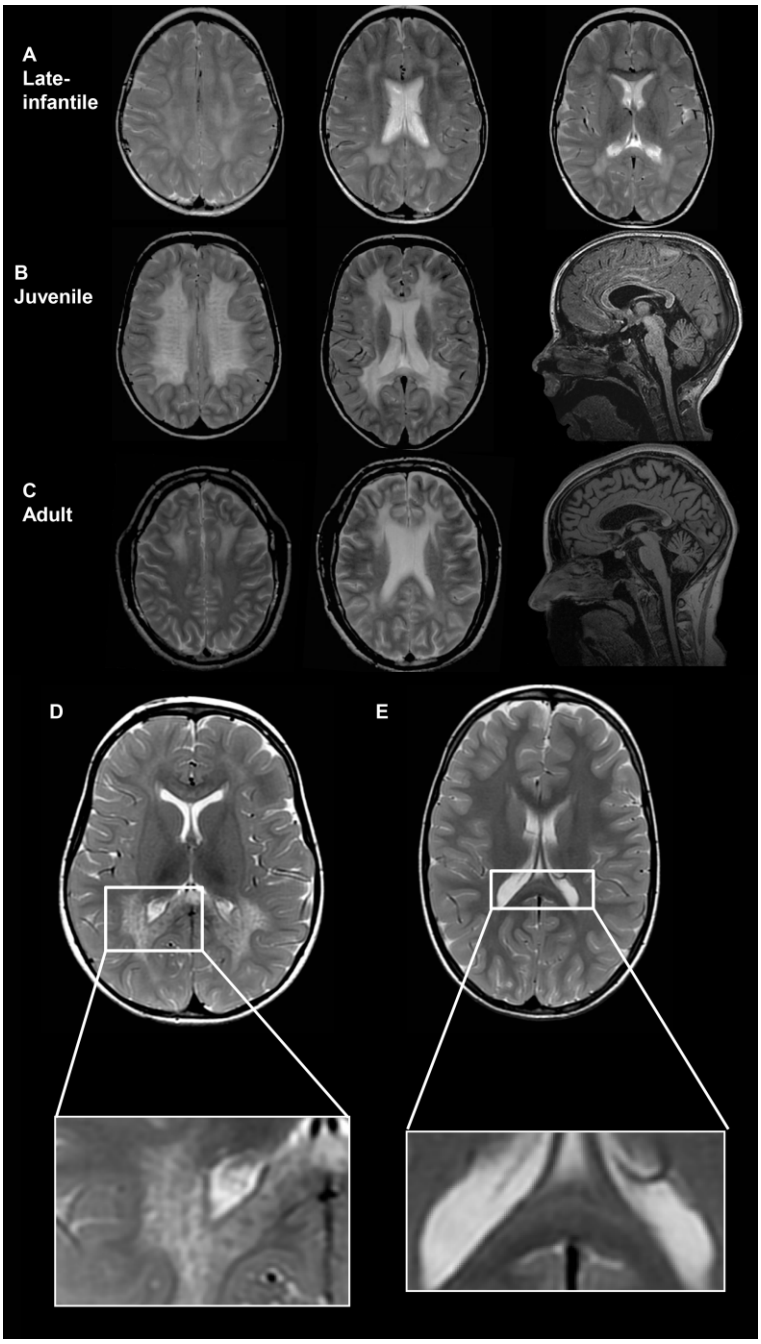

Figure 4. Axial T2-weighted and sagittal T1 weighted MR images of patients with late-infantile (A), juvenile (B) and adult (C) MLD. (A) shows parietooccipital predominance, involvement of the splenium and the periventricular white matter. In (B) the typical pattern of radiating stripes with bands of normal signal intensity in between is shown. This aspect is highlighted in (D). In the adult patient (C) there is frontal predominance and cerebellar

atrophy. In (E) involvement of the splenium is shown in a very early disease stage in a presymptomatic juvenile MLD patient.

Quantitative MRI

Quantitative MRI techniques can provide additional information about the physiology and pathophysiology of brain tissue next to the anatomical information provided by MRI. MRS is a technique providing chemical information on certain metabolites in the brain.[33] In MLD, spectra are characterized by decreased NAA, elevated myo-inositol (Ins) and choline-containing compounds (Cho) in the abnormal white matter.[34,35] We describe the relationship between clinical outcome of patients and their baseline white matter spectra in **chapter 7**. Diffusion tensor imaging (DTI) is a MRI technique that quantifies the direction and magnitude of water diffusion in the brain.[36] There are different diffusion parameters describing different degrees of displacement of water molecules. Fractional anisotropy (FA) and mean diffusivity (MD) are a combined measure of axial diffusivity (AD) and radial diffusivity (RD). Since the displacement of water molecules is impeded by cellular microstructures surrounding it, pathological processes such as demyelination, loss of axonal integrity and inflammatory processes can modulate the direction of diffusion. In **chapter 8**, we study DTI parameters in MLD patients in order to gain insight into the organization and structure of brain tissue and how this is affected by the disease.

Treatment

Enzyme replacement therapy

Animal models of intrathecal continuous infusion of ASA showed reversal of sulfatide storage, suggesting that this may be efficacious for the treatment of MLD.[37] However, the rapid demyelination that occurs in the early onset forms is unlikely to be sufficiently halted by ERT. Intravenous administration of the enzyme has been found not to be effective due to the inability to cross the blood brain barrier. Currently, the IDEAMLD phase I/II clinical trial

(NCT01510028) uses multiple intrathecal infusions with recombinant ASA for patients with the late-infantile subtype. The trial has been completed in January 2017, preliminary results are expected soon.

Hematopoietic Cell Transplantation

The theory behind hematopoietic cell transplantation (HCT) is that monocytic cells of bone marrow or umbilical cord blood cells (from a donor) cross the blood brain barrier and differentiate into macrophages, which start producing ASA to cross-correct the enzyme deficiency. Still, its exact mechanism is not yet fully understood. Prior to the transfusion of donor cells, patients undergo myeloablative chemotherapy which makes it a complicated procedure with the risk of infections and post-transplant complications such as graft versus host disease (GvHD). Treatment related mortality was estimated to be 10-15%, but is nowadays, with less toxic induction protocols, considerably lower, at least in children.[38] PNS involvement does not seem to be influenced by HCT, which hampers motor function in a substantial part of transplanted patients.[12,39] Another important issue is the time it takes for the donor cells to replace resident tissue. This can take 6 to 12 months, whilst disease progression continues. Unfortunately, this makes HCT ineffective for patients with the late-infantile form, because disease progression in these patients is too fast. Presymptomatic juvenile and adult patients are good candidates for HCT. In ambiguous cases, patients with mild symptoms at neurological examination or mild cognitive decline, the decision whether or not to transplant is often difficult. We assessed the efficacy of HCT in our patient cohort in order to formulate decision guidelines for the treatment of HCT, which is described in **chapter 5.**

Hematopoietic Stem Cell-Gene Therapy

It is evident that effective treatment for all clinical subtypes of MLD is needed. Recently, HCT and gene therapy have been combined into lentiviral vector-mediated hematopoietic stem cell gene therapy (HSC-GT).[40] Bone marrow-derived CD34+ HSCs are transduced with a

clinical grade lentiviral vector, which introduces a functional *ARSA* gene into the HSCs. This results in supraphysiological expression of the *ARSA* gene throughout the HSCs. After myeloablative conditioning, the HSCs are infused and thought to mediate cross correction of CNS and PNS resident cells.[40] The corrected cells secrete *ARSA* protein which is endocytosed by neighboring cells for therapeutic correction.[3] It is therefore not necessary to transfer the normal *ARSA* cDNA to all of the cells in the CNS.[3] There were concerns for an oncogenic potential of lentiviral vectors and possible negative effects of supraphysiological expression of *ARSA*, but animal and clinical studies so far report no increased incidence of neoplasms.[3] Longer follow up studies are needed to gain insight on long-term effects of supraphysiological levels of ASA enzyme on the other sulfatases and sulfatide levels.

Scope of this thesis

This thesis provides a broad overview of MLD, by studying both its natural course and its course after treatment. In **chapter 2**, the disease and its current treatment options are reviewed. **Chapter 3** presents late juvenile and adult cases, initially diagnosed with a psychiatric disorder. In **chapter 4**, we describe the effect of intrathecal baclofen treatment for spasticity in MLD. The efficacy of HCT compared to the natural course of the disease is analyzed in **chapter 5**. In **chapter 6**, the inflammatory response and oligodendrocyte numbers in brain tissue between transplanted and non-transplanted patients is compared. In **chapter 7** we focus on the possible value of MRS parameters at diagnosis in predicting eventual clinical outcome. In **chapter 8**, we use DTI parameters to gain more insight into brain microstructure abnormalities in MLD. The association between gallbladder polyps and carcinoma and MLD is presented in **chapter 9**. A summary of these findings, their possible implications and future perspectives are discussed in **chapter 10.**

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I

Clinical aspects

Chapter 2

Metachromatic Leukodystrophy: disease spectrum and approaches for treatment

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Abstract

Metachromatic leukodystrophy is an inherited lysosomal disorder caused by recessive mutations in *ARSA* encoding arylsulfatase A. Low activity of arylsulfatase A results in the accumulation of sulfatides in the central and peripheral nervous system leading to demyelination. The disease is classified in a late-infantile, juvenile and adult onset type based on the age of onset, all characterized by a variety of neurological symptoms, which eventually lead to death if untreated. There is no curative treatment for all types and stages. This review discusses diagnostic process and efficacy of current and possible future therapies such as hematopoietic stem cell transplantation, enzyme replacement therapy and gene therapy. A systematic evaluation regarding the efficacy of hematopoietic stem cell transplantation and a longer follow-up period for gene therapy are needed to come to a general conclusion and improve treatment options for metachromatic leukodystrophy.

Introduction

In this review, the pathology, diagnosis and possible treatment of metachromatic leukodystrophy MLD (250100), a rare disorder with an estimated birth prevalence of 1.4-1.8 per 100.000,[1] is described. At present no curative treatment is available for all types of MLD. This is an emerging field in which several clinical trials looking for a possible cure for this devastating disease are ongoing. Recently published data on patient care and treatment are discussed.

Metachromatic leukodystrophy (MLD) is an autosomal recessive inherited lysosomal disorder caused by mutations in the *ARSA* gene located on chromosome 22q13.33, resulting in a deficiency of the enzyme arylsulfatase A (ASA). Some mutations result in pseudodeficiency alleles[2] that result in 10-15% of normal enzyme activity, which is sufficient to physiologically hydrolyze sulfatides and does not lead to disease symptoms.[2] This implicates that sulfatide degradation can function normally in the presence of only 10-15% functional ASA enzyme; which is an important consideration for the development of treatment options for MLD. Mutations in *PSAP,* encoding prosaposin, an activator protein of ASA, also lead to MLD (249900), but are rare.[3] In multiple sulfatase deficiency (272200), caused by mutations in the sulfatase-modifying factor-1 gene (*SUMF1*)[4], the function of the whole family of sulfatase enzymes is affected, leading to symptoms of MLD in addition to features of various mucopolysaccharidoses.[5]

ASA is essential for sulfatide metabolism through the hydrolysis of the 3-O ester bond of galactosyl and lactosyl sulfatides.[1] Its deficiency results in the accumulation of sulfatides into lysosomal storage deposits in the central and peripheral nervous system, which exhibit accumulation of sulfatides and metachromatic staining characteristics.[6] In the nervous system, sulfatides accumulates in the oligodendrocytes, Schwann cells, phagocytes, astrocytes and also neurons (figure 1).[3] Sulfatides are the most abundant sphingolipids in myelin, accounting for 4% of its composition. They have important functions in the

maintenance of myelin.[2] Their accumulation leads to demyelination. It has been shown in vitro that sulfatide loading triggers inflammatory cytokines, which are thought to be involved in apoptosis.[7]

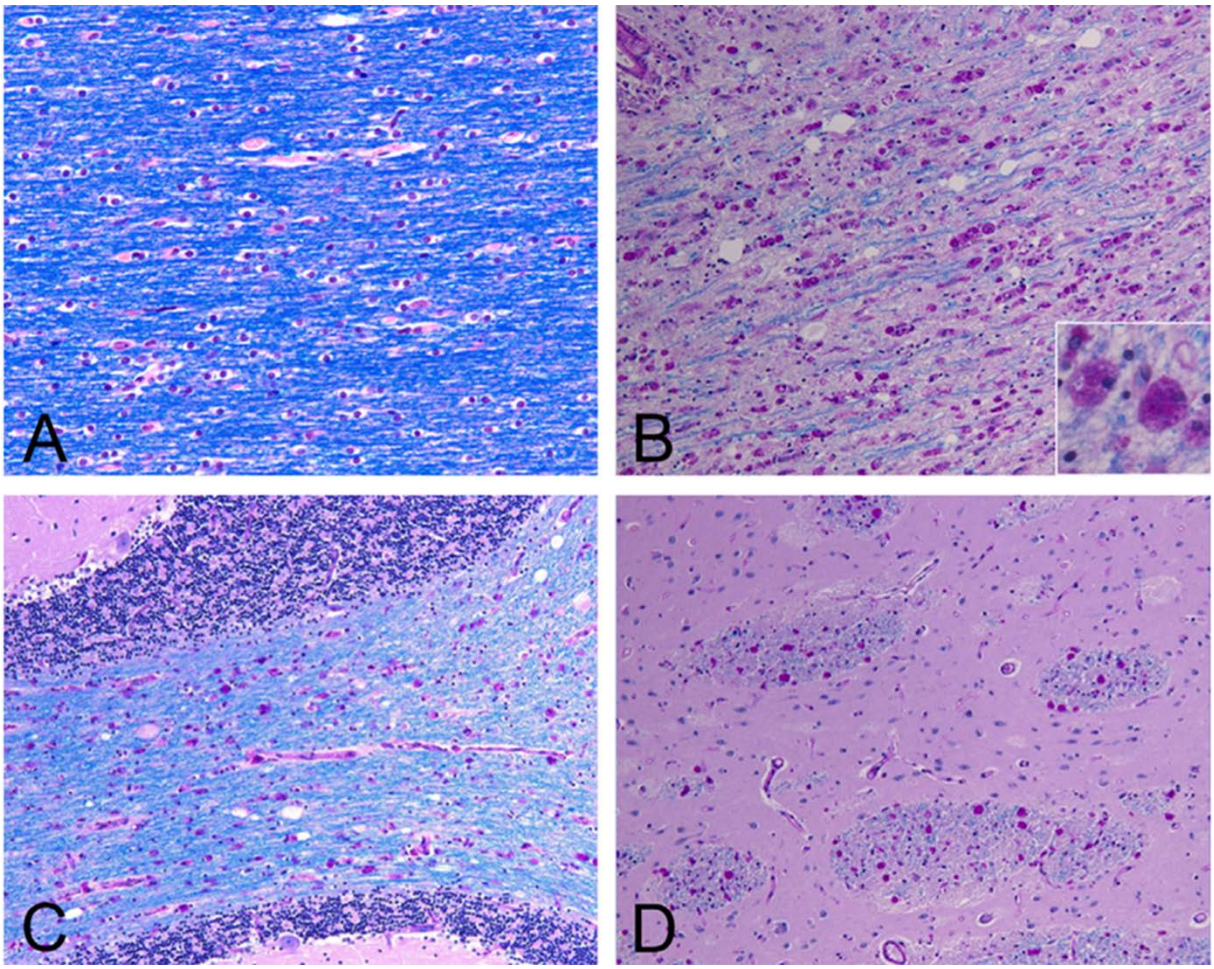

Figure 1: Klüver-PAS staining of white matter in a control (A, 200x) and a patient with MLD (B-C, 100x). (B) demonstrates loss of the normally blue-stained myelin and enlarged macrophages accumulating sulfatides (see also inlay). (C) demonstrates relatively spared white matter in the cerebellum. (D) shows pencil fibers in the basal nuclei, again with myelin loss and storage cells, within relatively spared grey matter.

Clinical presentation

Metachromatic Leukodystrophy is divided into three clinical subtypes, based on the age of onset of the first symptoms. These can be deterioration in motor or cognitive function or behavioral problems, depending on the clinical subtype. The disease inevitably ends in a decerebrated state and eventually death. Its course and duration are however highly variable, depending on the age of onset of the first symptoms.

The late infantile form has its onset before 30 months and is characterized by rapid progression of psychomotor regression resulting in ataxia and weakness with areflexia.[1] Some children have only signs of a progressive peripheral neuropathy during several months, before central nervous system involvement becomes apparent. As the disease progresses patients start suffering from dysphagia and drooling and feeding via gastrostomy usually becomes necessary. Seizures are common. Painful spasms and general irritability are particularly challenging. Death occurs within a few years after the onset of symptoms. The late infantile form is genetically characterized by homozygosity or compound heterozygosity for alleles that do not allow the synthesis of functional enzyme, resulting in rapid accumulation of sulfatides and rapid disease progression.[8]

In the juvenile variant, symptoms start between 2.5 and 16 years of age. The disease often begins with deterioration of school performance or behavior abnormalities. The first neurological signs are often ataxia and a mild pyramidal syndrome, leading to gait problems. Peripheral nerve damage may result in reduced deep tendon reflexes.[9] In the beginning, disease progression is slower than in the infantile onset form, but once the neurological signs become more evident, the decline is rapid and patients eventually lose all skills.[10] Spasticity becomes prominent, and many patients also develop epilepsy. The end stage of the disease can last several years, and its duration is variable. Patients suffering from the juvenile type mostly carry one allele that allows for expression of low amounts of residual enzyme activity.[8]

The adult variant has an insidious onset after the age of 16 years. Intellectual and behavioral changes, such as memory deficits or emotional instability, are usually the first presenting symptoms.[9] Mild polyneuropathy develops in a later stage. Disease progression is generally slower than in the infantile and juvenile form. Death occurs within decades after disease onset. In the adult onset type, many patients carry two mild mutations, allowing for the expression of low amounts of functional enzyme, which delays the process of sulfatide accumulation and thereby the onset of the disease.[8] As more siblings in one family can be

affected, it is important to test all siblings of the index patient for MLD regardless of their age as the age of disease onset can vary, especially for the juvenile or adult type.

Non-neurological symptoms: Apart from the neurological symptoms the accumulation of sulfatides can also cause symptoms in visceral organs. Gallbladder involvement is seen most often, leading to symptoms such as thickening of the gallbladder wall, gallstones, cholecystitis, and a small or enlarged gallbladder.[11] The diagnosis of MLD usually precedes the onset of gallbladder involvement, although cases have been reported in which gallbladder polyposis is the first symptom prompting the diagnosis.[11] Relatively little is known about gallbladder involvement in long term surviving MLD patients. Other organs that can be affected are liver, pancreas, intestines and kidneys. Case reports have also been written about disease expression in the adrenal glands, lymph nodes and ovaries.[12]

Diagnosis

Magnetic Resonance Imaging: An important tool in establishing the diagnosis of MLD is MRI, which shows characteristic brain abnormalities. Demyelination in MLD leads to bilateral symmetric abnormal T2 signal hyperintensity starting in the corpus callosum and then involving the periventricular white matter. In the infantile form, the disease usually starts in the splenium of the corpus callosum and the parietooccipital white matter, in the adult form, in the rostrum and frontal white matter (figure 2). The subcortical fibers are usually spared.[13] In severe disease there is often involvement of the projection fibers, cerebellar white matter, basal ganglia and thalami which have a decreased signal intensity on T2-weighted images, probably as a result of accumulation of metal or other breakdown products in the brain.[14] Typical for MLD is a pattern of radiating stripes with bands of normal signal intensity within the abnormal white matter,[15] the so called “tigroid-pattern”. This is also seen in globoid cell leukodystrophy (Krabbe’s disease) and infantile GM1 gangliosidosis. Histopathological techniques confirmed that the stripes are related to perivascular preservation of myelin.[15]

Eichler et al[14] developed a scoring system for the MRI abnormalities in MLD which can, when combined with clinical parameters, be used as a measure of disease severity. It takes into account extent and severity of abnormal white matter signal, involvement of projection fibers and basal ganglia and atrophy.[14] Staging (mild, moderate or severe) is based on the total

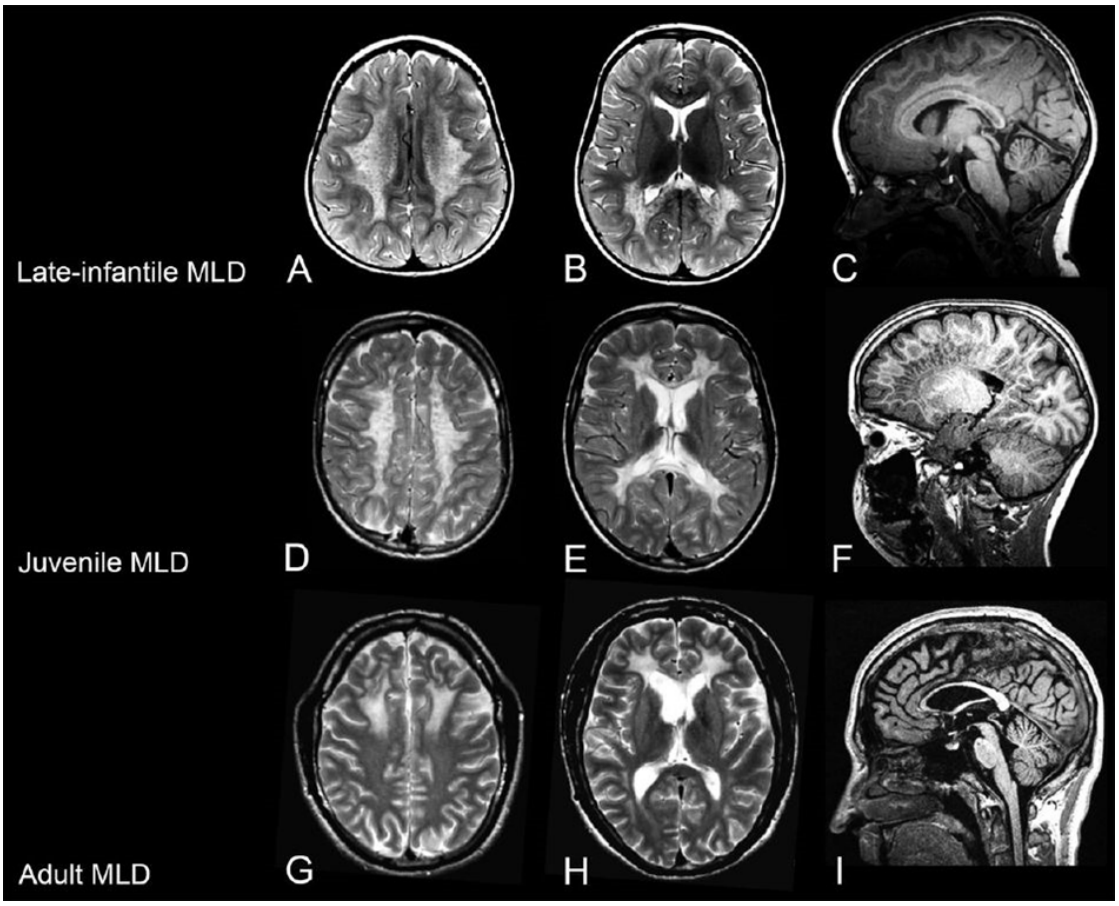

Figure 2: Axial T2-weighted (A, B, D, E, G, H) and sagittal T1-weighted (C, F, I) MR images of three patients with MLD. (A-C): 2-year-old patient with late-infantile MLD. Involvement of the periventricular white matter and centrum semiovale with parietooccipital predominance and involvement of the splenium. U fibers are spared. (D-F): 7-year-old patient with juvenile MLD. (F) shows the typical pattern of radiating stripes with bands of normal signal intensity in between. U fibers are spared. (G-I): 28-year-old patient with adult MLD. In addition to the white matter signal abnormalities with frontal predominance, there is mild supratentorial atrophy (G, H).

amount of points. The amount of demyelinated white matter can also be quantified as demyelination load and is correlated with disease duration and deterioration of gross motor function.[16] Proton magnetic resonance spectroscopy (^{1}H-MRS) can be used to gain insight into chemical information in addition to the anatomic information provided by MRI.[17] In MLD, ^{1}H-MRS is characterized by a low N- acetylasparate (NAA) level and elevated myo-inositol (figure 3).[17] The low NAA level can be explained by the diffuse neuronal loss that is seen in

MLD. The high myo-inositol level has been attributed to reactive gliosis, characteristic for MLD.[18] Assadi et al[18] furthermore found an increased lactate to creatine ratio, which is likely due to oligodendrocyte injury limiting lactate transport into axons.

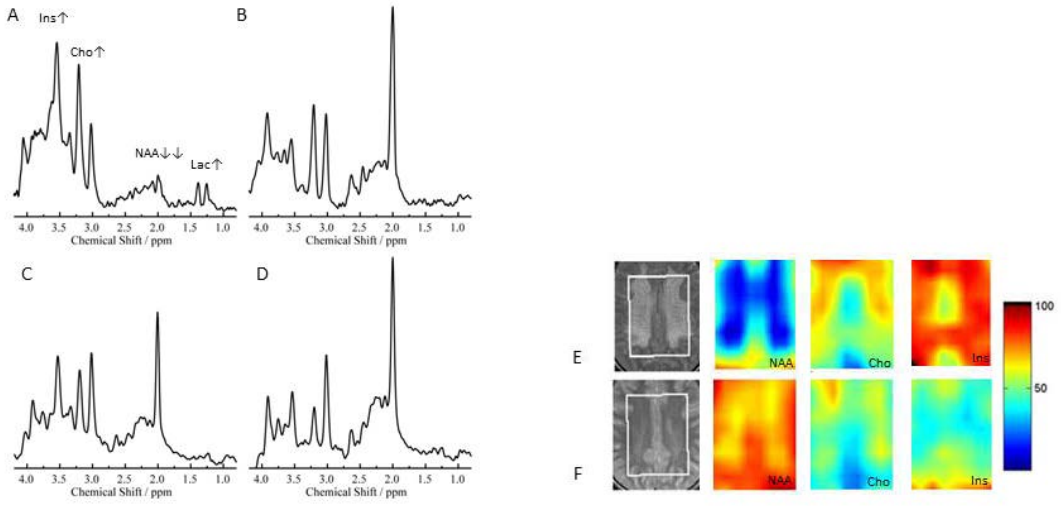

Figure 3: ^{1}H-MRS of frontal and parietal white (A) and grey (C) matter in an 8-year-old MLD patient. ^{1}H-MRS of frontal and parietal white (B) and grey (D) matter in a healthy 9-year-old control. The color maps are obtained from the same patient (E) and control (F) from the frontal and parietal central white matter and represent the concentrations of N-acetylasparate, choline and myo-inositol. Decrease of NAA and elevation of myo-inositol is typical for MLD.

Biochemical and genetic diagnosis: Diagnosing MLD consists of a combination of biochemical procedures and genetic analysis.[1] The biochemical procedure consists of measuring ASA enzymatic activity in leucocytes from whole blood.[19] Sulfatide excretion in urine can be measured when the levels of ASA enzymatic activity are normal in a child with typical clinical presentation and MRI and also when there is doubt about pseudodeficiency.

Mutation analysis is becoming an increasingly important tool in diagnosing MLD. Over 150 mutations have been reported in the *ARSA* gene. Two mutations occur more frequently; one is the 459+1G>A splice-site mutation which is associated with late-infantile onset, the other one is the p.Pro426Leu missense mutation frequently found in the adult form.[19]

Pitfalls in diagnosis:

- Clinical pitfalls: In the initial phase of late-infantile MLD, symptoms can be similar to those found in Guillain-Barre syndrome or chronic inflammatory demyelinating polyneuropathy (CIDP).[20] The findings of reduced motor nerve conduction velocities and increased protein concentration in cerebrospinal fluid together with progressive gait abnormalities and hyperirritability can result in a wrong diagnosis, which is only revised when central nervous system signs as spasticity develop.[20] Odd behavior, depression or psychotic symptoms in adult patients are often wrongly attributed to a primary psychiatric disorder, as neurological signs usually appear later.
- Low ASA activity: the diagnosis of MLD cannot exclusively be based on the level of ASA activity, due to the presence of pseudodeficiency alleles in the population. In the case of pseudodeficiency alleles, ASA activity is low, which could be mistaken for MLD whilst the pseudodeficiency alleles do not lead to symptoms. Sulfatide excretion is normal in pseudodeficiency and can help with distinguishing the two, as well as mutation analysis. This is important in families with MLD and carrying pseudodeficiency alleles.
- Normal ASA activity: Patients with saposin B deficiency do suffer from MLD but have an *in vitro* ASA activity in the normal range.[19] In these cases, the measurement of sulfatide excretion in urine is helpful as it is elevated in saposin B deficiency. Molecular analysis of the *PSAP* gene can confirm the diagnosis. *In vitro* ASA activity is normal in these cases because it is performed in an assay with a water soluble artificial substrate, in which the hydrolysis does not depend on the presence of saposin B.[9]

Current treatment

At present, there is no curative treatment available for all patients with MLD. Hematopoietic stem cell transplantation (HSCT), gene therapy and enzyme replacement therapy have been extensively tested in mouse models. The positive results reported from the different animal

studies regarding HSCT, gene therapy and enzyme therapy have led to clinical trials investigating the efficacy of these approaches.

Hematopoietic stem cell transplantation (HSCT): Monocytic cells of bone marrow are able to cross the blood brain barrier, differentiate into microglial cells and deliver enzymes to oligodendrocytes and neurons to correct the enzyme deficiency. This promising procedure is at this moment the only treatment, which has proven to be able to stop the disease. One of the main problems regarding HSCT is the slow replacement of resident tissue compared to rapid progression of the disease. It can take 12 to 24 months until the disease stabilizes, which makes HSCT ineffective for patients with overt neurological symptoms or for those with the aggressive infantile onset type.[1] In these patients, who are already symptomatic at the time of transplantation, neurologic involvement continues to deteriorate.[21] Even in asymptomatic patients with the infantile onset type, neurological deterioration and progression of white matter abnormalities on MRI were reported,[22] suggesting that disease progression is too fast for HSCT to influence. When HSCT was performed in patients suffering from the juvenile and adult form of MLD, both cerebral demyelination as well as disease progression have been reported to be delayed or stopped.[23-25] Even improvements in motor and behavioral functions have been reported, together with a decrease of the white matter abnormalities seen on MRI.[26-28] However, also cases, in which the disease takes the natural course or even worsens, have been reported. Smith et al[29] report a case of a symptomatic patient with adult onset MLD in which HSCT does not halt disease progression and results in relentless cognitive decline. Hosson et al [30] describe 5 symptomatic patients with adult onset MLD who were treated with HSCT. Stabilization of the disease occurred only in one of the five patients who later also experienced disease progression. Levels of donor chimerism (and achieved enzyme level) are important, as mixed chimerism will dilute the post- HSCT enzyme level. In other lysosomal storage diseases, such as mucopolysaccaridosis(MPS) 1, the enzyme level achieved after HSCT is associated with long-term outcome, including development.[31,32]

Mesenchymal stem cells (MSC) are non-hematopoietic multipotent stem cell-like cells that are capable of differentiating into both mesenchymal and non-mesenchymal lineages. They have been found to be able to differentiate into neurons and astrocytes. Because of these capabilities, concomitant infusion of MSC with HSCT has been performed for a symptomatic patient with adult onset MLD by Meuleman et al.[23] She had a complete stabilization of her disease during the 40-month follow up period.

Apart from the uncertain long-term effects, HSCT is furthermore complicated by substantial risks of the procedure and post-transplant complications such as graft versus host disease (GvHD) and infections. Mortality is estimated to be 10 to 15%, improving over time. Another limitation of HSCT is the fact that the involvement of the peripheral nervous system does not seems to be influenced, causing severe motor impairment in a substantial part of transplanted patients.[7]

Umbilical cord blood transplantation (UBCT) is an alternative for bone marrow transplantation with the advantage of quicker availability, less GvHD, lower risk of morbidity and mortality and better correction of enzymatic deficiency.[24] Furthermore, UCBT is associated with higher rates of full-donor chimerism compared to mixed chimerism in HSCT or sibling transplantation.[31] Martin et al [21] report a study of 27 children with both infantile and juvenile onset treated with UCBT. In asymptomatic patients at the time of transplantation, UCBT resulted in successful replacement of the missing enzyme and disease stabilization. In the group of children with moderate to severe symptoms (N= 19), 7 children died. The surviving children of the symptomatic group did not benefit from UCBT. Cable et al [33] report a 5-year follow up of three affected siblings with juvenile MLD after an UCBT. The patient who was symptomatic at the time of the transplant deteriorated and now remains in a vegetative state. The other two patients, who were asymptomatic at the time of transplant stabilized within a year after UCBT and have remained stable regarding neurological examination, neuroimaging, nerve conduction studies and neuropsychological evaluations.

Presymptomatic patients with the juvenile and adult form are good candidates for HSCT. Late-infantile patients often deteriorate, even if transplantation has been performed before the first symptoms.[22] In mildly symptomatic patients, the decision whether or not to perform HSCT is difficult. Better knowledge of the natural history of MLD will help in predicting clinical course in individual patients. One approach is by Kehrer et al [34] who modified the Gross Motor Function Classification (GMFC) system for MLD, which can be used as a robust and easy to use classification system to evaluate gross motor function from 18 months onwards. Both late infantile as well as juvenile patients who have just lost the independency of unsupported walking have a probability of more than 60% to have no locomotion or sitting without support within one year.[34] This is an important criterion in the decision process of whether to perform a HSCT or not, since it takes around 6-18 months before the donor cells become functional.

Although the first approach of using HSCT as a treatment for MLD was published in 1985 (Bayever, Lancet 1985), we are still short of a systematic analysis of its effectiveness of HSCT. Such a study is further complicated by the different protocols that are being used for HSCT worldwide. Therefore, no standard decision criteria exist for the utilization of HSCT. To develop these, we need a systematic overview of the long-term effects and outcome of HSCT in a large sample of patients.

Enzyme replacement therapy: The rationale for enzyme replacement therapy (ERT) is that extracellular lysosomal enzymes are taken up by cells and transported by endocytic receptors to the lysosome where they become active.[35] Traditionally, ERT is done by intravenous injection of an enzyme and has shown to be effective for several lysosomal disorders without involvement of the CNS such as Gaucher disease type 1, Fabry disease, mucopolysaccharidosis type I, II and VI and Pompe disease.[35]

Animal studies

The concept of enzyme replacement therapy has been proven to be effective in improving the function of the nervous system in several preclinical studies. An ASA deficient knock out mouse model has been generated, which indeed accumulated sulfatides. However, these mice did not develop demyelination, which was thought to be due to an insufficient buildup of sulfatides.[6] Transgenic mice were generated and crossed with ASA deficient mice resulting in an aggravated disease phenotype. In these mice, sulfatides were increased about two to threefold and demyelination in both peripheral and central nervous system was seen.[8]

Matzner et al [36] were the first to provide proof of principle of enzyme replacement therapy when they reported a decline of sulfatide storage in kidney and peripheral nerves following intravenous injection of rhASA in ASA knockout mice. Furthermore, a reduction of storage in the central nervous system was noted, which could not clearly be explained since the brain did not acquire enzyme levels of more than 0.1% of wild type levels due to the impermeability of the blood brain barrier. Stroobants et al [35] reported a preclinical experiment in which the blood brain barrier of mice was bypassed by continuous infusion of rhASA into the brain ventricles using miniature osmotic pumps. They found an improvement in nervous system function and reported no adverse immunological effects. Furthermore, a reduction in sulfatide storage in the brain and spinal cord of mice was observed following intrathecal ERT.[6] This suggests that rhASA is able to cross brain capillaries in a cell culture model of the blood brain barrier. The hydrolyzation of the storage material, however, seemed to be inversely dependent on the amount of accumulated sulfatides.

Table 1. Outcome after treatment

Study	Number of patients	Infantile MLD	Juvenile MLD	Adult MLD	Presymptomatic	Symptomatic	Type of intervention	Clinical Outcome	MRI	Survival	Follow up
van Egmond (2013)	1		1			1	UCBT	Improving motor+ behavioral functions, stable cognition	Improvement white matter abnormalities	1/1	27 months
Krageloh-Mann (2012)	1		1			1	HSCT	Stable cognitive function, progression neuropathy	16m post HSCT: increase white matter abnormalities, 24 months post: scores similar to pre HSCT	1/1	10 years
Hosson (2011)	5			5		5	HSCT	Continious deterioration (*N*=4) and stabilization (*N*=1)	Stable (*N*=1). Progression white matter abnormalities (*N*=4).	4/5	9 years
Ding (2011)	1		1		1		HSCT	No MLD symptoms	Halt of demyelinization + progress of myelination	1/1	8 years
Smith (2010)	1			1		1	HSCT	Cognitive decline	Persistent abnormalities+ progressive volume loss	1/1	11 years
Meuleman (2008)	1			1		1	HSCT with MSC infusion	Stabilization of neurological symptoms	Stable cerebral lesions	1/1	40 months
Bredius (2007)	1	1			1		HSCT	Continious deterioration	Progression of white matter abnormalities	1/1	2 years
Martin (2013)	27	10	17		8	19	UCBT	Asymptomatic children:stabilization. Symptomatic:deterioration	Improvement in LOES scores in 16/19 patients	20/27	5.1 years
Cable (2011)	3		3		2	1	UCBT	Symptomatic child:worsening. Other 2:stabilization	Progression of white matter abnormalities (*N*=1). Resolvement of white matter abnormalities 1 year post HSCT (*N*=2)	3/3	5 years
Biffi (2013)	3	3			3		HSC gene therapy	Halt of disease manifestation	Stable small area of hyperintensity *(N*=2).Normal myelination progression (*N*=1).	3/3	18-24 months

Clinical studies

In MLD, ERT has been administered intravenously but was found not to be effective, due to the inability of the enzyme to cross the blood brain barrier and thereby the inability to reach the nervous tissue (unpublished data). Different routes of administration, such as intracerebral agent delivery, are currently being tested in clinical trials to overcome this limitation. (http://www.clinicaltrial.gov/NCT01510028). The patients receive intracerebral injections of recombinant human arylsulfatase A every other week for 40 weeks. This trial is open for patients with the late infantile form who are still able to ambulate with help. In this selected group, the effectiveness of the treatment can be evaluated quickly due to the rapid progression of the disease. Moreover, no other treatment option is available for this group of patients. Even if the enzyme reaches the brain, this remains an intensive procedure with the risk of complications.

Gene therapy: In gene therapy, the goal is to genetically modify autologous hematopoietic stem cells (HSC) to express the *ARSA* gene.[1] Cells can also be modified to overexpress *ARSA* leading to a supraphysiological amount of enzyme.

Animal studies

This method has successfully been tested in experiments using the knock out animal model of MLD.[24] Through the use of retroviruses new genes can be integrated into the host cell genome. Various viral vectors, such as adeno-associated viral (AAV), lentiviral (LV) and retroviral (RV) vectors, have been used and have been found suitable for clinical trials of gene therapy.[24] In MLD, lentiviral vectors were successful in generating overexpression of the *ARSA* gene.[2] Efficient gene marking of mouse and human HSC is possible, with full maintenance of stem cell properties and transgene expression.[1] Biffi et al [37] transplanted HSCs transduced with a lentiviral vector carrying the *ARSA* cDNA in MLD mice. They report that enzyme activity was reconstituted in the hematopoietic system and development of central nervous system and peripheral nervous system disease manifestations was

prevented and corrected. Another approach is introducing the normal gene directly in the CNS. Colle et al [38] demonstrate the safety of intracerebral injection of AAV2-5 vector encoding human *ARSA,* which results in expression and activity of recombinant ASA enzyme in the brain of non-human primates. Piguet et al [39] compared the intracerebral injection of AAVrh.10cu *ARSA* vector with AAV5-PGK-*ARSA* vector and found the AAVrh.10vector to both result in a more robust and diffuse expression of ASA enzyme as well as in a correction of sulfatide accumulation in brain.

Clinical studies

Biffi et al [40] performed a phase I/II clinical trial in which they treated three presymptomatic infantile MLD patients with HSC-gene therapy. The autologous HSCs were transduced ex vivo with *ARSA* encoding LVs and reinfused after the patients had been treated with a myeloablative regimen. One year after HSC-GT, functional ASA was isolated from cerebrospinal fluid from all three patients; levels and activity were comparable to healthy donors. Moreover, disease manifestation was halted for the follow up times, ranging from 18-24 months. This was based on findings on MRI and evaluation of cognitive and motor skills. No evidence for activation of a nearby oncogene by an insertion was found. An advantage of this autologous gene-transduced HSCT is that supra –normal levels of the enzyme can be reached. In the future, better predictable gene-transduction efficacy and better predictable engraftment of transduced cells may further optimize treatment. At the moment a clinical trial of intracerebral gene therapy is performed in Paris by Aubourg et al. AAVhr.10 is used to transfer the *ARSA* cDNA coding for the ASA enzyme. Patients with an early onset MLD type are eligible, aged between 6 months and 4 years. The interval between the first symptoms and inclusion must be 12 months or less. Advantages of these approaches, when compared to allogeneic HSCT, are less transplant related morbidity and no risk of GVHD,[40] limitations the uncertainty about the risk of mutagenesis of cancer.[2] When vectors are integrated genome wide, they risk being integrated in the vicinity of proto-oncogenes, triggering their expression and thereby neoplasia. Another point of caution is the possible effect that the

overexpression of the ASA enzyme may have on the other sulfatases and the closely regulated sulfatide levels.[37]

Table 1 provides a summary of the results of clinical studies regarding HSCT and HSC gene therapy.

Other forms of therapy

Small molecule based therapies: Small molecules can cross the blood brain barrier.[2] Specific small molecules are able to rescue misfolded proteins. This could possibly enhance the level of the available mutant ASA, thereby increasing residual ASA enzymatic activity.[2] Pharmacological chaperones (PCs) are small molecules that can enhance the level of the misfolded-prone mutant enzymes. Proteostasis regulators (PRs) are small molecules that improve the protein folding capacity of cells. When these two classes of small molecules are used together, the misfolded enzyme can be guided to a folded state, maintaining its structural ability.[2] Through high throughput screening (HTS) assays the small molecules that function as PCs or PRs can be identified. Small molecules for mutant ASAs have been identified by HTS assays using patient derived cells. This might allow the identification of potential drugs for MLD.[41] Another way in which small molecules can be used is by manipulating downstream pathways that are initiated or disturbed by the enzyme defect; the so called pathogenic cascades.[2]

Warfarin administration: Vitamin K has been shown to play a role in the modulation of sphingolipid synthesis.[18] It is thought to control the rate-limiting step in the production of sphingolipids and the conversion of cerebrosides to sulfatides.[18] Warfarin as a vitamin K antagonist is hypothesized to reduce the formation of sulfatides. This is currently being tested in a clinical trial for which children with MLD, aged 1-10 years, were included who had received and failed a HSCT or were excluded from the treatment (http://clinicaltrials.gov/ct2/show/NCT00683189).

Symptomatic treatment

In patients not eligible for HSCT, treatment should be focused on creating the greatest possible comfort for both patients and parents. Feeding via gastrostomy usually relieves discomfort and struggling and prevents aspiration pneumonia. Painful spasms are common and need to be treated with injection of botulinume toxine or (intrathecal) baclofen. Communication, even when speech is lost, can be assisted through electronically devices. Genetic counseling and psychological support are important for the whole family.

Conclusion

Metachromatic leukodystrophy is a devastating disease for which at present no curative treatment is available for many patients. Despite the use of HSCT for leukodystrophies for decades now, the effectiveness of this treatment is still under debate. In general, HSCT does not seem to be beneficial for patients with overt neurological symptoms or the aggressive late infantile form of MLD. Inconsistent results have been reported for asymptomatic patients. A systematic evaluation of the effectiveness of HSCT is needed in order to be able to decide for which patients HSCT is beneficial. Standardized treatment protocols and longer follow-up will help to come to a conclusion.

Innovative strategies such as gene therapy and enzyme replacement therapy are now being tested in clinical trials. Gene therapy has the potential to produce more effective levels of enzyme by generating autologous hematopoietic cells that overexpress the ARSA gene, than through HSCT. The preliminary results of the first clinical trial regarding this type of therapy are promising. However, further evaluation regarding the safety and long-term effects of this approach are needed. If the current ongoing clinical trials studying enzyme replacement therapy are able to overcome its main limitation and cross the blood brain barrier, this type of therapy would not only be promising for MLD but also for several other lysosomal diseases. A combination of these approaches will hopefully lead to a satisfying treatment of patients suffering from MLD.

Practice Points:

- The diagnosis of MLD cannot be always based on the level of ASA activity, but may need confirmation by mutation analysis or urinary sulfatide excretion due to the possibility of pseudodeficiency.
- If clinical presentation and MRI suggest MLD, but ASA activity is normal, the measurement of sulfatide excretion in urine and mutation analysis of *PSAP* is necessary to come to a definite diagnosis.
- A scoring system for brain abnormalities, in combination with clinical parameters, can be used to measure disease severity.

Research agenda:

- A systematic evaluation of the effectiveness of HSCT
- Longer follow up on the preliminary results of gene therapy and intracerebral enzyme replacement therapy

Summary

MLD is a severe storage disease caused by deficiency of the lysosomal enzyme arylsulfatase A, resulting in accumulation of sulfatides in the central and peripheral nervous system. A late-infantile, juvenile and adult onset type are distinguished based on the age of onset of the disease. The diagnosis of MLD is established through MRI and the detection of levels of ASA enzymatic activity in leukocytes, accompanied by mutation analysis and, in selected cases, measurement of sulfatide excretion in the urine. Brain MRI is characterized by widespread white matter changes with T2-hyperintense signal starting in the corpus callosum and periventricular and central white matter, but sparing subcortical fibers.

There are several pitfalls in the diagnostic process: resemblance of the presenting symptoms of MLD to those found in Guillain-Barré syndrome or chronic inflammatory demyelinating polyneuropathy (CIDP) in young children, the pseudodeficiency alleles with no symptoms but

low ASA activity, and patients with saposin B deficiency who are symptomatic with normal ASA activity. No curative treatment is available for all types of MLD. This review focuses on current therapeutic approaches as HSCT, but also on possible future therapies that are now being evaluated in clinical trials such as enzyme therapy and gene therapy. The preliminary results of the gene therapy trial are promising, but more information is needed regarding the safety and long-term outcomes of this therapy. HSCT does not seem to be beneficial for overtly symptomatic patients or patients with the aggressive late-infantile onset type. The results for asymptomatic juvenile and adult patients are more encouraging. In order to come to a valid conclusion, a systematic evaluation including a larger patient sample, and longer follow-up periods is necessary.

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Chapter 3

Slowly progressive psychiatric symptoms: Think metachromatic leukodystrophy

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Summary

Metachromatic leukodystrophy (MLD) is an inherited neurodegenerative demyelinating disorder. Its recognition may be challenging, especially when patients present with psychiatric symptoms. Early diagnosis is crucial to allow life-saving treatment, hematopoietic cell transplantation (HCT)**.** We describe the clinical course of 4 MLD patients who were first evaluated by a psychiatrist. When MLD was eventually diagnosed, HCT was no longer possible in 2 patients due to disease progression between first symptoms and correct diagnosis. In the other 2 patients, diagnosis was made just in time to perform HCT, which halted disease progression.

Introduction

Metachromatic leukodystrophy (MLD) is a lysosomal disorder caused by biallelic mutations in *ARSA*, resulting in arylsulfatase A (ASA) deficiency and sulfatide accumulation in the central and peripheral nervous system. Sulfatides are major myelin lipids, and their accumulation leads to demyelination. MLD is one of the most common leukodystrophies with an estimated incidence of 1.4-1.8 per 100.000. Greenfield first described its pathological features – widespread demyelination and neuroglial sclerosis – in 1933. Numerous clinical and pathological studies have broadened our knowledge of this disease.[1]

Based on age at onset, MLD is divided into three clinical subtypes: late-infantile (onset before 30 months), juvenile (2.5 to 16 years) and adult (after 16 years. Level of residual ASA activity is correlated to the subtype, with (almost) no activity resulting in the late-infantile type. This form presents with rapid psychomotor regression, ataxia and weakness, sometimes also areflexia due to severe peripheral neuropathy.[2] Spasticity, dysphagia and seizures follow. The juvenile form often presents with deterioration in school performance due to disturbed attention, reduced processing speed, impaired executive functioning and behavioral abnormalities. First neurological signs are slowly progressive ataxia and a pyramidal syndrome. The adult form is characterized by intellectual and behavioural changes such as memory deficits and emotional instability.[2] Mild polyneuropathy usually develops as the disease progresses. With early disease onset, disease progression is fast; in later onset forms, deterioration is insidious. Eventually, all acquired skills are lost and patients die, depending on the clinical subtype, within a few years or decades after first symptoms.

Diagnosis of MLD is made through clinical presentation, typical brain MRI abnormalities (Figure 1), measurement of ASA activity in leukocytes, sulfatide levels in urine and *ARSA* mutation analysis.[1] MRI shows bilateral symmetric abnormal hyperintense T2 signal changes starting in the corpus callosum, subsequently involving the periventricular white matter, before spreading to the central and subcortical white matter.[1]

Still, diagnosing MLD may be challenging in juvenile and adult-onset patients when psychiatric symptoms precede the neurological signs, and this is not rare: Hyde et al describe 129 cases, half of whom with a disease onset between 10 and 30 years. Fifty-three percent of these presented with psychotic symptoms.[3]

In 1985, the first hematopoietic cell transplantation (HCT) for MLD was performed in the USA.[4] As for other lysosomal storage disorders, HCT is supposed to be effective because donor macrophages, a source of lysosomal enzymes, migrate to the recipient's brain to produce the missing enzyme there, cross-correcting the deficiency. Since then, treatment has greatly been optimized by reduced conditioning regimens, decreased treatment related mortality (now estimated at less than 10% for children), the usage of umbilical cord cells and better knowledge about correct timing. The decision whether HCT is appropriate is based on cognitive function (IQ >75) and neurological examination (able to walk without support). HCT has now been proven to be able to halt or even prevent the disease, but only when performed before disease is too advanced.[5] This is mainly due to the fact that it takes 6 to 12 months after transplantation until donor cells become effective, months in which the disease still progresses. This underlines the essence of timely diagnosis, as exemplified by 4 cases.

Case reports

Patient 1 (MLD-58) had typical motor and cognitive development, but always problems with social interaction. His premorbid IQ (age 9), was in the superior range (verbal IQ 119, performance IQ 130 on the Wechsler Intelligence Scale for Children-III (WISC-III)). At age 12, his school performance dramatically declined, from regular to special education within one year, leading to the diagnosis of Asperger syndrome. Eventually, his behavior became so aggressive and disinhibited that admission to a psychiatric ward was necessary at age 13. His motor function remained typical. Coinciding with an exacerbation of behavioral problems, a discrepancy between performance (81) and verbal (111) IQ (WISC-III) led to referral to a neurologist. MRI, at age 13, revealing white matter abnormalities suggesting MLD, which

was confirmed by biochemical and genetic analyses. Despite the delay between first symptoms and diagnosis, he was still a candidate for HCT with a total IQ of 92 and only minor abnormalities at neurological examination. Now, 2.5 years after HCT, his cognitive abilities have stabilised after an initial further decline (verbal IQ (73), performance IQ (65)). Motor function remained typical. His behavioral problems, treated with risperidone since age 13, are now well controlled.

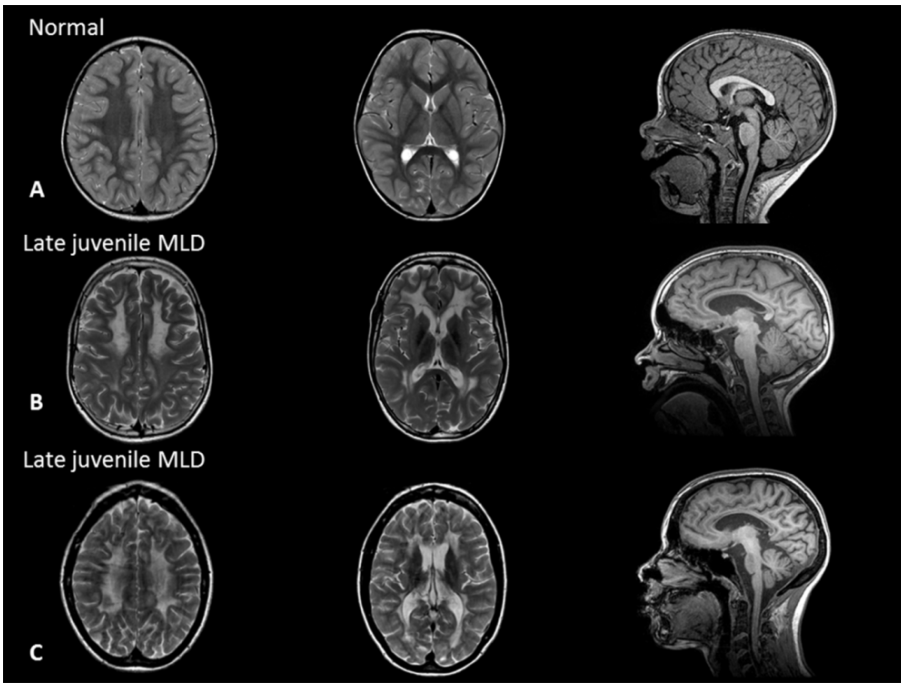

Figure 1: Axial T2-weighted and sagittal T1-weighted MR images of (A) a healthy adolescent control, and MLD-60 (B) and MLD-61 (C), both with late juvenile onset. (B) shows white matter abnormalities with frontal predominance and extensive periventricular white matter abnormalities. In (C) the typical radial oriented striped pattern of low signal intensity throughout the diffuse high signal intensity on T2-weighted images is shown, and involvement and atrophy of the corpus callosum.

Patient 2 (MLD-60) had a typical early development. At 8 years, her cognitive function gradually declined; she could no longer learn new tasks and even lost previously gained skills as reading, resulting in special education. Her behavior became increasingly aggressive and disinhibited. The diagnosis attention deficit and hyperactivity disorder (ADHD), was made, for which she was treated with methylphenidate and risperidone. At age 12 she developed repetitive motor and vocal tics. Her IQ was tested after parents noticed that her 2 years younger sister outran her in tasks (both cognitive as daily living such as getting dressed). An IQ drop from 70 to 55 (WISC-III) led to a brain MRI suggestive for MLD (Figure 1B). At age 13, diagnosis was confirmed biochemically and genetically. Although neurological examination was without major abnormalities, cognitive function had declined up

to a point (IQ 52) where HCT was judged to be no longer beneficial. Now, 1.5 year after diagnosis, motor function is starting to decline. She can still walk independently, but drags both feet, and fine motor movements are increasingly difficult for her. Her tics are progressive, and cognitive decline continues.

Patient 3 (MLD-61) had typical milestones and school career, but started to deteriorate, initially cognitively, at age 13. Within a period of 2 years she dropped from A level schooling to vocational level. As the cognitive deterioration developed soon after a major life event, this was initially interpreted as reactive to this incident, resulting in a period of 8 years with relentless cognitive decline. Eventually, her father insisted on referral to a neurologist., Her cognitive function had declined to an IQ of 60 on the Wechsler Adult Intelligence Scale (WAIS)-III. This, in combination with her abnormal neurological examination (hyperreflexia, muscle weakness and ataxia), resulted in an MRI at age 21 suggestive for MLD (Figure 1C). In hindsight, an MRI would have been justified when it became clear that the cognitive deterioration was progressive. HCT was no longer an option. Now, 2 years after diagnosis, she is wheelchair dependent with increasing spasticity and severely affected cognitive function. In her symptom-free brother, the diagnosis of MLD was also confirmed, and he was eligible for HCT.

Patient 4 (MLD-2) was already symptomatic at diagnosis at age 27, suffering from delusions and aggressive behavior and disinhibited behavior. As his older brother had been diagnosed with MLD many years earlier, diagnosis was made relatively fast. Because his treating physicians were unaware of HCT as possible treatment for MLD, he was not considered for HCT until one year after diagnosis. Meanwhile his increasing dangerous conduct resulted in compulsory admission to a psychiatric clinic. He was treated with risperidone, pipamperone and valproate. At time of his HCT evaluation, neurological examination showed no major abnormalities, and cognitive function was in the acceptable range (IQ 72, WAIS-III). After HCT, his behavior improved considerably with valproate, lithium and cognitive therapy,

allowing him to live at home. He still encounters concentration problems and fatigue. With an IQ of 76 (WAIS-III), 3 years after HCT, his overall cognitive function remained stable.

Discussion

These cases illustrate that, especially early on in the disease, when motor function is still intact, it is challenging to distinguish MLD from a primary psychiatric disorder as late juvenile and adult MLD patients have an insidious disease onset and often present with psychiatric symptoms. Initial symptoms can be similar to a first presentation of schizophrenia, depression, learning difficulties, ADHD or autism spectrum disorder.[6] Despite the acknowledgement of these types of presentation already in 1975[7] and several case reports since[8], knowledge about this differential diagnosis is not widespread, although during the last 40 years, we have achieved a much better understanding of pathophysiology and natural history, allowing it to evolve from an untreatable towards a treatable condition. Thanks to HCT, we now have the possibility to greatly alter both quality of life and life expectancy. Awareness of this diagnosis and the current therapeutic options among child and adult psychiatrists is therefore crucial to allow for correct and early patient identification and to avoid other potential pitfalls as deferral in testing (asymptomatic) siblings of already diagnosed patients and delay in referral to specialized centers.

There are clues to this diagnosis. All our cases had typical initial development, followed by a period of regression, first insidious, then overt. Concretely, the combination of an initially typically developing child with a clear change of behavior together with (in the beginning mild) cognitive deterioration should prompt diagnostic evaluation for neurometabolic disorders.[9] This should include both neurological examination and brain MRI. Psychiatric and neurological symptoms together are even more suggestive of a degenerative disorder and warrant prompt referral to a neurologist. Though a discrepancy between verbal and

performance IQ is also seen in other conditions, its combination with the afore mentioned symptoms is especially suspect.

The patients for whom HCT was no longer a possibility illustrate how crucial it is to be aware of this diagnosis and to properly identify patients who are likely to present first to psychiatrists and psychologists. Siblings of a patient (including older ones) should always be tested, making presymptomatic treatment possible. For patients who are no longer candidates for HCT, correct diagnosis is also essential to provide appropriate treatment and genetic counselling.

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II

Treatment

Chapter 4

Intrathecal baclofen: course of treatment in metachromatic leukodystrophy versus spastic cerebral palsy

Nikki van der Veldt, Diane F van Rappard, Laura A van de Pol, Marjo S. van der Knaap, Willem JR van Ouwerkerk, Jules G Becher, Nicole I Wolf, Annemieke I Buizer

Abstract

Aim To describe the course of treatment with intrathecal baclofen (ITB) in children and young adults with metachromatic leukodystrophy (MLD) compared to spastic cerebral palsy (SCP).

Method All MLD patients in our center on ITB treatment since a minimum of six months were included (4 male, 6 female, mean age 10y 8m (range 6–24y)). Those with a predominantly spastic (SMLD; n=8) and dyskinetic (DMLD; n=2) motor impairment were compared. SMLD patients were compared with matched-control SCP patients (n=8). Treatment goals, programming mode, ITB dose, number of boluses and complications were registered at six months after baclofen pump implantation.

Results Treatment goals were mainly to improve care and sitting position and to reduce pain. At six months, pump programming mode and mean dose of ITB did not differ between groups. However, the number of boluses was significantly higher ($p = 0.03$) in SMLD compared to SCP. Complication rates related to baclofen pump and its catheter were similar in MLD and SCP.

Interpretation ITB treatment course in the first six months after pump implantation is mostly comparable between MLD and SCP. ITB is a feasible therapy to improve comfort and daily care in MLD patients and should therefore be considered early.

What this paper adds

- ITB is a feasible and safe therapy to improve comfort and daily care in children and youth with MLD.
- Course of ITB treatment in the first 6 months is mostly comparable between MLD and SCP.

Metachromatic leukodystrophy (MLD, OMIM 250100) is an autosomal recessive lysosomal disorder leading to progressive neurological decline in a previously healthy child or young adult. Increasing motor impairments such as spasticity and dyskinesia are challenging to treat and often lead to limitations in daily care and comfort. [1-3]

MLD is divided into three clinical subtypes, based on the age of onset: The late-infantile form starts before 30 months of age, the juvenile form before 16 years and the adult form thereafter. First symptoms and signs in younger patients consist of motor deterioration, older patients usually present with cognitive and psychiatric symptoms.[4]

Currently, there is no curative treatment for MLD. Hematopoietic cell transplantation (HCT) has led to encouraging results, once performed in an early stage of the disease, especially for juvenile and adult patients.[5,6] First results for a gene-therapy approach are promising.[7]

In the many patients for whom the diagnosis comes too late and who are are no longer candidates for HCT, relentless disease progression is inevitable. Decline in level of mobility can be classified by an adapted gross motor function classification (GMFC-MLD),[8] based on the gross motor function classification system (GMFCS) for children with cerebral palsy. [9] All juvenile MLD patients develop spasticity and often also a dyskinetic movement disorder, young patients rapidly, older patients more slowly. Sometimes, demyelinating peripheral neuropathy becomes so overwhelming as to effectively counteract spasticity. However, in our clinical experience, this is not frequent, certainly not in patients with the juvenile and adult forms. Spasticity, dyskinesia and pain caused by these movement disorders can limit optimal daily care, transfers and sitting.

The first step in treatment is oral spasmolytic medication as baclofen,[10,11] a GABA-agonist that inhibits neural transmission at the level of the spinal cord, thereby leading to muscle relaxation.[12] When side effects occur (>10%) and problems persist, intrathecal baclofen therapy (ITB) is an alternative treatment. It delivers the baclofen locally to the spinal fluid by an intrathecal catheter. To reach an adequate effect, the dose is increased in a stepwise manner in continuous delivery or, when repeated vanishing effect occurs, by a flexible program with periodic boluses.[13,14]

During the last decades, experience with ITB has been increasing, and it is now known as an effective therapy to treat spasticity related problems in daily life in spastic cerebral palsy (SCP).[15-17] Positive effects in dyskinetic cerebral palsy have also been reported, but current evidence is limited.[18] ITB has recently been described to have beneficial effects in treatment of spasticity in progressive neurological diseases like MLD,[16,19] and in our center, ITB is frequently used as therapy in children with cerebral palsy and progressive neurological disorders.

As little is known about the clinical course of ITB treatment in MLD, our aim is to describe the course of ITB treatment in juvenile MLD patients and contrast it with SCP patients. We were interested whether the progressive nature of the disease makes other dose adaptations necessary than normally applied in static encephalopathies in children and young adults.

Methods

Design

This study is a retrospective cross-sectional cohort study with matched-control SCP patients.

Setting and participants

All patients with juvenile MLD (diagnosis established by typical clinical and MRI findings, ASA activity and ARSA mutation analysis) who were treated with ITB in our center between February 2002 and December 2016, and had a baclofenpump for at least six months, were included. All children were non-walking (GMFC-MLD level 3 to 6).

For the SMLD group, a matched control group of patients with SCP treated with ITB was composed, out of all ITB patients treated in our center. This SCP group was matched with the SMLD group for age, sex and functional mobility level (non-walking, GMFCS 4 and 5). Only the MLD patients with a spastic movement disorder were matched with SCP patients, as patients with spasticity seem to require different dosing than dyskinetic patients,[20] and the DMLD group (n=2) was too small to match.

Patient characteristics	**SMLD**	**DMLD**	**SCP**
Sex (n) Male	4		4
Female	4	2	4
Clinical subtype (n)			
Late infantile	0	0	
Early juvenile	3	0	
Late juvenile	5	2	
Adult	0	0	
Type of predominant movement disorder (n)			
Spastic	8	0	8
Dyskinetic	0	2	0
Age at diagnosis			
Mean (range)	8y 0m (5y – 14y)	8y 0m (7y – 9y)	
Age when wheelchair dependent			
Mean (range)	9y 0m (6y – 17y)	8y 6m (8y – 9y)	
Age at pump implantation			
Mean (range)	11y 0m (6y – 24y)	9y 6m (9y – 10y)	11y 0m (5y – 26y)
GMFC-MLD /GMFCS level* (n)			
3	1	0	0
4	2	0	3
5	4	2	5
6	1	0	
Cathetertip (n)			
C4	0	2	0
Th4	5	0	3
Th10	3	0	5
Purpose of ITB (multiple goals possible) (n)			
Improve care	7	0	8
Decrease pain	1	2	2
Improve transfers	1	0	0
Improve sitting position	3	1	1
Prevent contractures	0	0	1
Complications (n)			
Pump infection **	0	0	1
Catheter disconnection ***	1	0	1

Table 1. Patient characteristics
CSF = cerebral spinal fluid; GMFC-MLD = gross motor function classification in metachromatic leukodystrophy; ITB = intrathecal baclofen; MLD = metachromatic leukodystrophy; SCP = spastic cerebral palsy.
* Score at pump implantation
** at 0.5 month: negative cultures, removal of the pump. Second implantation included in the study
*** One SMLD patient at 0.5 month: revision of the catheter; One SCP patient at 1 month: revision of the catheter.

Variables, data sources and measurement

Of all included patients we collected age, sex, age at pump implantation and height of catheter tip. In MLD, we noted clinical subtype, age at diagnosis and at wheelchair dependency, and whether spasticity (SMLD) or dyskinesia (DMLD) was the predominant motor impairment. Mobility status was scored via GMFC-MLD in MLD patients, and GMFCS in SCP patients. The goals of ITB were noted. Dose pump settings at six months after implantation, including dose of baclofen in micrograms (µg) per day and baclofen pump dosing mode (simple continuous or flexible program with bolus administration) were used as outcome parameter. The starting dose after pump implantation was used as first measurement (t = 0).

Data analysis

The correlation between age at pump implantation and baclofen dosage at six months was calculated. An Independent Samples T-Test was used to search for a difference in baclofen dosage between DMLD and SMLD and between SMLD and SCP patients at 6 months after pump implantation. For the mode of baclofen dose and number of boluses, the Chi Square test was used. $P < 0.05$ was considered statistically significant. Analyses were performed using SPSS version 22.0.

Ethics

The medical ethical committee of the VU University Medical Center Amsterdam approved the study.

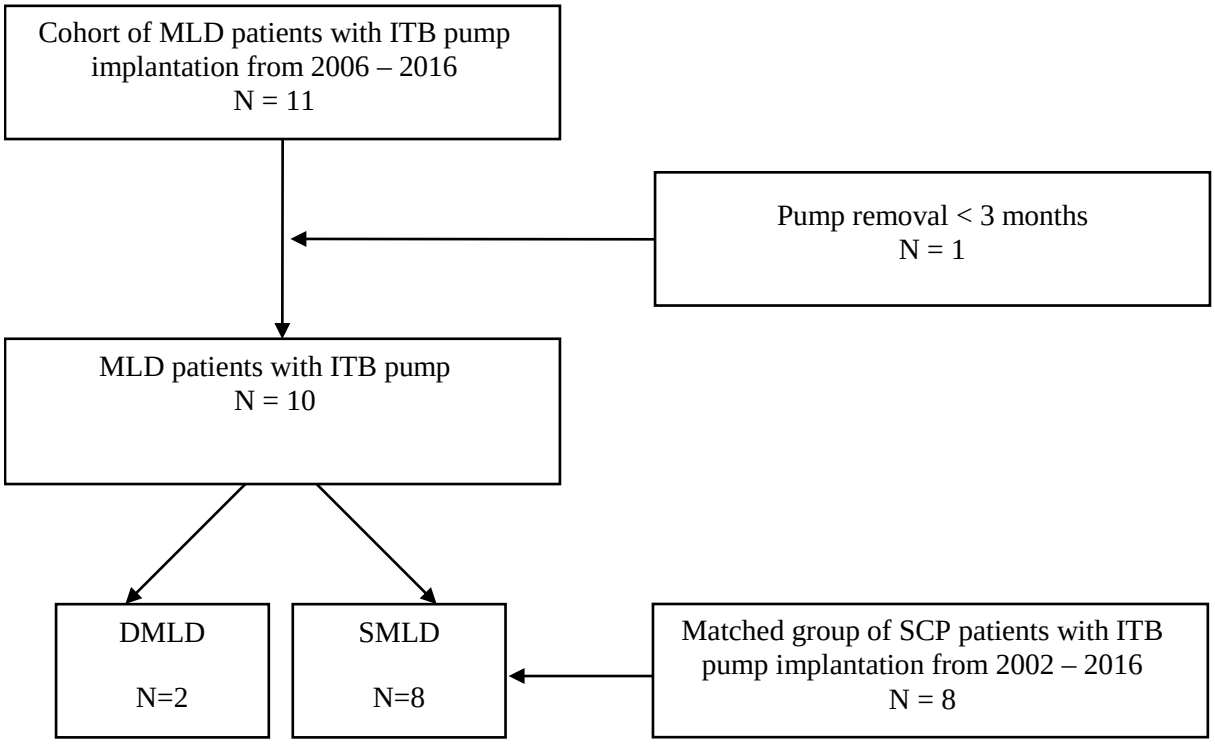

Figure 1. Study scheme
MLD = metachromatic leukodystrophy; ITB = intrathecal baclofen; SMLD = spastic MLD; DMLD = dyskinetic MLD; SCP = spastic cerebral palsy.

Results

Eleven patients with MLD were treated with ITB between 2006 and 2016 (Figure 1). One of them was excluded from the study because of removal of the baclofen pump due to infection with staphylococcus aureus within two weeks; reimplantation was followed by re-infection and definitive pump removal. Eight patients had SMLD, two DMLD. The eight matched SCP patients for the SMLD group had a pump implantation between 2002 and 2016. Patient characteristics are summarized in table 1. In one MLD patient and in one SCP patient, catheter disconnection occurred as a complication, necessitating operative revision. In one SCP patient, the baclofen pump became infected within 1 month after implantation, leading to pump removal. The patient was included in the study after reimplantation.

All MLD patients were wheelchair dependent within three years after diagnosis (mean 8y 10m, range 6y - 17y). Six out of eight patients with SMLD (aged 6 to 8 years) were started on

ITB therapy within two years after wheelchair dependency. In two older children (13 and 17 years old at the moment of wheelchair dependency), ITB treatment started seven years later. In both patients with DMLD, ITB therapy started one year after wheelchair dependency.

Results

Metachromatic leukodystrophy

In the eight SMLD patients, mean starting dose was 68µg per day (SD 23.60, range 40 – 100). Both DMLD patients started at 50µg per day. At six months, five out of eight SMLD patients and both DMLD patients had a flexible program with bolus administration. The mean dose in SMLD and DMLD at six months was 210µg (SD 119.97, range 70 – 428) and 308µg (SD 81.32, range 250 – 365), respectively (Figure 2).

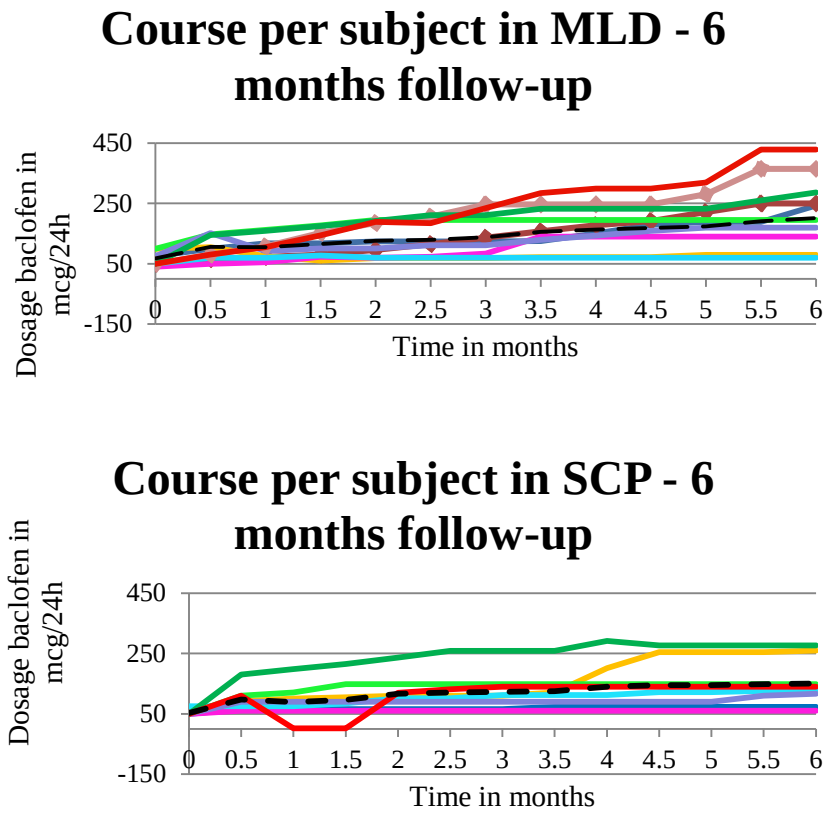

Figure 2. Course per subject in MLD and SCP group: 6 months follow up. MLD = metachromatic leukodystrophy; SMLD = metachromatic leukodystrophy with predominant spastic motor impairment; DMLD = metachromatic leukodystrophy with predominant dyskinetic motor impairment; SCP = spastic cerebral palsy.
Catheter disconnection (months after pump implantation): one SMLD patient at 0.5 month; one SCP patient at 1 month.
* DMLD **Mean of SMLD *** Mean of SCP

In patients who had a flexible program, mean dose of baclofen given by boluses was 112µg per day (SD 128.29, range 10 – 335) administered in 2.2 (SD 0.45, range 2 – 3) boluses in SMLD, and 140µg per day (SD 84.85, range 80 – 200) in 2.5 (SD 0.71, range 2 – 3) boluses in DMLD (Table 2). No correlation was found between age at pump implantation and baclofen dosage at six months (R = 0.33, p = 0.35).

	Baclofen in mcg per 24 hours (SD, range)	Dose of baclofen in boluses in mcg per 24 hours (SD, range)	Number of boluses (SD, range)
SMLD	210 (119.97, 70 – 428)	112 (128.29, 10 – 335)	2.2 (0.45, 2 – 3)
DMLD	308 (81.32, 250 – 365)	140 (84.85, 80 – 200)	2.5 (0.71, 2 – 3)
SCP	150 (79.37, 60 – 277)	35 (33.91, 10 – 85)	1.5 (1.00, 1 – 3)

Table 2. Results per group at 6 months after starting ITB
SMLD = metachromatic leukodystrophy with predominant spastic motor impairment; DMLD = metachromatic leukodystrophy with predominant dyskinetic motor impairment; SCP = spastic cerebral palsy; ITB = intrathecal baclofen.

Spastic cerebral palsy

In SCP, the starting dose was 67µg per day (SD 19.64, range 50 – 96). At six months, four had a simple continuous program and four had a flexible program with boluses added. Mean dose of baclofen was then 150µg per day (SD 79.37, range 60 – 277) (Figure 2). Of patients with a flexible program, mean dose of baclofen given by boluses was 35µg per day (SD 33.91, range 10 – 85), administered in an average of 1.5 boluses (SD 1.00, range 1 – 3) (Table 2). As in MLD, no correlation was found between age at pump implantation and baclofen dosage at six months (R = 0.41 , p = 0.32).

Comparison between groups

SMLD vs DMLD

At six months after initiating therapy, no statistically significant differences were found in the dose of baclofen between SMLD and DMLD patients (p = 0.32). The difference in simple continuous or flexible program with boluses was not significant between both groups (X(1) =

1.07, p = 0.30). In patients with a flexible program, the mean dosage of baclofen given in boluses (p = 0.79) and the number of boluses (X(1) = 0.63, p = 0.43) did not differ significantly between groups.

SMLD vs SCP

No statistically significant differences were found in the dose of baclofen between SMLD and SCP patients (p = 0.26) after six months. There was also no difference in whether patients had a simple continuous or flexible program with boluses (X(1) = 0.25, p = 0.61). The number of boluses (X(2) = 6.98, p = 0.03) was significantly higher in the SMLD group. The mean dosage of baclofen given in boluses (p = 0.29) did not differ significantly between groups.

Discussion

In this study, we showed that ITB is a feasible therapy in MLD patients with either predominant spastic or dyskinetic motor impairment, with a treatment course comparable to SCP. The complication rate in MLD was comparable to SCP patients and similar to rates reported in previous studies.[21,22]

The dosing in the first six months of ITB after pump implantation was mostly comparable in SMLD and SCP. Only the bolus number administered in flexible programming mode was higher in the SMLD group compared to SCP, indicating that more flexible programming may be more effective in improving care and comfort in SMLD than static administration of baclofen. As relevant spasticity in MLD usually occurs rather rapidly after wheelchair dependency, especially in younger patients, ITB should be considered early in the disease course.

Goals of treatment were mainly to improve care, improve sitting position and reduce pain. In this study, we did not formally evaluate whether goals were reached; still, families reported clear improvements on follow-up visits. A previous study on the effect of ITB on activities of daily life in SCP, DCP and progressive neurological disorders showed that caregivers were

generally satisfied with the improvement in comfort and daily care. However, the group with progressive neurological disorders showed less improvement in comfort during ITB treatment than the two CP groups. This study counted only two MLD patients.[4]

A limitation of this study is the small number of patients and the short follow-up time. To obtain more evidence on optimal treatment programs and efficacy of ITB in MLD, future research should address treatment course and outcomes in larger cohorts. This could likely be achieved by studying larger clinical cohorts with systematic gathering of data in multicenter studies, for example by centers of expertise participating in the European Reference Network for Rare Neurological Diseases (ERN-RND). It is important to also address goal achievement and outcomes at the level of activities as well as body structure and function, at the same time taking into account that MLD is a progressive disorder with inherent decline of function despite treatment.

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Chapter 5

Efficacy of Hematopoietic Cell Transplantation in metachromatic leukodystrophy: The Dutch experience

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Metachromatic leukodystrophy (MLD) is a neurodegenerative disorder caused by deficiency of arylsulfatase A,[1] leading to sulfatide accumulation and subsequent demyelination of the central and peripheral nervous system.[2,3]

MLD is divided into 3 subtypes, based on the age of onset, late-infantile (< 30 months), juvenile (2.5-16 years) and adult (>16 years). With early disease onset, progression is fast and motor signs are prominent, in contrast to later forms with insidious onset of cognitive deterioration.[6] Eventually, all acquired skills are lost and patients die. Hematopoietic cell transplantation (HCT) is a possible treatment, but systematic outcome data are lacking, due to the use of different eligibility criteria and protocols worldwide.[6-12] In order to assess HCT efficacy, we evaluated all 35 consecutive MLD patients presenting between 2004 and 2015 in our department, the Dutch Leukodystrophy Referral Center (Figure 1A).

Patients with a total intelligence quotient (IQ) above 70 and without gross neurological signs (i.e. ambulation without support, no dysphagia) were considered HCT candidates (Tables 1 and 2). HCT was performed at the University Medical Center Utrecht (UMCU; Blood and Marrow Transplantation Program) according to international protocols.[13] Patients received HCT from either a HLA identical sibling (n=3; noncarrier) or from an unrelated umbilical cord blood (n=10) donor (with a minimum match of 4 out of 6 HLA-loci) after fludarabine (160mg/m2) + busulfan (targeted to cumulative exposure of 90mg*h/L); thymoglobuline was added in cord blood recipients. For details, see supplemental Methods (available on the *Blood* Web site).

Transplanted patients were followed for a mean duration of 4.7 years, assessments including neurological examination, cognitive function, brain magnetic resonance imaging (MRI) rated by the MLD-Loes score,[14] measurement of arylsulfatase A activity, and assessment of nerve conduction velocity. Gross motor function (GMF) was scored according to a classification developed for MLD.[15]

After 5 years, follow-up intervals were adapted to clinical status. Follow-up of nontransplanted patients (mean, 4.6 years) consisted of neurological examination, in some cases, assessment of nerve conduction velocity, and MRI, the intervals depending on clinical condition. Two composite survival endpoints were analysed; intervention-free survival (IFS) and activities of daily living-compromise free survival (AFS).

There was no transplantation-related mortality. All patients engrafted and achieved full donor chimerism. Three symptomatic patients (23%; 1 late-infantile,1 juvenile,1 adult) died due to disease progression, all within 1 year after HCT. Eight nontransplanted patients (36%) died 22 to 72 months after diagnosis. Overall survival at latest assessment was 76.9% for transplanted and 63.6% for non-transplanted patients (Figure 1B). One patient experienced acute, 3 chronic graft-versus-host disease (1 extensive). All were effectively successfully treated with corticosteroids and came off immunosuppressive therapy.

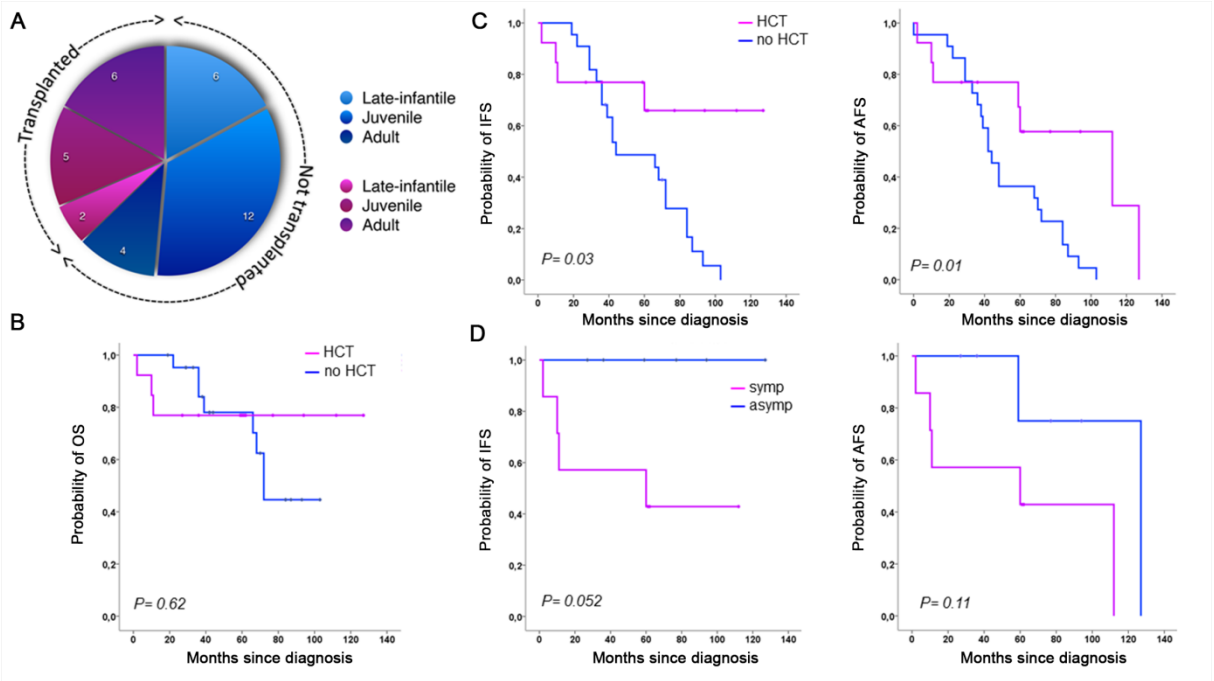

Figure 1: Patient cohort and outcome after HCT. (A) 13 transplanted patients (magenta shades, 6 asymptomatic (diagnosed because of an affected sibling), mean age 14.4 years, range 2-35 years) and 22 non-transplanted patients (blue shades, mean age 6.5 years, range 2-32 years). Four patients were referred from other European countries (Belgium, Denmark and Luxemburg); the remainder came from the Netherlands. (B) Overall survival probability for transplanted and non-transplanted patients. (C) Probability of IFS and AFS for transplanted and non-transplanted patients. (D) Probability of IFS and AFS for symptomatic (n=7) and presymptomatic (n=6) transplanted patients.

IFS (whereby death, wheelchair dependency, gastrostomy and intrathecal baclofen treatment were regarded as events) was 69.2% for transplanted and 9.1% for non-transplanted patients (*P*=.03; Figure 1C). Symptomatic transplanted patients had lower estimated IFS (42.9%) than presymptomatic transplanted patients (100%) at HCT (*P*=.052; Figure 1D). AFS was defined as no occurrence of death, motor (clinically relevant peripheral neuropathy, spasticity or ataxia, gross motor function ≥ 3) or cognitive (IQ decline ≥ 6 points) deterioration. Transplanted patients had higher AFS (46.2%) than nontransplanted patients (0%; *P*=.01; Figure 1C). Symptomatic transplanted patients had an AFS probability of 28.6%, versus 66.7% for presymptomatic transplanted patients (*P*= .11; Figure 1D). The only surviving late-infantile patient had very limited motor function 3 years after HCT, due to progressive neuropathy. Motor function remained preserved in 2 out of 4 surviving juvenile and 5 adult patients. Progressive neuropathy hampered motor function in 2 juvenile patients. Regarding the adult patients, none showed signs of spasticity or ataxia, and when present (n = 2), polyneuropathy was mild and did not interfere with motor function. In the entire transplanted group, nerve conduction remained normal in 1, stabilized in 8 and further decreased in 2 patients. In comparison, in the nontransplanted patients, all late-infantile and all 13 juvenile patients deteriorated from intact motor function within 16 months to hardly any remaining motor function. In only 2 patients (adult onset) did motor function remained intact for the duration of follow up (Tables 1 and 2).

Table 1: Evolution with and without HCT

Patient ID	MLD type	Follow-up since HCT (months)	GMF pre HCT	GMF post HCT*	Cognition pre HCT (age in years/months)	Cognition post HCT (age in years)	MRI pre HCT	MRI post HCT*	Deceased (age in years)
	Transplanted patients								
MLD-50	Late-infantile	10	2	6	DQ 105 (25m)	NA	2	20	yes (3y)
MLD-45	Late-infantile	60	2	5	DQ 101 (35m)	70 (4y)	3	8	no
MLD-16	Juvenile	36	0	0	104 (6y)	NA	0	0	no
MLD-37	Juvenile	127	0	2	DQ 116 (26m)	95 (6y)	2	4	no
MLD-4	Juvenile	112	1	1	110 (5y)	56 (10y)	4	8	no
MLD-53	Juvenile	11	1	6	74 (7y)	NA	20	25	yes (8y)
MLD-14	Juvenile	61	1	1	94 (14y)	93 (17y)	12	11	no
MLD-21	Adult	27	0	0	100 (17y)	95 (18y)	10	10	no
MLD-30	Adult	94	0	1	104 (19y)	107 (21y)	7	11	no
MLD-41	Adult	77	0	0	91 (25y)	89 (27y)	12	13	no
MLD-15	Adult	59	0	0	87 (35y)	73 (40y)	11	14	no
MLD-51	Adult	2	1	2	61 (22y)	NA	16	24	yes (22y)
MLD-2	Adult	62	0	0	72 (28y)	76 (31y)	13	12	no

Patient ID	MLD type	Follow-up (months)	GMF at diagnosis	GMF latest assessment*	Cognition at diagnosis (age in years)	MRI at diagnosis	MRI at latest assessment *	Deceased
	Non-transplanted patients							
MLD-8	Late- infantile	39	6	6	NA	4	NA	yes (5y)
MLD 20	Late-infantile	36	6	6	NA	10	NA	yes (6y)
MLD-26	Late-infantile	72	5	6	NA	17	NA	yes (8y)
MLD-34	Late-infantile	36	6	6	NA	12	NA	yes (4y)
MLD-40	Late-infantile	19	6	6	NA	8	NA	no
MLD-57	Late-infantile	29	4b	6	NA	13	NA	no
MLD-5	Juvenile	42	1	6	71 (7y)	22	26	no
MLD-6	Juvenile	42	1	6	65 (9y)	21	23	no
MLD-10	Juvenile	84	1	5	NA	18	NA	no
MLD-11	Juvenile	84	1	5	NA	14	NA	no
MLD-12	Juvenile	93	1	6	68 (6y)	12	NA	no
MLD-17	Juvenile	72	1	6	66 (6y)	14	NA	yes (12y)
MLD-18	Juvenile	48	1	6	75 (6y)	14	NA	yes (12y)
MLD-22	Juvenile	44	1	6	65 (15y)	22	NA	no
MLD-29	Juvenile	22	1	6	61 (7y)	18	NA	yes (8y)
MLD-33	Juvenile	68	NA	6	NA	15	25	yes (11y)
MLD-38	Juvenile	29	1	5	NA	17	NA	no
MLD-39	Juvenile	33	1	4b	63 (7y)	15	NA	no
MLD-54	Juvenile	87	1	5	NA	17	19	no
MLD-25	Adult	103	1	3	50 (22y)	19	23	no
MLD-32	Adult	70	0	0	NA	16	16	no
MLD-56	Adult	38	1	2	53 (32y)	13	17	no

Abbreviations: GMF, gross motor function; MRI, magnetic resonance imaging; DQ, developmental quotient; NA, not available.

* GMF and MRI post HCT are scored at latest follow-up assessment.

Cognitive function (Tables 1 and 2) remained unchanged in 6 transplanted patients. One late-infantile, 1 juvenile and 1 adult patient showed clear cognitive deterioration after HCT. For the evaluable nontransplanted patients at diagnosis (n = 10), all IQs were low. At follow-up, cognition was not formally tested, but, clinically, patients continued to deteriorate.

Other neurological symptoms were more severe in nontransplanted patients. Epilepsy developed in half (11 of 21; 52%) versus 1 of 13 (8%) of the transplanted group. Additionally, severe spasticity was a frequent problem in nontransplanted patients with 9 of 21 (43%) juvenile patients needing intrathecal baclofen treatment. At latest assessment, 17 of 21 (81%) evaluable non-transplanted patients required feeding via gastrostomy while only 1 transplanted patient (late-infantile) required gastrostomy 5 years after HCT. Brain MRI (Tables 1 and 2) improved in 5 of 10 (50%) surviving transplanted patients, after initial deterioration, and stabilized in the remainder. In all nontransplanted patients, but one adult, MRI deteriorated over time.

In summary, the unique characteristics of this study are the comparison of disease evolution in transplanted patients with the natural course of patients no longer eligible for HCT diagnosed within the same period. We used consistent decision guidelines, and all transplantations were performed in a single center. Our data show that, under these conditions, HCT is a safe procedure for pre- and early symptomatic MLD patients with the juvenile or adult type, resulting in disease stabilization and high disease burden-free survival, with even the suggestion of some brain repair, reflected by improvement of brain MRI abnormalities, confirming earlier findings.[6,16-19] For late-infantile and more advanced patients, results are not encouraging, suggesting that HCT in those at best delays disease progression. Our study shows that motor and cognitive functions are good predictors of outcome. Clearly, affected motor (inability to walk without support) and cognitive (IQ below 75) function resulted in no benefit of HCT. Brain MRI abnormalities were more severe and extensive in the patients rejected for HCT than in successfully transplanted patients, suggesting that an MRI score above 15 is associated with an unsuccessful outcome. As HCT

remains an intensive treatment and initial neurological worsening is to be expected, it should not be considered if the disease has progressed beyond a certain stage. In these patients, HCT negatively impacts their life and that of their families at a time, which should be cherished before the inevitable frank disease progression sets in.

Limitations of our study are its retrospective character and the inevitable selection bias resulting from the fact that nontransplanted patients were more severely affected than the transplanted patients at diagnosis. In addition, some issues remain: 3 patients (2 with the juvenile form) showed cognitive deterioration, despite presymptomatic HCT, and despite relatively stable white matter changes, suggesting neuronal involvement perhaps less amenable to treatment with HCT. Peripheral nerve involvement seemed unaltered, despite normal circulating enzyme levels after transplantation. Lastly, especially for the slowly progressive adult forms, follow-up needs to be longer to fully evaluate effects of HCT.

As the best moment for HCT is as early as possible and before clinical disease onset, it is of utmost importance to test all siblings of an index case, including older ones. For more advanced and late-infantile patients, results are discouraging. For the majority of patients evaluated, HCT was no longer an option neither did they qualify for treatment trials, emphasizing the need of earlier diagnosis and better treatment strategies.

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Supplementary data

Methods

Clinical parameters

Gross motor function (GMF) for MLD: GMF consists of seven different levels and can be applied to children aged 18 months and older.[1] All patients were scored by a child neurologist (MSK or NIW).

GMF for MLD

Level 0	Walking without support with quality of performance normal for age
Level 1	Walking without support but with reduced quality of performance, i.e. instability when standing or walking
Level 2	Walking with support. Walking without support not possible (fewer than five steps)
Level 3	sitting without support **and** locomotion such as crawling or rolling. Walking with or without support not possible
Level 4	(a) Sitting without support but no locomotion **or**
	(b) Sitting without support not possible, but locomotion such as crawling or rolling
Level 5	No locomotion nor sitting without support, but head control is possible
Level 6	Loss of any locomotion as well as loss of any head and trunk control

NCV: Motor NCV of the anterior tibial nerve was considered abnormal if conduction velocity was below the mean (± SD) reference values. Pediatric reference values were applied for children ≤16 years (2-4 years 49.8±5.79 m/s, 4-16 years 47.9±9.2 m/s).[2] For adults, we used local reference values, derived from published cohorts.[3]

Evaluation of cognitive function: patients received a developmental evaluation or IQ testing prior to HCT with the exception of ten children (six with late-infantile MLD, table 1, main text) already clearly affected so that HCT was evidently no longer an option. One adult patient declined HCT (MLD-32) and was therefore not tested.

Depending on patients' ages and proficiencies, cognitive function was evaluated using the Dutch versions of the Bayley Scales of Infant Development-II (BSID-II-NL; < 48 months), the Wechsler Intelligence Scale for Children-III (WISC-III-NL; 6–18 years)[4], the Wechsler

Nonverbal Scale of Ability (WNV-NL; 4–22 years) or the Wechsler Adult Intelligence Scale-III (WAIS-III-NL; ≥ 18 years). As a consequence, outcomes of cognitive function could not be immediately correlated. Especially the predictive validity of the BSID-II-NL for later cognitive function in terms of Wechsler IQs is poor and should therefore be interpreted with care.[5]

MRI evolution: For all patients, brain magnetic resonance imaging (MRI) was available at the time of diagnosis. Transplanted patients underwent an MRI at 6 months after HCT, thereafter every 1 year or on indication. Axial T2-weighted images were scored using the MLD Loes score.[6]

Statistical analyses

Kaplan-Meier estimates were used to depict outcome probabilities for the main and other outcomes of interest. Descriptive statistics were used to describe primary and secondary endpoints, using SPSS version 22.0.

HCT procedure

All patients received a myeloablative conditioning regimen of busulfan (75 to 90 mg*h/L) and antithymocyte globulin (ATG) (10 to 2.5 mg/kg/day, day -8 till -4) combined with either cyclofosfamide (200 mg/kg total) or fludarabine (160 mg/m2 or 40 mg/m2 from day -5 till -1). All patients received cyclosporine (target range 200–250 μg/L,) as Graft versus Host Disease (GvHD) prophylaxis. Prednisone was given to cord blood recipients from day 0 until day 28, 1 mg/kg per day, as well as methotrexate 10 mg/m2 at days +1, +3 and +6. The average total nucleated cell dose was 2.26 x 10^5 cells/kg, with a median of 2 x 10^5/kg CD34+ dose (range 0.2–3.3 x 10^5/kg).

Engraftment

Median interval to neutrophil engraftment was 19 days (range 14–45 days). Median time to platelet engraftment was 45 days (range 28–91 days). Neutrophil engraftment was defined as the first of three consecutive days with an absolute neutrophil count > 500. Platelet engraftment was defined as the first of three consecutive days of an absolute thrombocyte count of > 50.

Chimerism analysis was done by variable number of tandem repeats (VNTR) by polymerase chain reaction (PCR) after engraftment, at day 60, and subsequently every year post HCT. Full donor chimerism was defined as ≥ 95% donor derived hematopoietic stem cells, mixed

chimerism if 10% to 94% donor derived hematopoietic cells were of donor origin, and autologous recovery if ≤ 10% of hematopoietic cells were donor derived. GvHD was scored according to standard criteria.[7] ASA activity was measured at each follow-up and regarded as normal if within the range of normal according to the institutional reference range, and low if below the lower limit of normal but not within disease range.

Results

At latest assessment all patients were full chimeras. ASA activity at latest assessment was in the normal range for 11/13 patients. In 2 patients, ASA activity was low (36 and 38 nmol/mg/17h), but substantially higher than the disease range (0–11 nmol/h/mg). The sibling donor for both patients was heterozygous for the pseudodeficiency allele, resulting in this low activity, as no matched unrelated donor could be found.

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Chapter 6

Donor macrophages and remyelination in metachromatic leukodystrophy

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In preparation

Abstract

In metachromatic leukodystrophy (MLD), a lysosomal storage disorder,[1] hematopoietic cell transplantation (HCT), when performed early, stops brain demyelination and even allows remyelination, thereby halting white matter degeneration.[2-5] However, it remains unknown how disease stabilization is achieved. Brain tissue of eight patients with MLD, obtained at autopsy, was investigated for macrophage activation and polarization, myelin content and numbers of oligodendrocytes and their precursors. Additionally, sulfatide storage and digestion were assessed. Two of the patients had received HCT 10 to 12 months before death. We show that in brain tissue of transplanted MLD patients, metabolically competent donor macrophages are present and distributed throughout the white matter. Compared to untreated patients, these macrophages are activated and preferentially express markers of an M2 phenotype that supports oligodendrocyte survival and differentiation. The numbers of oligodendrocyte precursors and, even more, mature oligodendrocytes are increased in transplanted patients. Beyond cross-correction of enzyme deficiency, transplanted activated macrophages may play a neuroprotective role for resident oligodendrocytes, thereby enabling remyelination. These results support the importance of modulation of inflammation for oligodendrocyte survival and myelin restoration in MLD, which could be exploited for better therapeutic outcome.

Introduction

Metachromatic leukodystrophy (MLD, MIM 250100) is a devastating inherited white matter (WM) disorder caused by biallelic mutations in *ARSA*, leading to deficient activity of arylsulfatase A (ASA), a lysosomal enzyme digesting sulfatides (figure 1a).[1] Sulfatides are enriched in myelin sheaths; their accumulation in MLD causes demyelination and inhibits oligodendrocyte differentiation from precursor cells.[6] Depending on the age at onset, MLD is divided into late-infantile (onset before 30 months of age), juvenile (onset between 30 months and 16 years of age) and adult (onset after 16 years of age) forms. If MLD is diagnosed early, its later-onset forms (juvenile and adult) can be treated by allogenic hematopoietic cell transplantation (HCT).[2,3] Ex vivo gene therapy with ASA overexpression has been shown to treat the late-infantile form when done early, before symptoms develop.[7] HCT halts further demyelination, and in some patients brain white matter abnormalities even improve on MRI (figure 1b, c).[4,5] Mechanisms of HCT action in MLD, however, are not yet fully understood. HCT is supposed to provide cross-correction of deficient enzyme levels by secreting ASA from donor cells migrated into the brain, thus restoring sulfatide degradation, but its exact mechanism remains elusive.[8] Until HCT becomes effective, there is an estimated six to twelve month delay possibly causing treatment failure in the rapidly progressive late-infantile form and in juvenile cases presenting with significant disability and MRI abnormalities. Using tissue of children with MLD, two of whom HCT-treated[2] and six untreated, we ascertained the presence of metabolically competent cells in the brain following HCT and compared inflammatory response and oligodendrocyte numbers in the two groups.

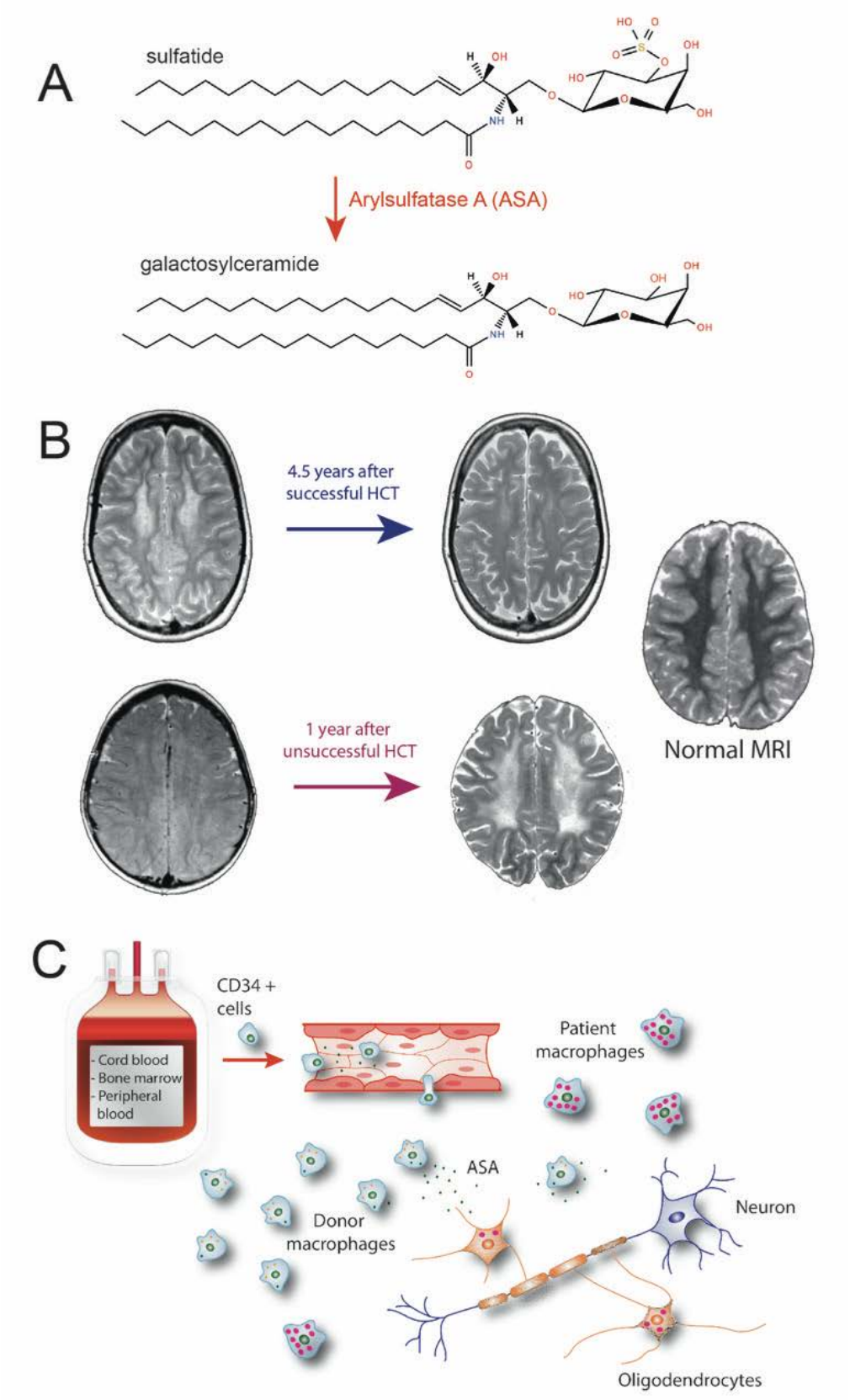

Figure 1. MLD at a glance. (A) shows the biochemical reaction impaired in MLD, the desulfation of 3-0-sulfogalactosyl glycolipids ("sulfatides") to galactosylceramide. (B) T2-weighted MRIs demonstrating the improvement of brain white matter abnormalities in a successfully transplanted patient, compared with patient 1 whose HCT was not successful. (C) illustrates the effect of HCT in MLD as interpreted so far.

Methods

The study was approved by the IRB of the VU University Medical Center, with informed parental consent. Brain autopsies of patients 1, 2 and 3 were performed within six hours post-mortem in our center; brain tissue of patients 4 to 8 was obtained through the NIH NeuroBioBank *(*https://neurobiobank.nih.gov*).*

Tissue staining

Six-µm-thick formalin-fixed paraffin-embedded tissue sections were routinely stained with Haematoxylin&Eosin and Toluidine blue. Immunohistochemistry was done as described[9] using antibodies targeting the microglia/macrophages markers CD68 (1:1600 Dako, M0814) and CD45 (1:100 Dako, 0701), the myelin marker proteolipid protein (PLP, 1:3000, Biorad, MCA839G), and the M2 marker CD163 (1:300, Cell Marque, MRQ-26).[10] Five-µm-thick frozen tissue sections were stained with Oil red O and with antibodies against M1 marker CD40 (1:500, Dako, ab13545) and CD64 (1:250, Abcam, ab104273)[11,12], M2 marker mannose receptor (CD206, 1:500, Dako, ab125028)[10] and oligodendrocyte lineage-specific marker Olig2 (1:100, Millipore, AB9610) as described.[13] Frozen tissue was also used for double staining of the M1 and M2 markers CD40 and CD206, respectively. CD40 immunoreactivity was visualized with an Envision+ system HRP-labelled antibody and 3,3'-Diaminobenzidine (Dako, K4002, K3467), CD206 with liquid permanent red (1:100, Dako, K00640) after secondary incubation with biotinylated secondary antibody (1:100, Dako, E0432) followed by streptavidin with an alkaline phosphatase conjugate (1:100, Sigma-Aldrich, 11089161001). Sections were counterstained with haematoxylin. Fluorescence in situ hybridization (FISH) against chromosomes X and Y was performed using a XY CEP probe (Abbott, 05J10-051) and a FISH Accessory Kit (Dako, K5799).

Image acquisition and analysis

Pictures were taken with a Leica DM6000B microscope (Leica microsystems). The number of positive pixels was quantified using the colour deconvolution plugin for imaging software ImageJ.[14,15] Total cell number were counted with the ImageJ cell counter. Oligodendrocytes

were classified as mature or precursor cells (OPC) based on Olig2 intensity as validated before.[16]

Statistical analysis

Statistical analysis was performed with GraphPad Prism v7.0a. Data are displayed as mean ± standard error of the mean. An unpaired t-test or the nonparametric Mann-Whitney U-test was performed to evaluate differences between non-transplanted and HCT-treated patients (considered significant when $p < 0.05$).

Results

Patients

Patient 1 presented at age 2 years with peripheral demyelinating polyneuropathy and mildly delayed myelination on brain MRI (figure 1b). Absent ASA activity and mutations c.245C>T, p.(Pro82Leu) and c.1168C>T, p.(Arg390Trp) in *ARSA* confirmed the diagnosis of late-infantile MLD. Cognitive function was age-adequate. Because there was no clear CNS involvement, HCT from a mismatched (5 out of 6 matched) unrelated female cord blood donor was performed, unfortunately followed by disease progression albeit rapid successful engraftment and full donor chimerism. He died one year after HCT. Patient 2 was diagnosed at age 7 years with gait abnormalities due to mild spasticity and ataxia. MRI showed extensive white matter abnormalities. Diagnosis of juvenile MLD was confirmed by low ASA activity; *ARSA* mutation analysis revealed c.830delTCinsAA, p.(Ile277Lys) and c.1277C>T, p.(Pro426Leu). Total IQ was 74. HCT was performed with a fully matched unrelated male cord blood donor. Despite fast successful engraftment and full donor chimerism, the disease rapidly progressed. She died one year after diagnosis. Patient 3 (female) was 26 months old when diagnosed with late-infantile MLD because of motor regression, confirmed by absent ASA activity and mutations c.245C>T, p.(Pro82Leu) and c.287C>T, p.(Ser96Phe) in *ARSA*. MRI showed extensive white matter abnormalities with sparing of the direct subcortical white

matter and the typical hypointense stripes within the abnormal white matter. She continued to deteriorate and died three years after diagnosis from disease progression. Patient 4 (NBB5505) died at age 3 years from late-infantile MLD, after having been diagnosed at age 22 months because of delayed acquisition of independent walking, with low ASA activity and mutations c.841G>T, p.(Asp281Tyr) and c.1004A>T, p.(Asp355Val) in *ARSA*. He deceased because of disease progression in the context of feeding difficulties and a respiratory infection. Patient 5 (NBB3308) died at age 21 years, patient 6 (NBB1144) at age 12 years, patient 7 **(**NBB5381) at age 7 years and patient 8 (NBB5509) at age 6 years. Regarding these patients, no further information was available.

Histopathology

In the two transplanted patients, donor macrophages had successfully reached the brain. Histopathology showed metabolically competent macrophages able to degrade sulfatides to cholesterol (orthochromatic and Oil red O-positive cells) next to macrophages loaded with sulfatides (metachromatic cells). FISH studies for X and Y chromosomes confirmed the presence of donor cells in the transplanted brains (figure 2).

In most WM areas of transplanted patients, the number of CD45-positive activated microglia/macrophages was greatly elevated compared to untreated individuals (figure 3). Notably, the total number of microglia/macrophages, as assessed by CD68, did not significantly differ in treated versus untreated patients, suggesting that HCT is associated with a more robust microglia/macrophage activation rather than with increased proliferation of these cells. We then questioned whether HCT had an effect on the microglia/macrophages phenotype. In vitro, these cells can be polarized towards opposite states of a spectrum, one being pro-inflammatory (M1) and the other anti-inflammatory (M2). In transplanted patients, macrophage expression of M2-associated proteins was significantly higher than in untreated patients in all WM areas examined.

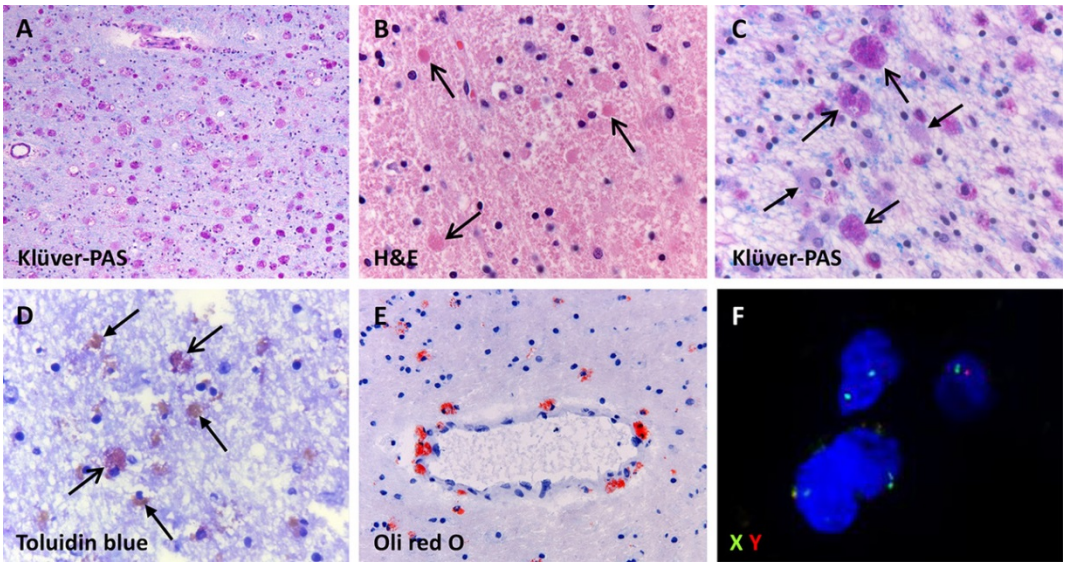

Figure 2. Donor cells reach the brain of transplanted MLD patients. (A, patient 5) Stain with Klüver (blue dye for myelin) and periodic acid Schiff (PAS, pink, stain for sulfatides in macrophages) of the cerebral periventricular white matter shows loss of myelin and abundance of cells loaded with PAS-positive granular material. (B, patient 1) Hematoxylin&Eosin stain of the frontal subcortical white matter shows presence of macrophages with intense eosinophilic cytoplasm (arrows) next to macrophages loaded with clearer granular material. (C, patient 1) A Klüver-PAS stain of the same region confirms the presence of a double population of macrophages, more (open arrows) and less (closed arrows) intensely PAS-positive. (D, patient 2) Toluidine stain of the parietal white matter reveals that only a subset of macrophages is metachromatic (purple, i.e. loaded with sulfatides), the remaining being orthochromatic (brown). (E, patient 1) Metabolic competence of a subset of macrophages is confirmed by their ability to digest sulfatides to cholesterol, as shown in this Oil Red-O stain for neutral fats. (F, patient 1) FISH against the X and Y chromosomes confirms cells of both sexes in the brain of this transplanted child.

Looking at oligodendrocytes, we found that numbers of both oligodendrocyte precursors and mature myelin-forming oligodendrocytes was significantly higher in transplanted than untreated patients (figure 4). The amount of total myelin as assessed by PLP immunopositivity was unchanged, indicating that in the white matter of treated patients, oligodendrocytes and their precursors may survive despite a similar degree of myelin loss.

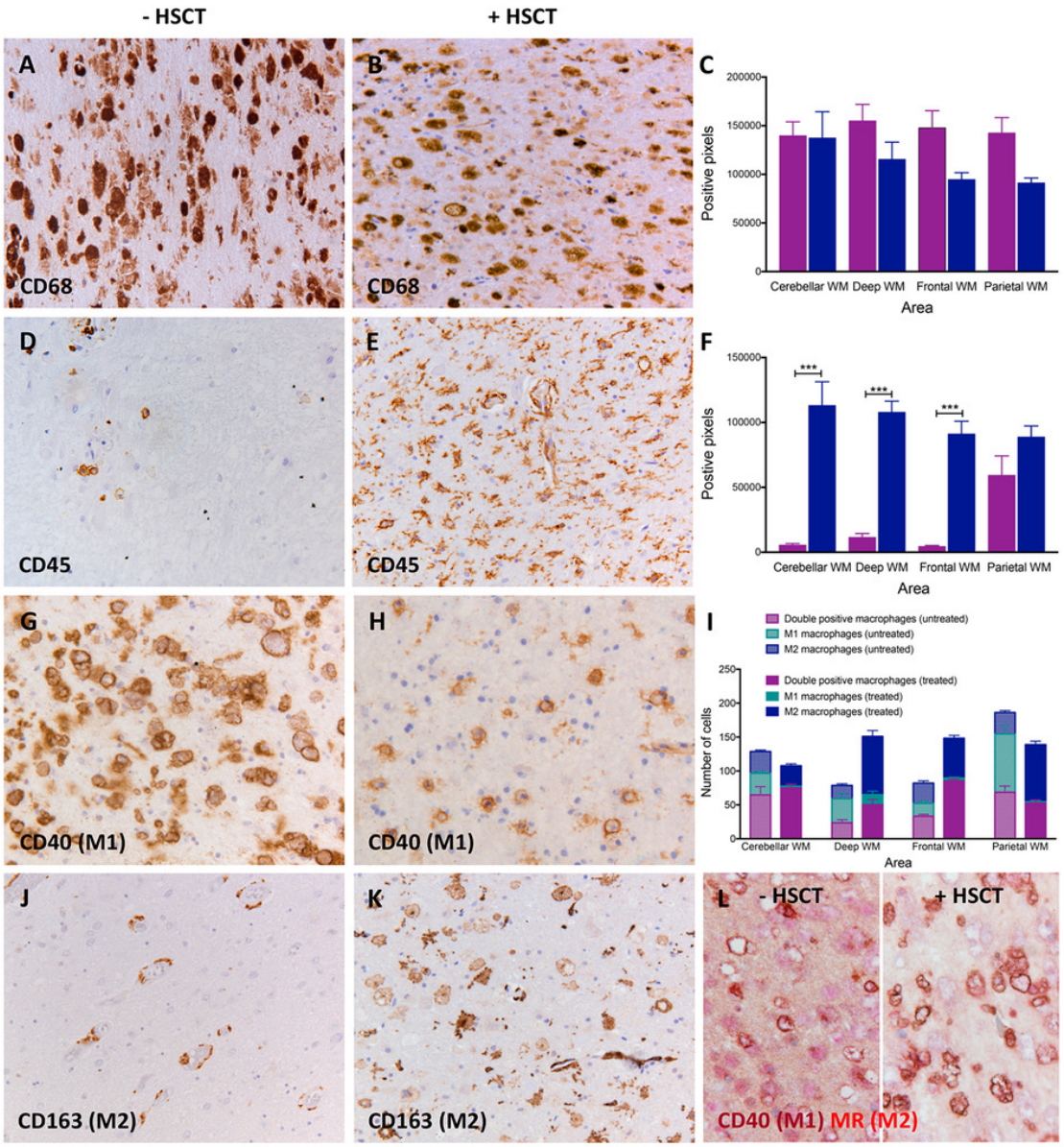

Figure 3. Robust microglia/macrophage activation and M2-like polarization in the white matter of transplanted MLD patients. (A-C) stain against the general macrophage marker CD68 shows similar abundance of immunopositive cells in transplanted (blue bars) and untreated patients (purple bars). (D-F) Stain against CD45, a marker for activated microglia/macrophages, reveals a strong microglia cell activation in the white matter of transplanted compared to untreated patients (color bars as in A-C). (G,H and J,K) Stain against the M1 marker CD40 shows more marked M1-polarization of microglia/macrophages in the brains of untreated compared to transplanted patients, whereas stain against the M2-marker CD163 shows the opposite. (I) Quantification of M1- versus M2- versus double polarized cells demonstrates lower numbers of M1-polarized cells and greater numbers of M2- and double polarized cells in treated compared to untreated patients.

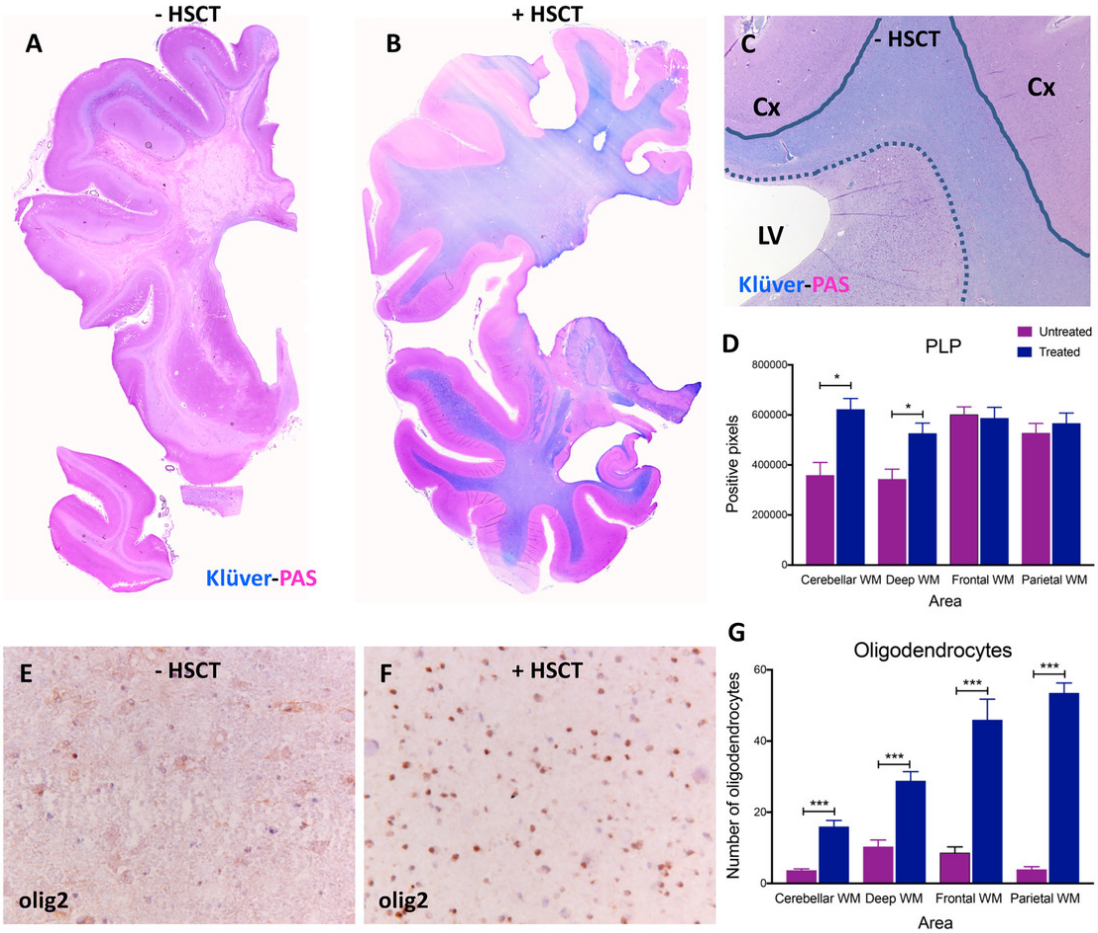

Figure 4. Transplantation prevents loss of white matter oligodendrocytes. Stain with Klüver-PAS of whole mount coronal brain slices of an untreated (A, patient 3) and a transplanted patient (B, patient 1) shows a variable degree of myelin loss in the periventricular and deep white matter, with relative sparing of the subcortical white matter and U-fibers. (C, patient 4) Klüver-PAS stain of an untreated patient shows the centrifugal progression of the demyelinating process, with the periventricular white matter (below the dotted line) containing less myelin than the subcortical white matter (between dotted and solid line). (D) Quantification of immunoreactivity against the myelin protein proteolipid protein (PLP) confirms that myelin loss is more marked in the deep white matter of untreated compared to transplanted patients, whereas myelin amounts in the subcortical frontal and parietal regions are comparable. (E-G) Stain against the oligodendrocyte lineage-specific marker olig2 shows marked loss of oligodendrocytes in untransplanted patients (E, patient 6), but preservation of cells in transplanted patients (F, patient 2). In treated patients (F), cells with more or less intense immunoreactivity are appreciable, corresponding to oligodendrocyte progenitors and mature cells. Quantification (G) confirms that oligodendrocyte numbers are much higher in the white matter of treated compared to untreated patients.

Discussion

In brain tissue of MLD patients, HCT leads to presence of metabolically competent macrophages, able to digest sulfatides, as expected. There was clear macrophage activation in transplanted patients, notably with a polarization of these macrophages towards an M2-like phenotype. Oligodendrocyte precursors and mature myelin-forming oligodendrocytes were present in higher numbers in brain tissue of transplanted than untreated patients,

suggesting an explanation for the improvement of MRI white matter changes in effective treatment.[4,5]

The obvious limitation of our study is that transplantation in the two HCT-treated patients was not sufficient to halt disease progression. Evidently, brain tissue of successfully treated MLD patients cannot be obtained. Nonetheless, we found key differences between treated and untreated patients that help explain the improvement of MRI WM abnormalities in successfully treated patients. In the WM, M1-like macrophages are considered detrimental whereas M2-like cells support regeneration. The potential of M2-like macrophages to sustain oligodendrocyte survival and remyelination was proven in vitro. Following demyelination, activated microglia signal to OPCs inducing them to proliferate and mature into myelin-forming cells.[17] M1-like macrophages dominate early after myelin loss and promote OPC proliferation. A switch to a M2-dominant phenotype is then necessary to bring about timely OPC differentiation. Consistent with this, M2-like macrophages predominate over M1-like cells in active multiple sclerosis lesions ongoing remyelination.[18] The findings of higher (mature) oligodendrocyte numbers in the WM of HCT-treated MLD patients suggests that HCT supports such M1-to-M2 switch resulting in a macrophage population that supports OPC survival and differentiation. In addition, ongoing myelin loss with abundance of myelin debris is a potent inhibitor of oligodendrocyte progenitor proliferation and maturation.[19] This myelin loss is moderated by metabolically competent macrophages. Both mechanisms are prerequisites for remyelination. Interestingly, in a *Plp1*-overexpressing mouse model for Pelizaeus-Merzbacher disease, the prototype hypomyelinating leukodystrophy,[20] transplantation of neural and oligodendrocyte progenitor cells is also able to modify CNS inflammation by microglia polarization towards an M2-like phenotype, resulting in remyelination and prolonged survival.[21] This indicates that, besides myelin restoration, modulation of inflammation may be necessary to promote clinical recovery.

Therapeutic strategies for MLD (and other lysosomal disorders) are changing: non-cellular options as enzyme replacement therapy and substrate inhibitors are being explored [22] and

gene therapy by autologous genetically manipulated HCT has been shown effective.[7,23] Likely, MLD treatment in the future will be multimodal. Our data point to additional beneficial effects of HCT beyond cross-correction of enzyme deficiency that could be further exploited in order to improve outcome, probably not only for MLD, but also for other leukodystrophies treated with HCT (e.g. Krabbe disease) and acquired white matter disorders as multiple sclerosis.

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III

Quantitative MRI

Chapter 7

Quantitative MR spectroscopic imaging in metachromatic leukodystrophy: value for prognosis and treatment

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Abstract

Objective: To determine whether proton magnetic resonance spectroscopic imaging is useful in predicting clinical course of patients with metachromatic leukodystrophy (MLD), an inherited white matter disorder treatable with hematopoietic cell transplantation (HCT).

Methods: 21 patients with juvenile or adult MLD (12 HCT treated) were compared to 16 controls in the same age range. Clinical outcome was determined as good, moderate, or poor. Metabolites were quantified in white matter, and significance of metabolite concentrations at baseline for outcome prediction was assessed using logistic regression analysis. Evolution of metabolic changes was assessed for patients with follow-up examinations.

Results: In this retrospective study, 16 patients with baseline scans were included, 5 with good, 3 with moderate, and 8 with poor outcome, and 16 controls. We observed significant group differences for all metabolite concentrations in white matter ($p<0.001$). Compared with controls, patients had decreased N-acetylaspartate and glutamate, and increased myo-inositol and lactate, most pronounced in patients with poor outcome (post-hoc, all $p<0.05$).

Logistic regression showed complete separation of data. Creatine could distinguish poor from moderate and good outcome, the sum of glutamate and glutamine could distinguish good from moderate and poor outcome, and N-acetylaspartate could distinguish all outcome groups.

For 13 patients (8 with baseline scans) one or more follow-up examinations were evaluated, revealing stabilisation or even partial normalisation of metabolites in patients with moderate and good outcome, clearly visible in the ratio choline/N-acetylaspartate.

Conclusion: In MLD, quantitative spectroscopic imaging at baseline is predictive for outcome and aids in determining eligibility for HCT.

Introduction

Metachromatic leukodystrophy (MLD, Online Mendilian Inheritance in Man (OMIM) 250100) is an autosomal recessive lysosomal disorder with deficient activity of arylsulfatase A (ASA), essential for sulfatide degradation.[1] Sulfatides are major myelin components; their accumulation in MLD results in demyelination and neurological decline.[2]

MLD has 3 clinical subtypes, based on age of onset. The late-infantile form starts < 30 months of age, the juvenile form between 2.5 and 16 years and the adult form thereafter.[1] Hematopoietic cell transplantation (HCT) shows promising results, especially for patients with juvenile and adult MLD in early disease stages.[3-5] Eligibility for HCT is currently based on neurological examination and cognitive function; the degree of MRI abnormalities[6] is also prognostic.[7,8] Proton magnetic resonance spectroscopy (^{1}H-MRS) provides additional metabolic information, as either single voxel MRS or MR spectroscopic imaging (MRSI).[9] MLD spectra are characterized by decreased N-acetylaspartate (NAA), elevated myo-inositol (Ins) and choline-containing compounds (Cho) in abnormal white matter (WM).[10,11]

This study investigates the relationship between clinical outcome in patients with juvenile or adult MLD and WM metabolite concentrations at baseline. All examinations at diagnosis at the same scanner using two-dimensional (2D) MRSI, for simultaneous acquisition of spectra in multiple regions of a single slab[9] were defined as baseline scans. For patients with follow-up examinations, we additionally studied longitudinal evolution of metabolites.

Methods

Patients and control subjects

In this retrospective study, approved by the institutional review board, we included 21 MLD patients (12 juvenile, 9 adult) and 16 subjects who served as controls (same age range; median 10.6 y, range 4.2-29.6 y; undergoing MRI for reasons like mild developmental delay, tics, headache; they had normal MRI and neurological examination) who underwent quantitative MR imaging including MRSI between January 2007 and April 2013. One patient

was described before.[12] MLD was diagnosed by brain MRI, ASA activity and *ARSA* mutation analysis. Patient characteristics and number of examinations at the same scanner are summarized in Table 1.

Table 1. Characteristics of MLD patients

MLD code	MLD type	Age / y [a]	Baseline scan [b]	Follow-up scans [b]	Eligible for HCT	Treated with HCT	Outcome
37	Juvenile	6.4	No	5	Yes	Yes	Moderate
16	Juvenile	6.5	Yes	1	Yes	Yes	Good
39	Juvenile	7.0	Yes	0	No	No	Poor
29	Juvenile	7.1	Yes	0	No	No	Poor
53	Juvenile	7.1	Yes	1	No	Yes	Poor
5	Juvenile	7.2	Yes	0	No	No	Poor
4	Juvenile	8.1	No	1	Yes	Yes	Moderate
33	Juvenile	8.1	No	1	No	No	Poor
6	Juvenile	8.6	Yes	0	No	No	Poor
54	Juvenile	12.5	Yes	0	No	No	Poor
14	Juvenile	14.1	Yes	3	Yes	Yes	Good
22	Juvenile	15.1	Yes	0	No	No	Poor
21	Adult	17.8	Yes	1	Yes	Yes	Good
30	Adult	20.1	No	5	Yes	Yes	Good
51	Adult	22.5	Yes	0	No	Yes	Poor
41	Adult	25.4	Yes	2	Yes	Yes	Good
32	Adult	26.9	Yes	1	Yes	No	Moderate
2	Adult	28.4	Yes	3	Yes	Yes	Good
46	Adult	28.8	No	1	Yes	Yes	Good
23	Adult	32.5	Yes	0	No	No	Moderate
15	Adult	35.2	Yes	2	Yes	Yes	Moderate

a) Age at first examination at 1.5T Siemens Sonata.
b) At 1.5T Siemens Sonata.

Eleven patients were eligible for HCT (four juvenile, seven adult); twelve received HCT: ten eligible (one eligible adult patient declined HCT), and two initially classified as non-eligible, after careful consideration and discussion with parents. HCT was performed as previously described.[7]

For 16 patients baseline data at the 1.5T Siemens Sonata (Erlangen, Germany) were available, and follow-up data for eight of these. The dedian age at baseline scan was 14.6 y

(range 6.5-35.3 y). At latest clinical follow-up, five (all eligible and treated) had good outcome, three (two eligible, one treated) moderate and eight poor outcome (all non-eligible, two treated, three deceased).

For five additional patients, MRI scans at diagnosis were obtained with a different protocol at another scanner or in another hospital. For these patients, only follow-up data after HCT or diagnosis were available, (median interval 3.3 y (range 11 months -14.8 years) until first follow-up, median age at first follow-up 8.1 years, range 6.4-28.8 years). Two adult patients (eligible and treated) had good outcome at latest follow-up, two juvenile patients (eligible and treated) had moderate outcome and one juvenile patient (non-eligible and untreated) had poor outcome (now deceased).

Clinical status

Motor function was scored by the MLD Gross Motor Function (MLD-GMF).[13] Cognitive function was determined through developmental or IQ testing and clinically estimated in evidently non-eligible patients. Depending on age and proficiencies, cognitive function was assessed using the Wechsler Intelligence Scale for Children-III (6–18 years), the Wechsler Nonverbal Scale of Ability (4–22 years) or the Wechsler Adult Intelligence Scale-III (≥ 18 y).[7] Median follow-up of surviving transplanted patients was 7.7 years (range 4-16.9 years), of surviving non-transplanted patients 4.1 years (range 2.7-7.8 years). Clinical outcome at latest follow-up was defined as good (MLD-GMF ≤1, IQ deterioration ≤5 points), moderate (MLD-GMF 2-4, IQ deterioration >5 points) or poor (MLD-GMF ≥5 or deceased). While peripheral neuropathy contributed to a suboptimal outcome in two patients, their classification was not influenced by this. Accordingly, classification reflected central nervous system (CNS) involvement for all patients.

MR Data Acquisition

All subjects were examined at 1.5T (Siemens Sonata) with an eight-channel phased-array head-coil. MR imaging included axial T2-weighted fast spin-echo images (repetition time

(TR) 2450 ms, echo times (TE) 24/85 ms, 4 mm slice thickness, in-plane resolution 1 mm), axial fluid attenuated inversion recovery (FLAIR) images (TR/TE/ inversion time (TI) 9000/108/2500 ms, 5 mm slice thickness, in-plane resolution 1 mm), and sagittal 3D T1-weighted images using a 3D magnetization prepared rapid acquisition gradient echo sequence (TR/TE/TI 2700/5/950 ms, 1 mm isotropic voxels). MRSI was obtained with point resolved spectroscopy localization (TR/TE 3000/30 ms, 6 acquisitions with weighted phase-encoding) on a single 15 mm slice (field of view 160x160 mm^2, volume of interest (VOI) 80x100 mm^2, 16x16 phase-encodings, voxel size 10 x10x15 mm^3) centered onto the corpus callosum (Figure 1A). Unsuppressed water reference scans were obtained with head and body coil.[14]

MR Data Analysis

For quantification of metabolite concentrations measured by MRSI, we used the signal intensity of water in the unsuppressed reference scans obtained with head coil and body coil, i.e. the voxel-wise ratio SI_{body}/SI_{head} multiplied by the transmitter amplitude of the body coil.[15-17] All spectra within the VOI were quantified using LCModel.[18] Metabolite concentrations (in mmol/L VOI) were reported for creatine and phosphocreatine (Cr), NAA (including contributions of N-acetyl-aspartylglutamate), Cho, Ins, glutamate (Glu), and lactate (Lac). Because estimation of glutamine (Gln) was less reliable in the majority of spectra, indicated by Cramer Rao lower bounds (CRLB) above 20%,[18] we also reported Glx, which is the sum of Glu and Gln. Although the low concentration of Lac in controls and some patients inherently led to large CRLB values as well, we included all estimated Lac concentrations because they were necessary for comparison with patients in whom Lac was clearly detectable.

Lesions were outlined and quantified on FLAIR using the semi-automatic tool *clusterize*[19] and linearly registered to 3D T1 (Figure 1). Lesion masks in 3D T1 were filled with the signal

intensity of normal-appearing WM (NAWM) to ensure correct segmentation into grey matter (GM), WM and cerebrospinal fluid (CSF) with SIENAX from FMRIB's software library FSL.[20]

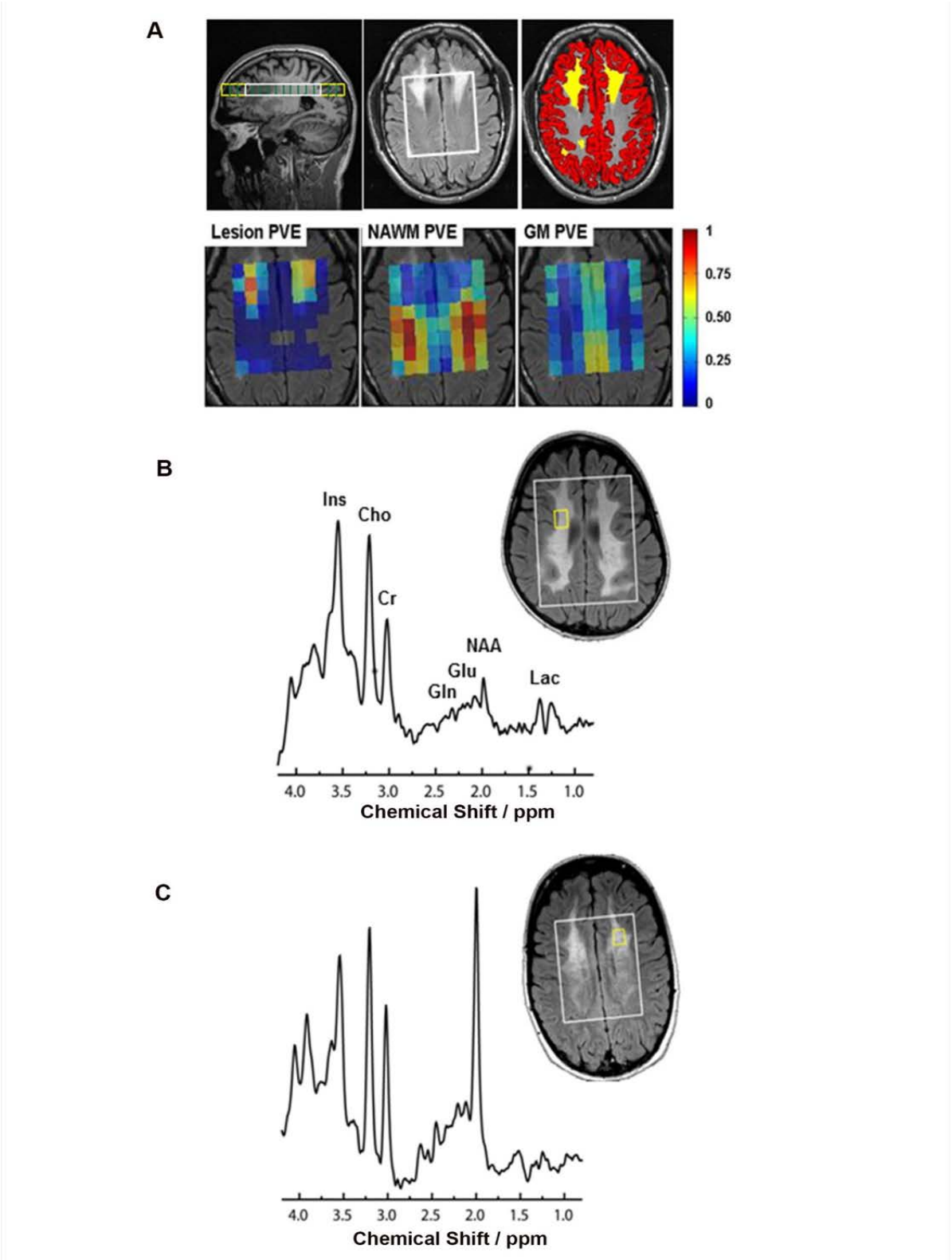

Figure 1: A: Top: sagittal 3DT1 weighted image showing position of CSI slab (field of view in yellow, PRESS VOI in white), original FLAIR image with rectangular PRESS VOI in white, FLAIR with lesions as outlined with clusterize (yellow), NAWM (gray scale) and cortical GM (red) as determined on lesion-filled 3DT1 weighted

image. Bottom: partial volume estimates (PVE) of respective tissue classes within MRSI grid. B,C: Spectra (1.5T, PRESS, TR/TE 3000/30 ms) of selected and indicated WM voxels in 2 patients at baseline. B: juvenile MLD, no HCT, poor outcome. NAA concentration in overall WM is 0.92 mM. C: juvenile MLD, with HCT, good outcome. NAA concentration is 5.9 mM. Indicated concentrations are based on analysis of all WM voxels.

Because the MRSI slab may include subcortical areas, these were segmented with FIRST, also part of FSL.[20] Extrapolation analysis combining the MRSI slab and partial volume estimates of CSF and tissue from segmentation resulted in concentrations for GM, overall WM, and NAWM and lesions separately. In this analysis concentrations in CSF are assumed negligible, and concentrations in mM indicate mmol/L tissue. Because of large variability between patients in lesion volume, our main analysis involved concentrations in overall WM (NAWM + lesions), unless otherwise indicated. Subcortical areas had no or only a minor contribution to the MRSI slab and were taken into account for determining the other tissue concentrations, but not further evaluated.

The quantitative analyses were not blinded for patient characteristics, but apart from the semi-automatic lesion segmentation, all analyses were user-independent.

Axial T2-weighted images were scored using the MLD-MRI score.[6]

Statistical analysis

We performed statistical analysis (using IBM SPSS V.22) for patients with baseline examinations and the control subjects. We first corrected for age-dependency: for each metabolite, we estimated the parameters a, b and tau based on a mono-exponential fit ($a+b \cdot \exp(-age / tau)$) through the control measures. For both control subjects and patients, we subtracted the predicted concentration at corresponding age from the observed concentration (residuals).[14] A general linear model was used for overall comparison of these age-corrected metabolite concentrations (residuals) at baseline. Post-hoc, we compared differences between all four groups, i.e. patients with good, moderate or poor outcome, and controls, with Dunnett's T3 (correcting for multiple comparisons in case of small groups with unequal variances). MRI scores and lesion volumes were similarly compared between patients. $p<0.05$ was considered significant.

For patients with baseline examinations, we intended to use logistic regression to determine which (age-corrected) metabolites at baseline explained outcome. However, data showed complete separation, as illustrated by scatterplots of age-corrected metabolite concentrations (shown in Results). Therefore, we distinguished groups based on visual comparison of scatterplots.

Spearman's rank correlations were determined between baseline metabolite concentrations and MLD-GMF at latest follow-up.

Results

Metabolite concentrations at baseline and clinical outcome

In all MLD patients at baseline, there were significant group differences in MRI score and lesion volume ($p \leq 0.001$). Patients with poor outcome had significantly larger lesion volumes than patients with moderate or good outcome, $p < 0.001$ (Table 2), such that overall WM represents predominantly lesions in poor outcome patients.

Table 2. Demographics and quantitative MR results of control subjects and MLD patients at baseline

	controls		good outcome		moderate outcome		poor outcome	
n - male/female	16	8m/8f	5	2m/3f	3	3f	8	1m/7f
age / y median	10.6		17.8		32.5		7.9	
range	4.2 - 29.6		6.5 - 28.4		26.9 - 35.3		7.1 - 22.5	
Cr / mM [a]	3.77 ± 0.38		4.80 ± 0.52 [b]		4.61 ± 0.58		3.12 ± 0.82 [c]	
NAA / mM [a]	6.99 ± 0.65		6.53 ± 0.98		5.56 ± 0.20 [b]		1.99 ± 0.73 [b,c,d]	
Cho / mM [a]	1.49 ± 0.21		2.11 ± 0.23 [b]		1.84 ± 0.24		1.67 ± 0.41	
Ins / mM [a]	2.95 ± 0.46		6.85 ± 1.41 [b]		7.25 ± 1.25		7.05 ± 1.88 [b]	
Glu / mM [a]	4.68 ± 0.50		4.80 ± 0.80		4.46 ± 0.75		1.68 ± 0.55 [b,c,d]	
Glx / mM [a]	6.88 ± 0.59		8.43 ± 0.38 [b]		6.50 ± 0.89		4.82 ± 1.28 [b,c]	
Lac / mM [a]	0.33 ± 0.08		0.59 ± 0.14		0.80 ± 0.40		1.57 ± 0.66 [b,c]	
MRI score [a]			9.4 ± 5.4		14.7 ± 3.2		20.1 ± 3.0 [c]	
lesion volume / mL [a]			32.5 ± 20.7		43.9 ± 11.8		111.7 ± 25.1 [c,d]	

Values are given as mean with standard deviation, unless indicated otherwise
Actual metabolite concentrations in overall WM (NAWM + lesions) are shown, but statistical comparisons were performed on age-corrected values (see methods).
a: group-wise GLM, $p < 0.001$; b,c,d: post-hoc Dunnett's T3, $p < 0.05$, b) compared to controls, c) compared to good outcome, d) compared to moderate outcome.

We observed significant group differences (p<0.001) for all investigated metabolites (Table 2, Figure 2). Compared with controls, differences were most pronounced in patients with poor outcome, for whom concentrations of NAA, Glu and Glx were severely reduced, while Ins and Lac were increased (all p<0.01). Patients with moderate outcome had decreased NAA (p<0.01), and patients with good outcome had increased Cr, Cho, Ins and Glx compared with controls (p<0.05). Differences in metabolite concentrations compared with control WM were most prominent in lesions, and less so in NAWM (data not shown).

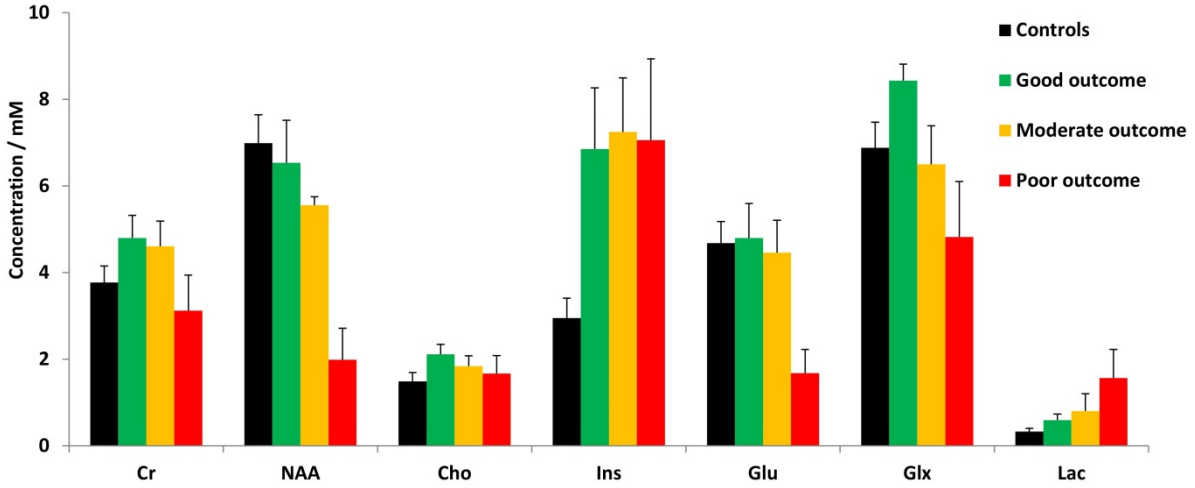

Figure 2: Mean metabolite concentrations in overall WM (NAWM+lesions) of control subjects (black, n=16) and MLD patients at baseline, categorized per clinical outcome: good outcome (green, n=5), moderate outcome (orange, n=3), poor outcome (red, n=8). Error bars indicate standard deviations. Based on age-corrected values, all metabolites showed significant overall group differences (p<0.001). See Table 2 for differences between groups with post-hoc Dunnett's T3 analysis.

Scatterplots for age-corrected concentrations of metabolites that were different between patient outcome groups (i.e. NAA, Cr, Glx and Lac) are shown in Figure 3. This figure illustrates that patients with poor outcome were completely separated from moderate and good outcome based on NAA and also on Cr. Vice versa, patients with good outcome were completely separated from moderate and poor outcome based on NAA and also on Glx. Despite prominent differences in mean Lac between groups, Lac could not uniquely distinguish the groups. Scatterplots for MRI score and lesion volume (Figure 3E, F) show that these parameters had less power to distinguish good and moderate outcome.

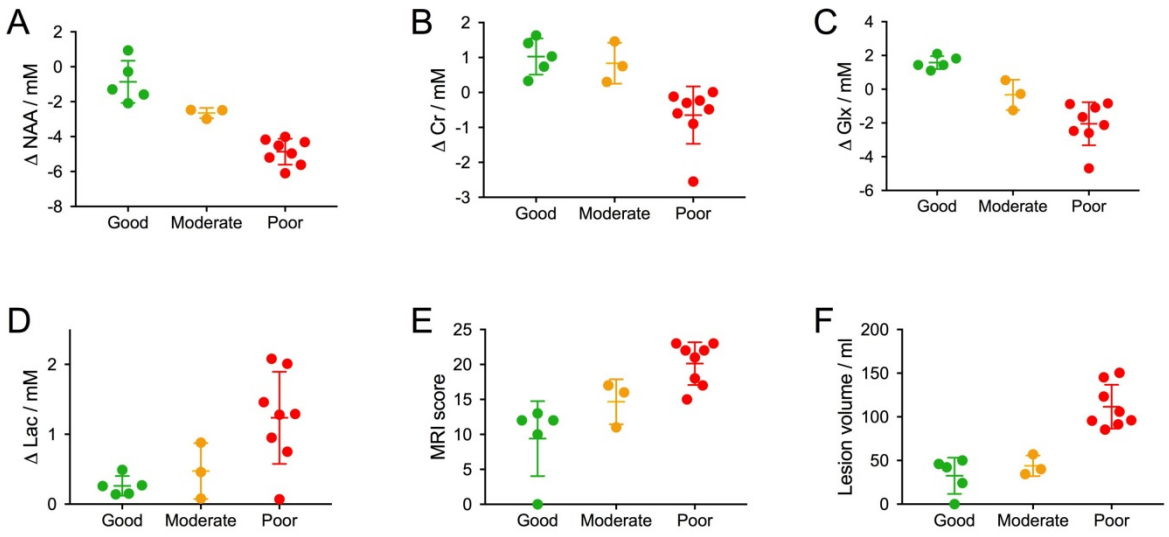

Figure 3: Scatterplots per clinical outcome. A-D: age-corrected WM metabolite concentrations. E: MRI score. F: lesion volume. Lines indicate mean and standard deviation.

The natural course of the disease inevitably results in poor outcome after a longer follow-up. To achieve insight into the pace of disease progression, we also evaluated outcome for non-transplanted patients two years after diagnosis, which altered outcome for only one patient (good 2 years after diagnosis, moderate at end of follow-up). In this case, the moderate outcome group contained only 2 patients, and was marginally separated from the good outcome group.

Spearman's rank correlations between metabolite concentrations at baseline and motor performance at latest follow-up showed a strong correlation between MLD-GMF and NAA ($r=-0.78$, $p<0.001$, Figure 4), as well as with age-corrected NAA ($r=-0.75$, $p=0.001$).

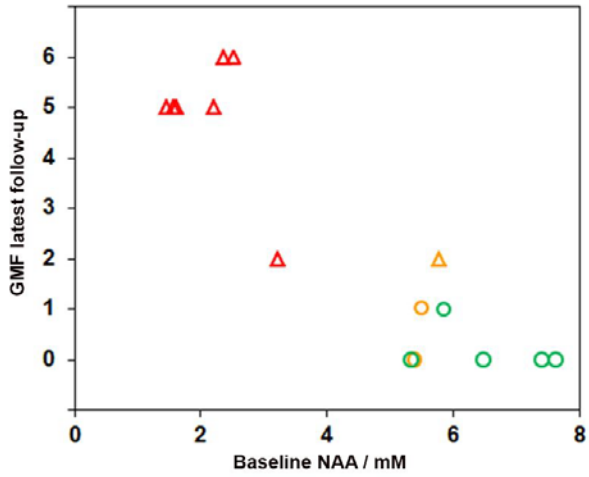

Figure 4: MLD-GMF score at latest follow up as function of WM NAA concentration at baseline. Spearman's rank correlation = -0.78 ($p<0.01$). Green, orange and red symbols correspond to patients with good, moderate and poor outcome, respectively. Non-eligible patients are indicated with triangles, and eligible patients with circles.

Longitudinal evaluation

Baseline and longitudinal WM concentrations of NAA, the concentration ratio Cho/NAA, MRI scores, and lesion volumes for all patients are shown in Figure 5. In general, absolute metabolite concentrations showed longitudinal fluctuations, for NAA as well as for Cr, Glx and all other metabolites (not shown), comparable to those observed in healthy control subjects.[17]

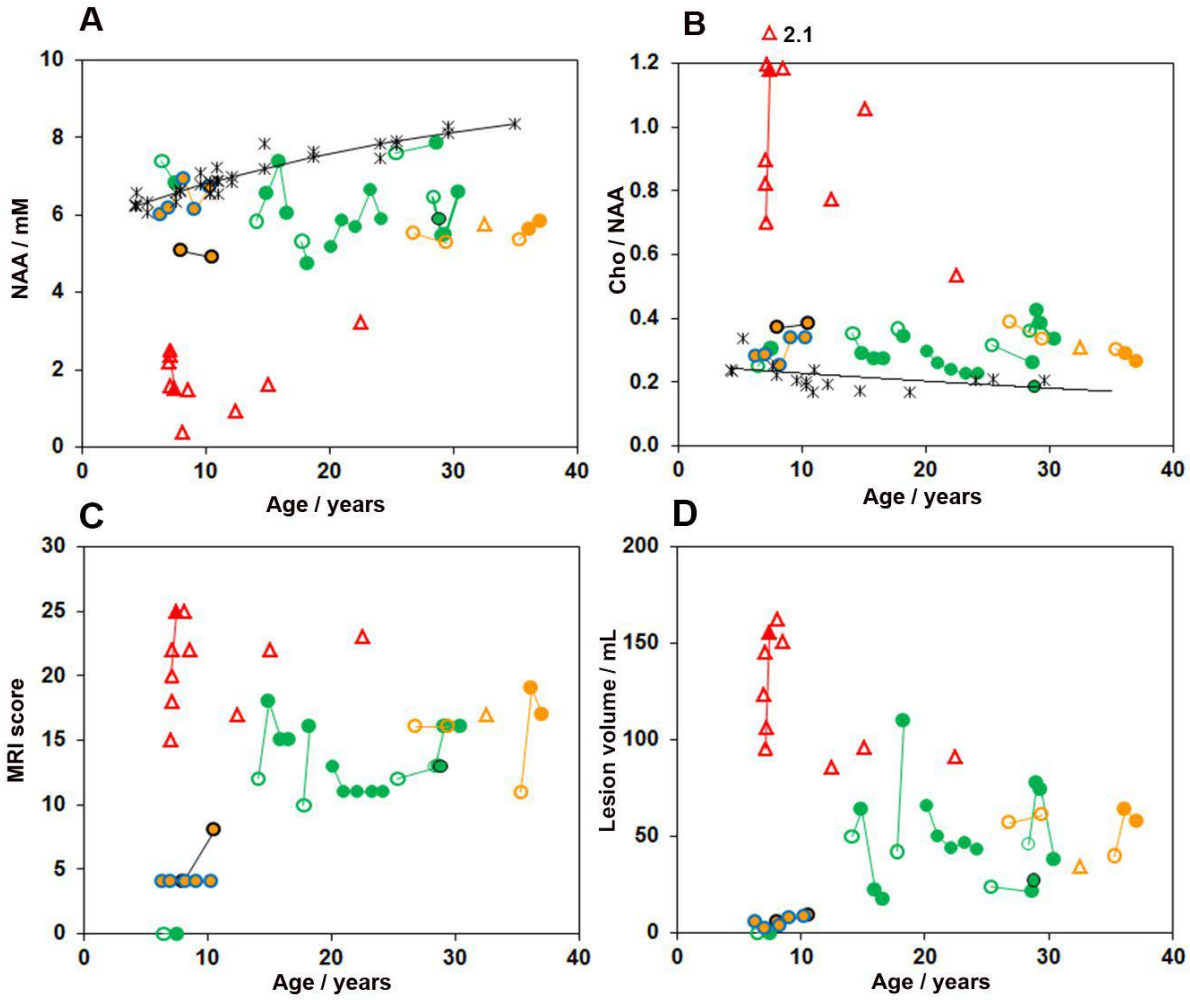

Figure 5: Longitudinal WM metabolite concentrations of (A) NAA, (B) concentration ratio Cho/NAA (a single extreme value of 2.1 outside the range is shown at the top border). C: longitudinal MRI score. D: longitudinal lesion volumes. Control values are indicated with black asterisks, and the black line is the mono-exponential fit visualizing the age-dependency. Green, orange and red symbols correspond to patients with good, moderate, and poor outcome, respectively. Non-eligible patients are indicated with triangles, and eligible patients with circles. After patients are transplanted, symbols are filled. To distinguish overlapping symbols (patients of the same age with similar scores), symbols are outlined and connected with different colors. The treated eligible patient (good outcome) that was described before had her first scan at the age of 14.1 y.

Concentration ratios have higher reproducibility, and since we previously showed that Cho/NAA is sensitive for improvement after HCT,[12] we chose to display this ratio in figure 5B. Although this ratio slightly increased in some patients in the first examination after HCT, this was reversible in all patients with good outcome.

In adult patients with moderate outcome, we also observed ongoing decrease of Cho/NAA, but this ratio did not decrease in two juvenile patients with moderate outcome. Remarkably, in several patients with good or moderate outcome we observed a decrease of Cho/NAA, which did not coincide with a decrease in MRI score or lesion volume (Figure 5C,D). In the treated non-eligible patient who died 11 months post-HCT, metabolites and imaging markers all clearly deteriorated as both Cho/NAA and the MRI score and lesion volume strongly increased after HCT.

Discussion

Metabolite concentrations at baseline in relation to outcome

MRS is a powerful method to gain insight into brain metabolites, thereby contributing to understanding pathological processes. Reduced concentrations of NAA and Glu are associated with neuroaxonal damage.[9-11] Increased Lac reflects energy failure,[9] probably also macrophage/microglia activation,[21] and oligodendrocyte injury leading to limiting Lac transport into axons.[22] Cho is increased in regions of active demyelination.[9-11,23,24]

Using MRSI in juvenile and adult MLD, WM metabolite concentrations at diagnosis correlate well with outcome, with NAA as main explanatory variable. This is underlined by the strong association between baseline NAA concentrations and motor function at follow-up in our cohort, but also in late-infantile MLD,[25] indicating that preserved neuroaxonal function is a prerequisite for good or moderate clinical outcome. The same conclusion was drawn in an MRS study on outcome after HCT in patients with X-linked adrenoleukodystrophy.[26]

Patients with poor outcome had severely reduced concentrations of NAA, Glu and Glx, and increased Lac and Ins. In patients with moderate outcome, most metabolite concentrations were closer to normal. Compared with controls, Cho was increased only in patients with good outcome, possibly reflecting signs of active demyelination and/or increased glial density. This latter explanation is supported by the observation of increased Cr and Glx in patients with good outcome compared to controls. Because concentrations of NAA and Glu are similar in patients with good outcome and controls, it can be assumed that axonal density is preserved in an early disease stage. The increased Glx must be due to a higher concentration of Gln, which together with Cr suggest a higher glial density in this disease stage.[27]

It should be noted that these results were obtained from metabolite concentrations in overall WM using a combined analysis of tissue segmentation and 2D MRSI. However, since lesions in MLD start to develop in periventricular WM, it can be assumed that single voxel MRS in a periventricular WM region will give comparable results.

Longitudinal evolution

Even in some patients with good outcome, metabolic and imaging markers deteriorated in the first follow-up scan six months after transplantation, indicating ongoing disease activity and delayed treatment effect. Partial normalization of the Cho/NAA ratio in subsequent examinations in successfully treated patients implies reduction of demyelinating activity and some axonal tissue recovery. In several patients, Cho/NAA improvement indeed coincided with a reduction of MRI score and lesion volume, supporting our interpretation of tissue repair. However, in a few patients we could not observe a concomitant decrease of MRI score or lesion volume. This may be partly explained by the fact that the MRI score reflects WM damage at the level of the MRSI slab as well as WM involvements in other regions and atrophy of both supra- and infratentorial structures.

The small number of patients for each of the outcome groups is a limitation of this study, although the total patient number, given the fact that the study was performed in a single center on the same scanner, is rather large for a rare disorder. In addition, age distribution differed between groups, a fact which can hardly be prevented, because treatment eligibility and success are related to age of onset. Finally, the inevitable selection bias of this retrospective study results in an eventual poor outcome for all non-transplanted patients.

Despite these difficulties, this study shows that quantitative MRSI has high discriminative power regarding clinical outcome in MLD already at diagnosis with NAA as the most important parameter. Severely abnormal metabolite concentrations at baseline indicate low probability of good outcome. Therefore, results are helpful in deciding whether HCT will be beneficial, certainly for patients with borderline neurological and cognitive examination.

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Chapter 8

Diffusion tensor imaging in metachromatic leukodystrophy

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In revision

Abstract

Objective: We aimed to gain more insight into the pathomechanisms of metachromatic leukodystrophy (MLD), by comparing magnitude and direction of diffusion between patients and controls at diagnosis and during follow-up.

Methods: Four late-infantile, 16 juvenile and 8 adult onset MLD patients (of which 13 considered eligible for hematopoietic cell transplantation (HCT)) and 47 controls were examined using diffusion tensor imaging. Fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD) were quantified and compared between groups using tract-based spatial statistics (TBSS). Diffusion measures were determined for normal-appearing white matter (NAWM), corpus callosum, thalamus (all based on subject-wise segmentation), and pyramidal tracts, determined with probabilistic tractography. Measures were compared between HCT-eligible patients, non-eligible patients and controls using general linear model and permutation analyses (randomise) for TBSS data.

Results: In both patient groups FA was decreased and MD and RD increased throughout WM, while AD was decreased in NAWM and corpus callosum. In the thalamus no differences in FA were observed, but all diffusivities were increased in both patient groups. Differences were most pronounced between controls and patients non-eligible for HCT. Longitudinally, diffusion measures remained relatively stable for HCT-treated patients, but were progressively abnormal for non-eligible patients.

Interpretation: The observed diffusion measures confirm that brain microstructure is changed in MLD, reflecting different pathological processes including loss of myelin and sulfatide accumulation. The observation of both increased and decreased AD probably reflects a balance between myelin and axonal loss versus intracellular storage in macrophages, depending on region and disease stage.

Introduction

Metachromatic leukodystrophy (MLD, OMIM 250100) is an autosomal recessive lysosomal disorder caused by mutations in *ARSA*. This results in deficiency of the enzyme arylsulfatase A (ASA), essential for sulfatide metabolism.[1] Sulfatides are major myelin lipids; their accumulation, mainly in membranes, leads to demyelination and subsequently storage in macrophages that cannot digest them.[1] MLD is a devastating disease: without treatment, eventually all acquired skills are lost and patients die.

MLD has three clinical subtypes, based on age of onset. The late-infantile form starts before 30 months, usually presenting with motor deterioration. The juvenile form presents with a combination of motor and cognitive decline before 16 years. The adult form begins with cognitive decline and psychiatric symptoms thereafter.[2] When performed early, hematopoietic cell therapy (HCT) has promising results, especially for juvenile and adult patients.[3,4]

Brain magnetic resonance imaging (MRI) in MLD is characterized by bilateral symmetric T2 signal hyperintensities, starting in the corpus callosum and subsequently involving the periventricular white matter (WM), followed by projection fibers and cerebellar WM.(5) Thalamic volume and signal intensity on T2-weighted images in the thalamus are decreased already at diagnosis.[6,7] Typical for MLD are stripes of low signal intensity throughout the hyperintense signal on T2-weighted images in the cerebral WM, related both to the accumulation of macrophages bursting with undigested lipids and to better preserved perivascular myelin.[8]

Brain diffusion tensor imaging (DTI) is based on the motion of water molecules, which is more restricted perpendicular to than along WM fibers, a feature termed diffusion anisotropy.[9] Magnitude and direction of diffusivity are determined by molecules, membranes and microtubules, and provide information about tissue composition and microstructure and its architectural organization.[10,11]

The tensor model is a relatively simple model using diffusion weighted images (DWI) obtained with one b-value. It results in axial diffusivity (AD), radial diffusivity (RD) and the derived fractional anisotropy (FA) and mean diffusivity (MD).(11) Often, RD is thought to be correlated to myelin degradation and AD to axonal degeneration or inflammation and gliosis,[12-17] but it is difficult to unequivocally associate the interpretation of diffusivity variations with specific biophysical changes.[18]

The precise pathomechanisms involved in MLD, such as importance of inflammation or how accumulated sulfatides lead to demyelination, are not completely understood. DTI is, taking into account its recognized limitations, a valuable tool to gain more insight into changes in tissue properties in MLD. We therefore compared diffusion measures (FA and the three diffusivities) between patients who were eligible for HCT, patients not eligible at time of diagnosis, and controls. HCT-eligible patients are typically in an early disease stage, while patients not eligible for HCT have more advanced disease with extensive demyelination of the WM. We also studied the longitudinal behavior of diffusion measures of both treated and untreated patients

Methods

Patients and control subjects: All 28 MLD patients (4 late-infantile, 16 juvenile, and 8 adult onset), visiting the Center for Childhood White Matter Disorders, who underwent a quantitative MRI protocol at time of diagnosis between January 2007 and April 2017 were included in this retrospective study, in addition to 47 control subjects in the same age range (Table 1), after informed consent.

Table 1. Subjects demographics

	Controls	All MLD patients	Eligible for HCT	Not eligible for HCT	Contrasts *p*
Number of subjects	47	28	13	15	
Male / female	23 / 24	9 / 19	6 / 7	3 / 12	(NS)
Age at first scan (mean , SD, y)	10.5 (5.3)	14.5 (9.5)	16.9(10.6)	12.4(8.3)	0.017[a]
Late-infantile /juvenile / adult		4 / 16 / 8	2 / 5 / 6	2 / 11 / 2	(NS)

[a] Post-hoc Dunnett's T3 revealed no significant pairwise group differences

The study was approved by the institutional review board. Diagnosis of MLD was established by brain MRI, ASA activity and *ARSA* mutation analysis.(4) Motor function was scored by the MLD Gross Motor Function (MLD-GMF) at baseline and at latest clinical follow up.[19] Cognitive function was evaluated through neuropsychological examination. Eligibility for HCT was based on patients' neurological examination (no major abnormalities and able to walk independently) and cognitive function (IQ>75). Treatment with HCT was performed as described before.[4] Characteristics of individual patients are described in Table 2. Thirteen patients were considered eligible for HCT, and 15 non-eligible for HCT. Fourteen patients received HCT (2 patients initially classified as non-eligible; one eligible patient declined HCT).[4] Follow-up MRI examinations were available for 17 patients (12 HCT-eligible, 5 non-eligible).

Table 2. Characteristics of MLD patients

MLD code	MLD type	Age/ y[a]	Baseline scan	Number of follow up scans	Eligible for HCT	HCT-treated
045	Late-infantile	2.0	1.5T	3 [b]	Yes	Yes
050	Late-infantile	2.1	1.5T	1 [b]	Yes	Yes
057	Late-infantile	2.4	3T	0	No	No
026	Late-infantile	2.6	1.5T	0	No	No
016	Juvenile	6.5	1.5T	3 [d]	Yes	Yes
039	Juvenile	7.0	1.5T	1 [c]	No	No
029	Juvenile	7.1	1.5T	0	No	No
053	Juvenile	7.1	1.5T	1 [b]	No	Yes
005	Juvenile	7.2	1.5T	1 [c]	No	No
065	Juvenile	7.4	3T	2 [c]	Yes	Yes
064	Juvenile	8.6	3T	0	No	No
006	Juvenile	8.6	1.5T	1 [c]	No	No
054	Juvenile	12.5	1.5T	1 [c]	No	No
060	Juvenile	13.1	3T	0	No	No
058	Juvenile	13.8	3T	3 [c]	Yes	Yes
014	Juvenile	14.1	1.5T	4 [d]	Yes	Yes
022	Juvenile	15.1	1.5T	0	No	No
067	Juvenile	17.6	3T	0	Yes	Yes
068	Juvenile	19.2	3T	0	No	No
061	Juvenile	20.1	3T	0	No	No
021	Adult	17.8	1.5T	3 [d]	Yes	Yes
051	Adult	22.5	1.5T	0	No	Yes
063	Adult	23.1	3T	1 [c]	Yes	Yes
041	Adult	25.4	1.5T	6 [d]	Yes	Yes
032	Adult	26.9	1.5T	2 [d]	Yes	No
002	Adult	28.4	1.5T	5 [d]	Yes	Yes
056	Adult	32.5	1.5T	0	No	No
015	Adult	35.2	1.5T	4 [d]	Yes	Yes

[a] Age at baseline examination
[b] follow-up examinations at 1.5T
[c] follow-up examinations at 3T
[d] follow-up examinations at both 1.5T and 3T

Control subjects at 1.5T had normal MRI and neurological examination. Controls at 3T had experienced a non-neurological trauma and were included in a previous study.[20]

Acquisition: Between January 2007 and April 2013, 19 patients and 20 controls were examined at 1.5T (Siemens Sonata, Erlangen, Germany). Between May 2013 and April 2017, 9 patients and 27 controls were examined at 3T (GE Signa HDxt and MR750, Milwaukee, WI).

Conventional imaging included sagittal 3-dimensional (3D)-T1 and axial FLAIR, using the same spatial resolution at both field strengths.[20,21] FLAIR imaging was not performed for control subjects at 3T. DTI was obtained with a multi-slice echo planar imaging sequence and isotropic 2.5x2.5x2.5mm^3 voxels. At 1.5T we obtained 1 b0 volume and 12 gradient directions with b-value 750s/mm^2, 2 acquisitions, TR/TE 6700/81 ms.(21) At 3T we obtained 5 b0 volumes and 30 gradient directions with b-value 750s/mm^2, 1 acquisition, TR/TE 5100/75 ms, and parallel imaging factor 2.[20]

Analysis: DTI data were analyzed using FMRIB's software library FSL after correction of distortion and subject motion. The diffusion tensor was fitted resulting in maps of FA, AD, RD and MD. Tract based spatial statistics (TBSS) was used to align FA images from all subjects into a common space and to create a mean FA skeleton. Each participants' aligned FA data were projected onto this skeleton and fed into voxel-wise cross participant statistics using randomise (see statistical analysis).[22]

Based on the regional differences found in the TBSS analyses we further analyzed diffusion measures in the following regions of interest (ROIs): normal-appearing white matter (NAWM, corpus callosum and thalamus in all subjects, and abnormal cerebral WM in patients. In addition, we analyzed the pyramidal tracts, which were determined for each subject by tractography between motor cortex and cerebral peduncles (see below).

To determine these ROIs in DTI subject space, we first outlined abnormal WM on 2D FLAIR images of patients using clusterize.[23] The mask of abnormal WM was registered to the corresponding 3DT1, and filled with signal intensity of NAWM. This 3DT1 image was then segmented with the FSL tools FAST [24] and FIRST [25] to obtain WM, grey matter (GM) and deep GM (DGM) structures, including the thalamus. DGM and abnormal WM were subtracted from the WM mask to obtain NAWM. ROIs for corpus callosum and cerebral peduncles were identified using the Johns Hopkins University (JHU) WM atlas defined in standard Montreal Neurological Institute (MNI) space.[26] The motor cortex was identified in

MNI space using the Automated Anatomical Labeling (AAL) atlas.[27] ROIs in MNI space were warped into 3DT1 subject space after linear and non-linear registration. All ROIs were subsequently registered to DTI subject space using nearest neighbor interpolation.

The ROIs of motor cortex and cerebral peduncles were used as seed and target for probabilistic tractography using the FSL tools bedpostx and probtrackx2 to obtain the left and right pyramidal tract.[28] Mean diffusion measures within the pyramidal tracts were determined by weighting the underlying FA and diffusivity maps by the probability of a voxel within the tract.

Statistical analysis: Statistical analyses were performed for HCT-eligible and non-eligible patients at baseline and control subjects. Groups were compared on demographic variables using ANOVA and chi-square tests, as appropriate. In the TBSS analysis FA, MD, AD and RD were compared between the three groups with nonparametric permutation analysis (randomise), using age and scanner as covariate. We considered a family-wise error corrected $p<0.05$ significant.

General linear model analyses including age and scanner as covariates were performed for a 3-group comparison of diffusion measures at baseline within selected ROIs. In case of main group effects, we performed post-hoc pairwise comparisons between groups, using Dunnett's T3.

The pyramidal tracts primarily regulate motor function. We therefore determined Spearman rank correlations between diffusion measures of all 28 patients at baseline and motor function, determined with MLD-GMF, at latest clinical follow-up. $P<0.05$ was considered significant.

Results

Baseline: Controls, HCT-eligible and non-eligible patients did not differ on demographic variables. Only for age a main group effect was detected (p 0.017), which was not reflected in post-hoc testing(Table 1).

In the TBSS analysis of all baseline examinations (see Fig. 1), FA was decreased in HCT-eligible and non-eligible patients compared to controls, and in non-eligible patients compared to eligible patients in almost the entire skeleton. An increase of MD and RD in both patient groups compared to controls was also observed in almost the whole skeleton. An increase of MD and RD in non-eligible patients compared to eligible patients was limited to a smaller part of the skeleton. An increase in AD in HCT-eligible patients compared to controls was restricted to part of the periventricular WM and the genu and splenium of the corpus callosum. In HCT-non-eligible patients compared to both controls and eligible patients, AD was increased mainly in the thalamus, but decreased in a large part of the skeleton, including the corpus callosum.

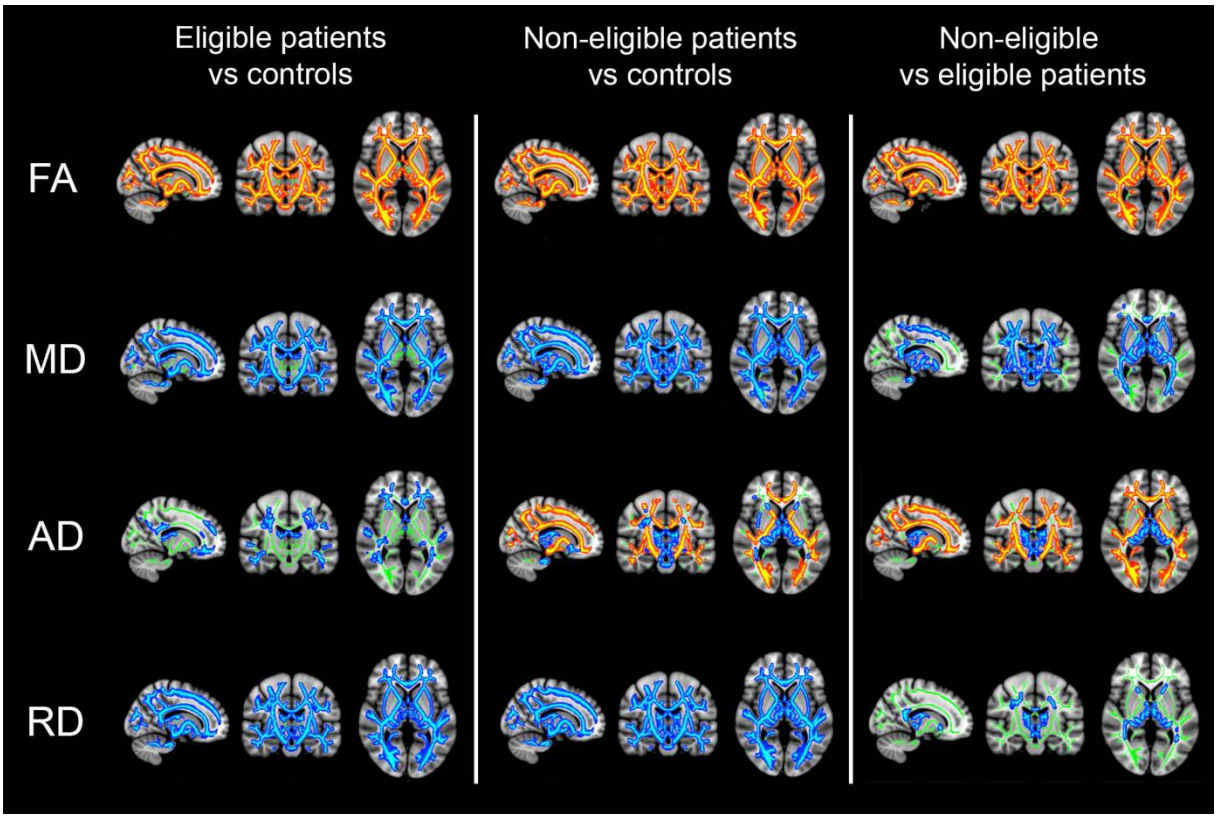

Figure 1. TBSS analysis for FA, MD, AD and RD comparing HCT-eligible patients vs control subjects (left column), non-eligible patients vs. control subjects (middle column) and non-eligible vs. eligible patients (right column). FA was decreased (orange-yellow) in eligible and non-eligible patients compared to controls and in non-eligible patients compared to eligible patients in almost the whole skeleton. MD and RD were increased (blue-lightblue) in both patient groups compared to controls, and in non-eligible patients compared to eligible patients. AD was increased in eligible patients compared to controls in parts of the skeleton. When comparing non-eligible patients to controls or to eligible patients, AD was increased mainly in the thalamus (blue-lightblue), and decreased in WM areas including the corpus callosum (orange-yellow). The part of the WM skeleton that does not differ between groups is indicated in green. A family-wise error corrected $p<0.05$ was considered significant.

In the selected ROIs (see Fig. 2), FA was decreased for both patient groups compared to controls in NAWM, corpus callosum and pyramidal tracts. Differences were most pronounced between controls and HCT-non-eligible patients. The relative and absolute decrease in FA was largest in corpus callosum. In patients, FA in abnormal WM was smaller than in NAWM, and lower in HCT-non-eligible patients than in eligible patients (not shown). Although the TBSS analysis showed group differences in FA in the skeletonized thalamus, there were no FA differences in the thalamus based on a ROI analysis.

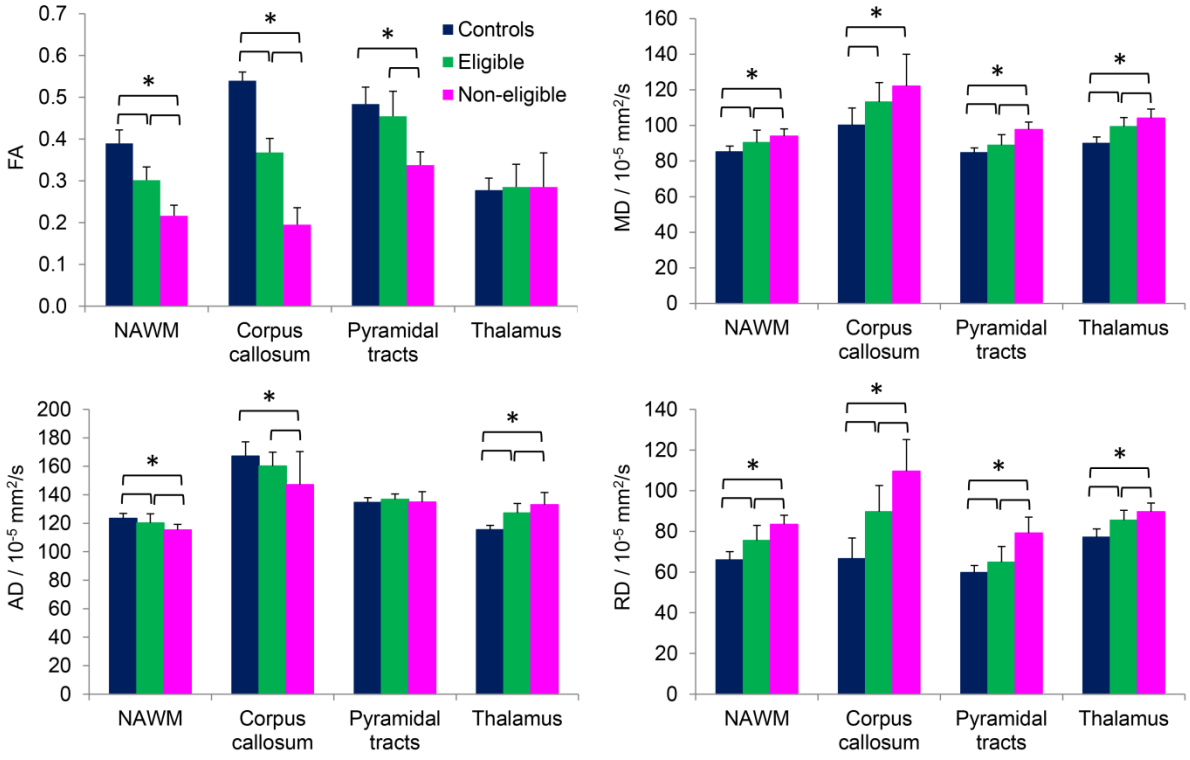

Figure 2. Mean values for FA, MD, AD and RD in NAWM, corpus callosum, pyramidal tracts and thalamus for control subjects (blue), eligible (green) and non-eligible (pink) patients. Error bars indicate standard deviations. Significant differences between groups are indicated with square brackets and a single asterisk (post-hoc Dunnett's T3, $p<0.05$).

Following the TBSS findings, MD and RD were increased in both patient groups in NAWM, corpus callosum, pyramidal tracts and thalamus, and differences were most pronounced

between controls and non-eligible patients. Differences in RD were larger than differences in MD, again with the corpus callosum showing most prominent differences between groups. MD and RD within abnormal WM were higher than in NAWM, but did not differ between patient groups (not shown).

In the pyramidal tracts, there were no group differences in AD. Following the TBSS findings, in NAWM and corpus callosum, AD was lower in both patient groups than in controls, whereas in the thalamus AD was higher in patients. Again, these differences were most pronounced between HCT-non-eligible patients and controls. AD within abnormal WM was higher than in NAWM, and lower in non-eligible patients than in eligible patients (not shown).

Spearman rank correlations with MLD-GMF were significant for FA (-0.84), MD (0.78) and RD (0.86), all $p<0.01$. Thus, low FA and high MD and RD of the pyramidal tracts at baseline indicate poor motor function at follow up.

Longitudinal evolvement of diffusion measures: Figure 3 shows longitudinal diffusion measures in selected ROIs. For each patient with follow-up measurements, symbols are connected by lines. The longitudinal variation indicates the actual course, but also the reproducibility of the measurement, including the effect of examinations at both field strengths for some patients. The effect of field strength can also be appreciated when comparing control subjects at 3T and 1.5T. Overall, measures remained relatively stable, especially for HCT-eligible patients after treatment. In non-eligible patients, values showed a progressively abnormal trend.

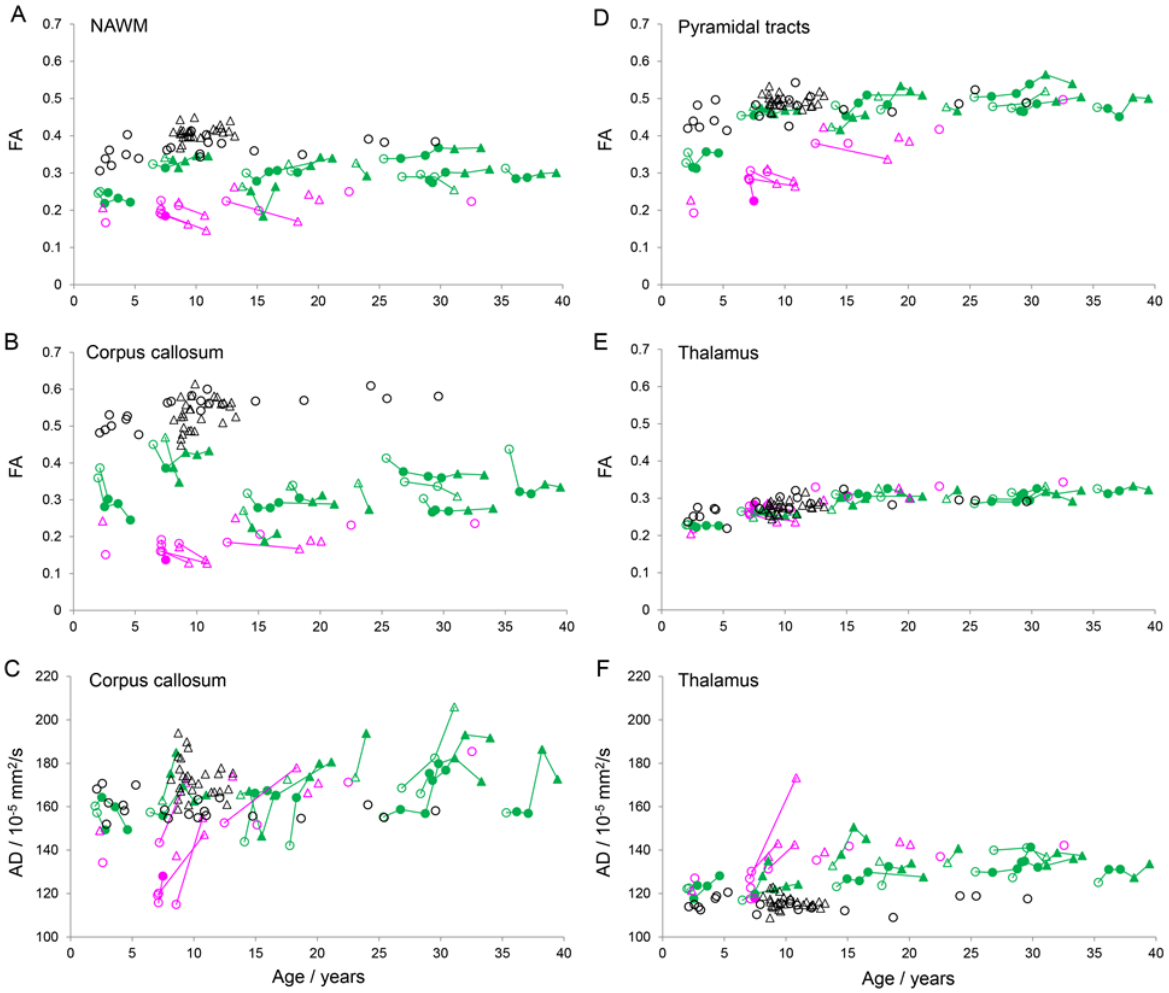

Figure 3. Longitudinal evolvement of (A) FA in NAWM, (B) FA and (C) AD in corpus callosum, (D) FA in pyramidal tracts, (E) FA and (F) AD in thalamus. Control values are indicated in black, HCT-eligible patients in green and non-eligible patients in pink. Data measured at 1.5T are indicated with a circle, data measured at 3T with a triangle. After transplantation, symbols are filled.

In NAWM, FA mildly fluctuated for treated eligible patients, while FA further decreased for HCT-non-eligible patients (Fig. 3A). Diffusivities remained relatively stable for all patients. In the corpus callosum, FA tended to decrease in the treated eligible patients, while the reduction in the non-eligible patients was marginal (Fig. 3B). However, MD, AD and RD longitudinally increased especially in non-eligible patients, which meant that AD, which was decreased at baseline, showed a pseudo-normalization (Fig. 3C).

In the pyramidal tracts, FA remained constant or slightly increased over time in most treated eligible patients, whereas FA slightly decreased in HCT-non-eligible patients (Fig. 3D). Diffusivities showed some longitudinal variability, but no clear trend was observed.

In the thalamus, in which FA did not differ between groups at baseline, FA remained stable in treated eligible patients, and showed a slight decrease in non-eligible patients (Fig. 3E). Diffusivities in treated eligible patients remained stable or showed a mild increase, whereas a larger increase was observed in non-eligible patients (as shown for AD in Fig. 3F).

Discussion

Using diffusion-weighted MRI, we compared magnitude and direction of diffusion between MLD patients and controls to gain insight into the microstructure of affected brain tissue. At baseline, FA was decreased and MD and RD were increased in MLD patients compared to controls throughout the WM, not only in the corpus callosum (affected early in the disease), but also in NAWM. FA measures of the thalamus did not differ between groups, but its components AD and RD were both increased in patients compared to controls. Whereas AD was increased in thalamus, it was unchanged in the pyramidal tracts and decreased in the corpus callosum and, to a lesser degree, in NAWM. All differences were most pronounced between controls and HCT-non-eligible patients.

Longitudinally, in treated HCT-eligible patients diffusion measures remained stable or showed only minor changes. FA remained constant or even tended to increase in NAWM, pyramidal tracts, and thalamus, whereas it slightly decreased in the corpus callosum. HCT-non-eligible patients had less follow-up examinations than eligible patients, but those available showed clear increases of RD and AD, causing a small FA reduction in all investigated regions. The treatment effect of HCT most likely influenced the longitudinal differences between treated eligible patients (approaching control values in NAWM and pyramidal tracts), and untreated non-eligible patients (increasingly abnormal values). This is in line with our observation that metabolite concentrations observed with magnetic resonance

spectroscopy partially normalized in successfully transplanted patients, whilst concentrations for non-treated patients further deteriorated.[29]

Limitations of this study were its retrospective character, a large age range of patients (inherent to the inclusion of patients with all disease types), and a limited age range of controls at 3T. The combination of 1.5T and 3T data also introduced some variability, although these differences were typically smaller than differences between controls and patients. Also, the diffusion tensor model itself has limitations. Most importantly, it reflects the underlying structural characteristics in a simplified manner, hampered by partial volume effects and crossing fibers.[18] Advanced multi-compartment diffusion models, such as the composite hindered and restricted model of diffusion (CHARMED), are more sensitive than the conventional ones.[30-32] However, since our study, ongoing since 2007, concerns a rare disease, application of these advanced diffusion models was not feasible.

These issues imply that we can merely hypothesize about the precise mechanism responsible for the observed differences rather than draw general conclusions because different cellular processes may lead to identical changes.[9-11] Since both animal[13,33,34] and human studies[35] have shown an increased RD parallel to myelin loss, our results of increased RD in WM suggest myelin loss in patients, in line with histopathological findings.[36] This is also supported by our observation that high RD and low FA in the pyramidal tracts at baseline indicate poor motor function at follow-up.

With regard to AD, animal and human studies provide discrepant results, correlating axonal damage to either an AD decrease[13,17] or increase [37,38], respectively. This reflects the difficulty in relating AD to underlying pathological processes. Our observation of opposing AD patterns in MLD suggests that different pathological mechanisms can cause either a decrease or an increase in AD, with the overall balance between these effects depending on brain region and disease stage. MLD is characterized by accumulation of sulfatides, major myelin lipids mainly synthesized by oligodendrocytes. Analytical studies of MLD patients' brain tissue showed that metachromatic deposits are mainly present in the WM, with a sulfatide content

up to eight times higher than normal, with relatively minor chemical GM changes.[36] Regarding WM, we assume that the massive intracellular sulfatide accumulation in swollen macrophages, in vain trying to digest these lipids, causes overall diffusion restriction and thereby AD reduction. Using conventional DWI, restricted diffusion in the outermost part of the demyelinated WM has indeed been described for single cases in relatively early disease stage.[39,40]However, as the disease progresses, axons are increasingly damaged. Based on previous observations in human studies, we expect that loss of both myelin and axons will lead to an AD increase.[37,38] Our results suggest that, particularly in the corpus callosum of non-eligible patients at baseline, diffusion restriction due to sulfatide accumulation in macrophages contributes more to the severely reduced AD values than increased diffusion due to myelin and axonal loss. Our longitudinal observation of an increase, and thereby a pseudo-normalization of AD, in the corpus callosum suggests that myelin and axonal loss likely becomes more prominent in progressive disease. The corpus callosum is, of the investigated ROIs, least hindered by limitations of the tensor model, suggesting that the interpretation of AD values is not much influenced by the presence of crossing fibers.

The thalamus is a DGM structure, in which sulfatide accumulation is much more limited than in WM.[36] In addition, the thalamus has a different microstructure than WM as it largely consists of neurons and contains few axons. In control subjects, this is mirrored by low thalamic FA, as the difference between AD and RD is much smaller than for a WM structure like the corpus callosum. In the thalamus of patients, we observed an increase of AD and RD of similar relative magnitude, which had hardly any effect on FA. This increase of both AD and RD probably implies an increase in extracellular space due to neuronal loss, which apparently dominates reductions in diffusivity due to storage material.

Overall, the observed changes of FA, RD, and especially AD indicate that MLD alters certain aspects of brain microstructure. These changes most likely reflect a multitude of pathological processes such as accumulation of metachromatic material, followed by myelin and axonal loss. The differences between untreated and treated patients indicate that diffusion

measures are positively affected by HCT, further emphasizing the beneficial effects of this intervention on WM and supporting the findings of other quantitative MR measures as proton MR spectroscopy.[29] Altogether, quantitative MR measures provide more insight into time-dependent disease mechanisms and might in the future aid in determining the right window for intervention.

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IV

Extra-neurological involvement

Chapter 9

Gallbladder and the risk of polyps and carcinoma in metachromatic leukodystrophy

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Abstract

Objectives To assess frequency of gallbladder polyposis and carcinoma in metachromatic leukodystrophy (MLD).

Methods We evaluated 34 MLD patients (average age 16.7 years, age range 2-39 years) screened for gallbladder abnormalities by ultrasound. In the case of cholecystectomy, findings at pathology were reviewed.

Results Only 8 of 34 (23%) patients had a normal gallbladder at ultrasound. Gallbladder polyps were visible in 8 (23%) patients. Cholecystectomy was performed in 11 (32%) patients. In these, pathology revealed various abnormalities, including hyperplastic polyps, intestinal metaplasia, prominent Rokitansky-Aschoff sinuses and sulfatide storage.

Conclusions Our results demonstrate that gallbladder involvement is the rule rather than the exception in MLD. The high prevalence of hyperplastic polyps, a known precancerous condition, and one death from gallbladder carcinoma at a young age suggest that MLD predisposes to neoplastic gallbladder abnormalities. As novel therapies for this patient group are emerging leading to increased life expectancy, we recommend screening for gallbladder abnormalities by ultrasound in order to prevent early death.

Introduction

Metachromatic leukodystrophy (MLD, OMIM #250100) is an autosomal recessive storage disorder caused by deficiency of the lysosomal enzyme arylsulfatase A. Consequently, sulfatides accumulate mainly in oligodendrocytes and Schwann cells in the central and peripheral nervous system, resulting in demyelination.[1] Onset is from infancy to adulthood, with the late-infantile form starting before 30 months of age, the juvenile form before 16 years and the adult form thereafter. First symptoms and signs in younger patients consist of motor deterioration, in older patients of cognitive decline and psychiatric symptoms. The disease relentlessly progresses and eventually all acquired skills are lost.[2] Hematopoietic stem cell transplantation (HSCT) is a possible treatment for selected patients, if MLD is diagnosed early;[3] gene therapy is emerging as a novel treatment option.[4] Both aim at halting or preventing the neurodegenerative process. Sulfatides also accumulate in visceral organs, including the gallbladder. Case reports describe complications such as hemobilia and cholecystitis[5-8] Histopathologic findings include macrophages filled with sulfatides, polyps, intestinal metaplasia and low-grade dysplasia. [9-13]

One of our MLD patients died at the age of 32 years from metastatic gallbladder carcinoma, a neoplasm usually occurring at an average age of 56-63 years.[14] Together with the report of another MLD patient who died of gallbladder carcinoma at age 18 years,[15] this observation raises the possibility of an increased vulnerability of MLD patients to develop this malignancy. We therefore started screening our patient group for gallbladder abnormalities by ultrasound. Retrospective evaluation of these data revealed a high rate of abnormal findings.

Patient ID	MLD type	Mutation 1		Mutation 2		Age at diagnosis (y)	Age at HSCT (y)	Age at US (y)	Symptoms leading to US	Ultrasound findings
		cDNA level	Protein level	cDNA level	Protein level					
MLD-59	Late-infantile	c.465+1G>A	p.?	c.465+1G>A	p.?	1	no HSCT	2	No	Sludge
MLD-50	Late- infantile	c.245C>T	p.Pro82Leu	c.1168C>T	p.Arg390Trp	2	2	2	No	Thickened wall
MLD-57	Late- infantile	c.112_1126del	p.Leu375fs	c.112_1126del	p.Leu375fs	2	no HSCT	3	No	Collapsed gallbladder, sludge
MLD-45	Late-infantile	c.459+1G>A	p.?	c.830_831delTCinsAA	p.Ile277Lys	1	2	6	No	Normal gallbladder
MLD-16	Juvenile	c.459+1G>A	p.?	c.536T>G	p.Ile179Ser	1 (sib)	7	9	No	Normal gallbladder
MLD-37	Juvenile	c.1073T>C	p.Leu358Pro	c.1277C>T	p.Pro426Leu	2	2	12	No	Thickened wall, sludge, possible polyps
MLD-23	Juvenile	c.1277C>T	p.Pro426Leu	Not found	n/a	4	4	21	No	Thickened wall
MLD-4	Juvenile	c.245C>T	p.Pro82leu	c.1144G>A	p.Glu382Lys	4	4	13	No	Normal gallbladder
MLD-38	Juvenile	c.582dup	p.Trp195fs	c.1277C>T	p.Pro426Leu	5	no HSCT	6	Colic-like pain	Thickened wall and sludge
MLD-17	Juvenile	c.1.1217_1255del 9	p.Ser406_Thr408del	c.1277C>T	p.Pro426Leu	6	no HSCT	7	Colic-like pain	Thickened wall, collapsed gallbladder, sludge, cholelithiasis
MLD-12	Juvenile	c.634G>C	p.Ala212Pro	c.1277C>T	p.Pro426Leu	6	no HSCT	13	Colic-like pain	Sludge
MLD-29	Juvenile	c.1372C>T	p. Gln548*	c.1277C>T	p.Pro426Leu	7	no HSCT	8	Colic-like pain	Sludge
MLD-39	Juvenile	c.459+1G>A	p.?	c.1277C>T	p.Pro426Leu	7	no HSCT	8	No	Sludge
MLD-5	Juvenile	c.459+1G>A	p.?	c.1277C>T	p.Pro426Leu	7	no HSCT	9	Vomiting	Sludge
MLD-10	Juvenile	n/a	n/a	n/a	n/a	7	no HSCT	12	No	Sludge
MLD-11	Juvenile	n/a	n/a	n/a	n/a	7	no HSCT	12	No	Sludge
MLD-6	Juvenile	c.459+1G>A	p.?	c.1277C>T	p.Pro426Leu	9	no HSCT	11	No	Thickened wall and cholelithiasis

MLD-62	Juvenile	c.1277C>T	p.Pro426Leu	c.1277C>T	p.Pro426Leu	9	no HSCT	27	No	Sludge, infiltrated aspect gallbladder bed
MLD-27	Juvenile	c.251G>A	p.Arg84Gln	c.251G>A	p.Ser96Phe	10	no HSCT	23	No	Multiple polyps (maximal diameter 6mm)
MLD-46	Juvenile	c.1277C>T	p.Pro426Leu	c.1277C>T	p.Pro426Leu	12	14	30	No	Polyp 8mm
MLD-54	Juvenile	c.1277C>T	p.Pro426Leu	c.1277C>T	p.Pro426Leu	12	no HSCT	18	No	Normal gallbladder
MLD-58	Juvenile	c.1277C>T	p.Pro426Leu	c.1277C>T	p.Pro426Leu	13	no HSCT	13	No	Multiple polyps (maximal diameter 13mm)
MLD-60	Juvenile	c.251G>A	p.Arg84Gln	c.287C>T	p.Ser96Phe	13	no HSCT	13	No	Multiple polyps (maximal diameter 6mm)
MLD-14	Juvenile	c.1277C>T	p.Pro426Leu	c.1277C>T	p.Pro426Leu	14	14	18	No	Normal gallbladder
MLD-35	Juvenile	c.1277C>T	p.Pro426Leu	c.1277C>T	p.Pro426Leu	14	no HSCT	25	No	Multiple polyps (maximal diameter 4mm)
MLD-61	Juvenile	c.1277C>T	p.Pro426Leu	c.1277C>T	p.Pro426Leu	21	no HSCT	21	No	Thickened wall, collapsed gallbladder, possible polyps
MLD-63	Adult	c.1277C>T	p.Pro426Leu	c.1277C>T	p.Pro426Leu	23	no HSCT	23	No	Thickened wall, polyps (maximal diameter 6mm)
MLD-21	Adult	c.635C>T	p.Ala212Val	c.1277C>T	p.Pro426Leu	17	17	20	No	Multiple polyps (maximal diameter 2·3mm)
MLD-30	Adult	c.1277C>T	p.Pro426Leu	c.1277C>T	p.Pro426Leu	18	19	26	No	Multiple polyps (maximal diameter 10mm)
MLD-49	Adult	c.1189C>T	p.His397Tyr	c.1277C>T	p.Pro426Leu	20	22	32	Colic-like pain	Dilatation of hepatic bile ducts
MLD-25	Adult	c.1194C>T	p.Phe398Phe	c.1194C>T	p.Phe398Phe	22	no HSCT	29	No	Normal gallbladder
MLD-41	Adult	c.459+1G>A	p.?	c.536T>G	p.Ile179Ser	25	25	26	Pancreatitis	Normal gallbladder
MLD-2	Adult	c.251G>A	p.Arg84Gln	c.287C>T	p.Ser96Phe	27	28	33	No	Thickened wall, collapsed gallbladder, possible polyps
MLD-32	Adult	c.536T>G	p.Ile170Ser	c.1171A>G	p.Ser391Gly	30	no HSCT	32	No	Normal gallbladder
MLD-15	Adult	c.251G>A	p.Arg84Gln	c.287C>T	p.Ser96Phe	35	35	39	No	Thickened and uneven wall, possible polyps

Table 1. Findings at gallbladder ultrasound.

Case report

A 20-year-old male (MLD-49 in table 1) was diagnosed with the adult type of MLD and received two years later an HSCT from an HLA-identical unrelated donor. The procedure was performed without complications.[16] At the time of transplantation his disease was already advanced: he had memory loss and behavioral changes, but his motor function was intact. He remained neurologically stable after HSCT during the entire follow up period. Nine years after the procedure, he presented with severe acute pain in the right upper abdomen. Ultrasound revealed dilatation of the intrahepatic and extrahepatic bile ducts and cholelithiasis. A large concrement in the cystic duct was seen on CT scan. Cytology of the endoscopic brushing revealed atypical cells suggesting adenocarcinoma. A cholecystectomy with bile duct resection and reconstruction with a hepaticojejunostomy was performed after microscopic pathology during the procedure was suspicious of malignancy. Definitive histological examination revealed adenocarcinoma.

Five months later, a control CT scan showed ascites and two lesions in the liver. A biopsy revealed metastasis of the gallbladder carcinoma. Because of the poor prognosis of metastatic disease and neurological impairment as a consequence of his MLD, palliative care was provided. He died 2 months later, 7 months after the diagnosis of gallbladder carcinoma.

Methods

At the Center for White Matter Disorders, VU University Medical Center, we follow almost all Dutch patients with MLD. Diagnosis of MLD was established by brain MRI, measurement of arylsulfatase A activity, which was in the disease range for all patients, and *ARSA* mutation analysis. After our index patient was diagnosed with gallbladder carcinoma, we added gallbladder ultrasound to the routine clinical care of all MLD patients. Six patients had already undergone gallbladder ultrasound for symptoms such as biliary colic, abdominal pain

or vomiting. Twenty-six patients had not reported abdominal symptoms when they were screened with ultrasound at one of their routine follow-up appointments or at diagnosis.

An experienced (pediatric) radiologist at the VU Medical Center performed ultrasound of the gallbladder in 30 cases; 4 patients were examined in 2 other centers. All examinations were carried out after at least 4 hours of fasting to ensure an optimally filled gallbladder. When polyps were detected, their maximal diameter was measured. Cholecystectomy was performed if polyps exceeded 5mm in patients having undergone HSCT or before planned HSCT. In the case of cholecystectomy, a pathologist experienced in neurometabolic disorders reviewed macroscopic and microscopic findings. In addition to routine hematoxylin and eosin stain, additional histochemical and immunohistochemical stains were used to identify macrophages containing sulfatides (metachromasia with toluidine blue), confirm intestinal metaplasia (Periodic acid-Schiff), and detect expression of the tumor suppressor gene product p53 (1:500, Dako).

We reviewed the radiologic and pathologic results, collected between 2009 and 2015, in our cohort. Symptoms possibly related to gallbladder abnormalities, such as biliary colic, vomiting, nausea, jaundice, anorexia and abdominal pain in the right upper quadrant were screened for during the regular visits and information was collected from patient charts.

Standard protocol approvals, registration and patient consents: the study received approval of the medical ethics review board of our hospital.

Results

Clinical characteristics and findings at ultrasound

We included 34 patients in our study. Table 2 shows the demographic characteristics of our cohort. Thirteen patients of our cohort had received allogeneic HSCT for treatment of MLD. Eight patients (24%) had symptoms possibly related to gallbladder abnormalities such as episodes of biliary colic, nonspecific abdominal pain or frequent vomiting. Intestinal bleeding,

as previously described due to papillomatosis,[9] was not encountered. Unequivocal diagnosis of biliary colic was difficult in some cases with advanced neurologic disease. Management was usually conservative, vomiting was treated with antiemetic drugs, resulting in some relief. One patient with gallstones was treated during 6 months with ursodeoxycholic acid, without effect on cholecystolithiasis or on frequent vomiting. Another had recurrent pancreatitis in the absence of gallstones on ultrasound after a HSCT procedure.

Characteristic	**Patients (n= 34)**
Age (mean, median, range) at US in years	16.7, 13 (2-39)
Sex- no. (%)	
Male	15 (44.1)
Female	19 (55.8)
Received HSCT- no. (%)	13 (38.2)
MLD type- no. (%)	
Late-infantile	4 (11.8)
Juvenile	22 (64.7)
Adult	8 (23.5)

Table 2. Demographic characteristics of patient cohort

Findings at gallbladder ultrasound are shown in figure 1 and table 1. A normal gallbladder was found in only 8of 34 (24%) patients. Eight (62%) of HSCT-treated patients had an abnormal gallbladder at ultrasound, showing possible polyps in 6 of them.

One patient had (asymptomatic) gallstones. Sludge was seen in 12 (35%) patients, one of whom was transplanted.

Increased thickness of the gallbladder wall up to 9mm (normal ≤ 3mm) [17,18] was seen in 10 (29%) patients. Eight patients (24%) had polyps with a diameter exceeding 5 mm in 6 patients, with a maximum of 13 mm (table1). Four (12%) patients had a small, collapsed gallbladder with a thickened wall. In this situation, polyps could not be ruled out.

Laparoscopic cholecystectomy was performed in 11 patients (32%). Indications for cholecystectomy (table 3) were polyps ≥ 5mm visible on ultrasound (n=5), impossibility to exclude polyps due to thickening of the gallbladder wall (n=4), severe biliary colics (n=1) and recurrent pancreatitis (n=1) (table 3). In the patient with recurrent pancreatitis, these episodes stopped after removal of the gallbladder, in which gallstones were found. One patient with advanced early-juvenile MLD had sludge and a thickened gallbladder wall with episodes of severe, biliary colics that ceased after cholecystectomy. There were no complications directly related to the procedure.

Pathology

Table 3 and figure 2 give an overview of histopathological findings in the 12 patients (11 + the index patient). In patient MLD-49, our index patient, histopathology was reviewed and revealed a poorly differentiated adenocarcinoma. The gallbladder wall was infiltrated by a proliferation of highly atypical epithelial cells arranged in small glands and strings, and there was evidence of perineural invasion (figure 2P). There were no cholesterol polyps.

Gross examination revealed presence of polyps in 7 patients (MLD-2, MLD-30, MLD-38, MLD-46, MLD-58, MLD-60, and MLD-63; figure 2), including 2 in whom these were not identified at ultrasound. Microscopically, the polyps were lined by normal to hyperplastic epithelium (figure 2 H). Multiple foci of intestinal metaplasia with clustered goblet cells were present in the polyps as well as in the mucosa of the wall (figure 2 I). In places, mild architectural and cytologic changes of the polyp epithelium were also seen, including nuclear hyperchromasia and stratification. Stain for p53 was focally positive in 2 patients (figure 2 N and O). There were no cholesterol polyps. In 3 HSCT-treated and 5 untreated patients

(including 2, MLD-58 and MLD-63, in whom cholecystectomy was performed prior to HSCT), premalignant changes (intestinal or gastric metaplasia and hyperplasia) were found.

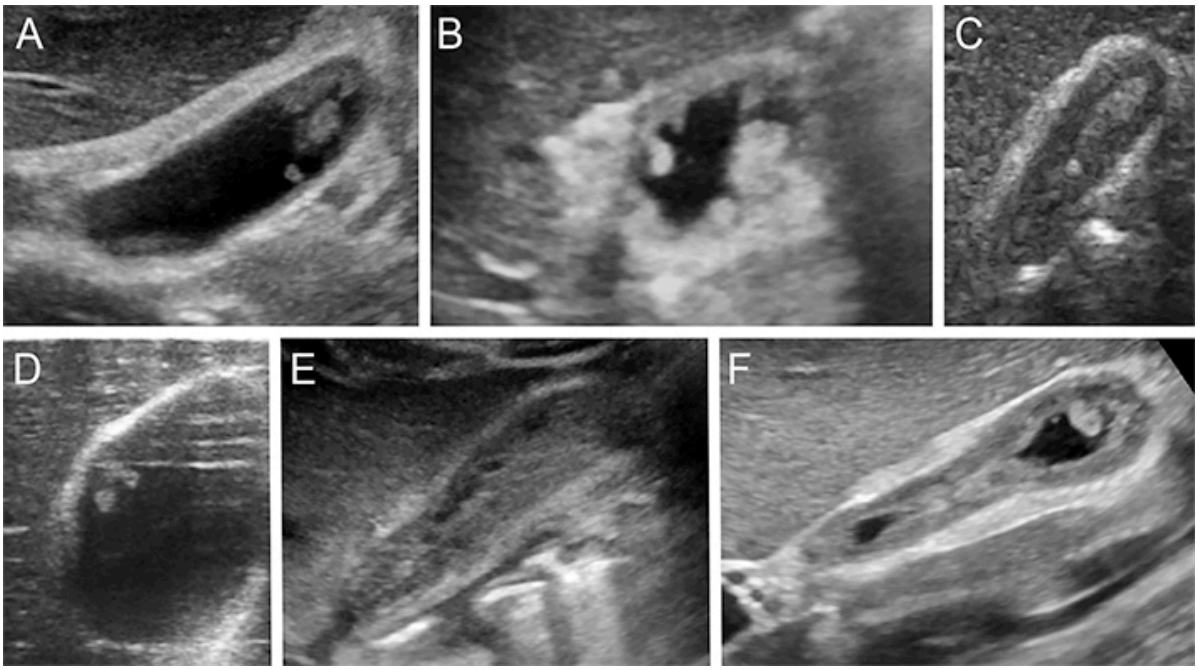

Figure 1. Gallbladder ultrasound findings in MLD**.** In (A), an 8mm polyp in the gallbladder of a transplanted patient (MLD-30) is depicted. (B) shows a 10mm polyp in the gallbladder of a patient before transplantation (MLD-58). Cholelithiasis is present in (C) in a non-transplanted patient (MLD-6). A gallbladder with multiple polyps (maximal diameter 4mm) in a non-transplanted patient (MLD-35) is shown in (D). (E) shows a gallbladder with thickened wall and sludge in a non-transplanted patient (MLD-38) with episodes of severe colic-like pain. Polyps are not discernible, but were present at histopathological examination. A small and contracted gallbladder with thickened wall and polyps in a non-transplanted patient is seen in (F) (MLD-63).

In 3 patients (MLD-2, MLD-37 and MLD-38) in whom ultrasound showed a thickened gallbladder wall, histology showed prominent Rokitansky-Aschoff sinuses with fibrosis and hyperplasia of the muscle wall. Prominent Rokitansky-Aschoff sinuses without hyperplasia of the muscle wall were also detected in 3 patients (MLD-46, MLD-60, MLD-63), in whom no wall thickening was detected at ultrasound. Besides cholelithiasis and scattered macrophages filled with storage material, histopathology of the patient with recurrent pancreatitis (MLD-41) showed normal findings.

The stroma of the polyps and mucosa of the wall contained foamy macrophages with periodic-acid-Schiff-positive metachromatic material (2 L and M). Of note, presence and extent of sulfatide storage differed between HSCT-treated and untreated patients. The

number of macrophages containing sulfatides was considerably higher in untreated patients, including those who underwent cholecystectomy before transplantation, whereas little (MLD-2, MLD-41 and MLD-37) or no storage material (MLD-46) was detected in HSCT-treated patients. There was no difference in incidence of other abnormalities between HSCT-treated and untreated patients.

Table 3: Indication for cholecystectomy and histopathological findings

Patient ID	MLD type	Age at cholecystectomy	Indication	Macroscopy	Histopathology	Storage material
MLD-38	Juvenile	7	Episodes with severe colic-like abdominal pain	Wall thickness 2mm, mucosa unrecognizable, hemorrhagic bile	Intestinal metaplasia, focal prominent Rokitansky- Aschoff sinuses, hyperplastic polyps	Numerous macrophages with metachromatic material in both polyp stroma and gallbladder wall
MLD-37	Juvenile	12	Possible polyps	Small gallbladder, wall thickness 3mm	Gastric and intestinal metaplasia, prominent Rokitansky-Aschoff sinuses, no polyps	Scattered macrophages with metachromatic material in gallbladder wall
MLD-46	Juvenile	30	Polyp	Serosa slightly hemorrhagic, wall thickness 4mm	Rokitansky-Aschoff sinuses, small finger like polyps	No metachromatic material-containing macrophages
MLD-60	Juvenile	13	Multiple polyps	Serosa slightly hemorrhagic, wall thickness 4mm	Intestinal metaplasia, focal prominent Rokitansky-Aschoff sinuses, hyperplastic polyps	Numerous macrophages with metachromatic material in both polyp stroma and gallbladder wall
MLD-58	Juvenile	13	Multiple polyps	Hemorrhagic bile	Intestinal metaplasia, hyperplastic polyps, hyperplasia	Numerous macrophages with metachromatic material in both polyp stroma and gallbladder wall
MLD-61	Juvenile	21	Possible polyps	Wall thickness 3mm, hemorrhagic bile	Intestinal metaplasia, hyperplasia, prominent Rokitansky-Aschoff sinuses	Numerous macrophages with metachromatic material in the gallbladder wall
MLD-63	Juvenile	23	Polyps	Several polyps, maximal diameter 5mm, wall thickness 3mm	Adenomyomatosis, focal prominent Rokitansky-Aschoff sinuses, hyperplastic, polyps, intestinal metaplasia	Numerous macrophages with metachromatic material in the polyp stroma
MLD-41	Adult	26	Recurrent pancreatitis	n/a	Cholelithiasis, no polyps	Scattered macrophages with metachromatic material in gallbladder wall
MLD-30	Adult	27	Multiple polyps	Several polyps, maximal diameter 10mm,wall thickness 3mm,	Polyps with villous aspect	Numerous macrophages in the polyp stroma
MLD-49	Adult	32	Suspected	n/a	Invasive poorly differentiated adenocarcinoma	n/a

			malignancy			
MLD-2	Adult	33	Possible polyps	Small gallbladder, bile completely absent, wall thickness 3mm	Hyperplastic polyps, prominent Rokitansky-Aschoff sinuses	Scattered macrophages with metachromatic material in both polyp stroma and gallbladder wall
MLD-15	Adult	39	Possible polyps	Small gallbladder, hardly any bile, wall thickness 4mm	Intestinal metaplasia, hyperplasia, prominent Rokitansky-Aschoff sinuses, no polyps	n/a

Discussion

In this first systematic evaluation of gallbladder involvement in MLD, we found significant abnormalities in a high percentage of patients (26/34, 76%). Gallbladder sludge was frequent, regardless of transplantation status. Although HSCT is a known predisposing factor for its formation, [19,20] this typically occurs within the first 6 months after HSCT, while most of our patients were at least 1 year post HSCT. Furthermore, the conditioning regimens (hypothesized to impair gallbladder contractility resulting in sludge) used for MLD patients are not as intensive as those used for most hematological conditions. We therefore assume that MLD itself and not HSCT causes sludge.

In one quarter, polyps were found by ultrasound, considerably more often than expected in a population of healthy children and young adults. The true prevalence of polyps in MLD is likely even higher, since in 2 patients polyps were detected only at histopathological examination while ultrasound only showed a thickened gallbladder wall. At histopathological examination, these polyps were all hyperplastic. In addition, intestinal metaplasia and hyperplasia of the gallbladder mucosa were present in 8 of 12 cases.

The exact prevalence of gallbladder polyps in children is unknown, but considered as extremely rare.[21] In healthy adults, irrespective of age, prevalence of gallbladder polyps varies between 3 and 7% at ultrasound, cholesterol polyps being by far the most frequent type at histologic examination.[22, 23] Gallbladder polyps are usually asymptomatic and as such an incidental finding, with about 75% are benign.[23, 24] They are, however, associated with an increased risk of evolving into carcinoma. This risk is substantially higher for hyperplastic than for cholesterol polyps, for polyps larger than 10 mm and for those associated with a thickened gallbladder wall.[23, 25]The evolution to gallbladder carcinoma, which takes approximately 10 to 15 years, progresses from metaplasia to dysplasia, carcinoma in situ and eventually invasive tumor.[26] The average age for dysplastic changes in the general population is 51.9 years, for localized gallbladder carcinoma 56.8 years and for advanced

carcinoma 62.9 years.[14] Because gallbladder carcinoma remains asymptomatic for a prolonged period,[14] it is usually diagnosed in an advanced stage with a high risk for metastases and dismal prognosis, reflected by the overall mean survival rate of only 6 months and a 5-year survival of 5%.[24] Current guidelines therefore recommend cholecystectomy for polyps larger than 10mm in otherwise healthy persons, and for polyps exceeding 5 mm in patients with conditions associated with increased risk of gallbladder carcinoma.[27-29]

Findings in our patient cohort confirm earlier single case reports of gallbladder abnormalities in MLD. Our results suggest a causal relationship between MLD and the development of gallbladder polyps and eventually carcinoma, because of the high incidence of abnormalities found, in contrast to the situation in otherwise healthy children and young adults in whom polyps are very rare and carcinoma virtually nonexistent.[14] It has been hypothesized that gallbladder polyposis in MLD is caused by the prolonged contact of gallbladder epithelium with sulfatides accumulating in bile.[5] The putative role of sulfatides in this process is uncertain. Of note , sulfatide expression is elevated in many human cancer cell lines and tissues, although their role in carcinogenesis is not yet understood.[30] After HSCT, the number of sulfatide-laden macrophages in the gallbladder wall was decreased, but not normal, consistent with a limited effect of HSCT on sulfatide accumulation in visceral organs. Presence and aspect of polyps were, however, grossly unchanged.

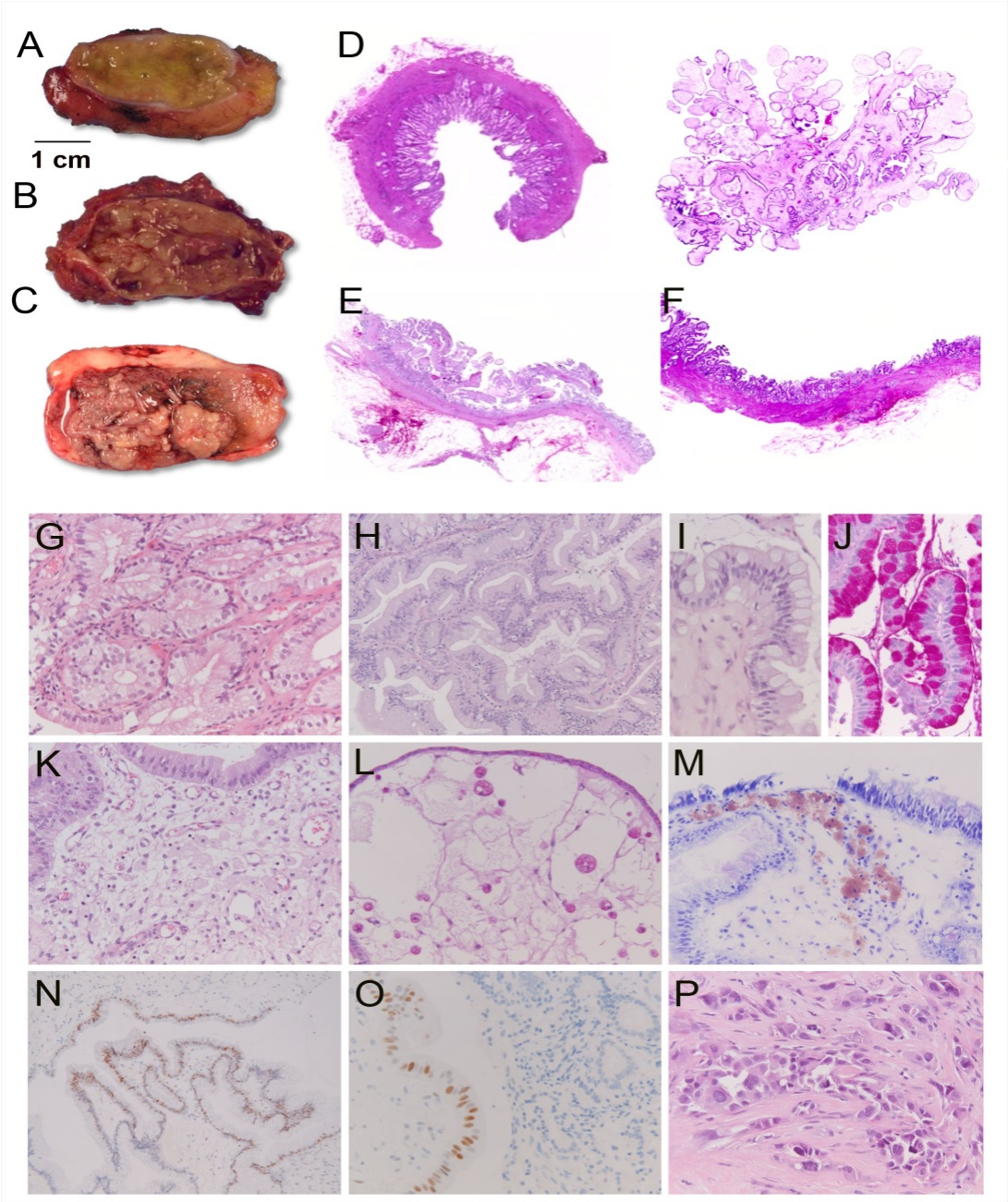

Figure 2. Pathology of the gallbladder in MLD**.** Gross inspection in (A-C) showing thickening of the gallbladder wall (A, MLD-37) and medium-sized (B, MLD 60) to large polyps (C, MLD-58). A whole mount preparation (D, MLD-37) demonstrates that thickening of the wall corresponds to prominent Rokitansky-Aschoff sinuses and muscle wall hyperplasia. (E-F) show the presence of small finger-shaped (E, MLD-60) or large polyps (F, MLD-58) protruding into the gallbladder lumen. In (G) H&E stain of patient MLD-37 shows normal mucosal epithelium. H&E stain in (H, MLD-60) depicting hyperplasia of the epithelium lining a polyp. H&E stain in (I, MLD-60) shows abundance of goblet cells in the epithelium, indicating intestinal metaplasia. The mucin contained in goblet cells is intensely PAS-positive in (J, MLD-60). H&E stain in (K) showing numerous foamy macrophages in the stroma of a polyp (MLD-58). In (L-M) the foamy macrophages are PAS-positive (L) and metachromatic (red) with the Toluidine blue stain (M), indicating that they contain sulfatides (MLD-58). In (N, MLD-58) p53 stain shows pathologic nuclear expression in the epithelium lining a polyp. In MLD-46, p53 stain shows pathologic nuclear expression (O, left), in comparison to normal absent expression in the epithelium of the wall (O, right). In (P, MLD-49) a proliferation of cytonuclear atypical epithelial cells in the gallbladder wall is shown.

With improved symptomatic treatment, the life expectancy of neurodegenerative disorders has increased over the last few decades. As treatments for MLD as HSCT and gene therapy are emerging, life expectancy of successfully treated patients will increase further and hopefully normalize. This implies that late or unusual disease complications will become more frequent. An additional risk factor for developing malignancies may be the exposure to the myelo-ablative and intensive immunosuppressive therapy that patients receive around HSCT although we did not find a higher incidence of premalignant abnormalities in HSCT-treated compared to untreated patients, with the limitation of short follow-up time, not reflecting potential long-term effects of immunosuppression.

Based on a previous report[15] and the present findings, we consider MLD a disease associated with an increased risk of gallbladder carcinoma. Consistent with this, we followed current guidelines for patients with increased risk of malignant transformation of gallbladder polyps [29] and performed cholecystectomy for polyps exceeding 5 mm in HSCT-treated patients and also in untreated patients in good clinical condition. Patients with thickened gallbladder wall in whom ultrasound cannot rule out the presence of polyps should also be considered candidates for cholecystectomy, as radiologic follow-up is predicted to be difficult. In patients with advanced neurologic involvement, decision to operate or closely follow abnormal findings is more challenging and should be made on an individual basis, also taking into account the reduction in quality of life due to symptomatic gallbladder involvement.

We propose adding ultrasound screening for gallbladder abnormalities to the standard clinical care of patients with MLD. Ultrasound is noninvasive and inexpensive and allows early detection of changes predisposing to gallbladder carcinoma, although one should bear in mind that it may miss polyps in patients with thickened wall or collapsed gallbladder. Patients at risk, as defined by polyps ≥ 5mm, (severe) thickening of the gallbladder wall or with symptomatic gallbladder involvement can be treated with laparoscopic cholecystectomy,

a relatively low risk procedure, in order to improve quality of life and avoid untimely death from a preventable cause.

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Chapter 10

Summary, discussion and future perspectives

Discussion and summary

Clinical aspects

In order for patients to be diagnosed in time for HCT, it is of great importance that the different possible presenting symptoms are recognized by physicians. We described in **chapter 3** that in juvenile and adult onset patients, psychiatric symptoms can precede neurological signs. The combination of an initially normal child with a clear change of behavior together with mild cognitive deterioration should prompt diagnostic evaluation for neurometabolic disorders. Early and correct diagnosis is not only essential to allow for HCT, a possible life saving treatment, but also for appropriate palliative treatment and genetic counseling. In addition, only through correct (and timely) diagnosis, siblings can be diagnosed while they are still presymptomatic and thus ideal candidates for HCT.

Treatment

MLD patients not treated with HCT invariably develop spasticity and often also a dyskinetic movement disorder, which is painful in many patients and can hamper daily care. Baclofen is a GABA-agonist that inhibits neural transmission at the spinal cord and is therefore frequently used to improve spasticity.[1] Intrathecal baclofen treatment (IBT) allows specific drug administration to tissues that are most responsible for spasticity with little exposure to the brain, thereby reducing side effects.[2] In **chapter 4** we studied ITB treatment in MLD patients to reduce spasticity and compared this to patients with spastic cerebral palsy (SCP). We showed that ITB is a safe and feasible therapy to improve comfort and daily care in MLD patients with both spastic and dyskinetic movement impairments. The treatment in MLD patients is comparable to SCP patients regarding baclofen dosage and complications. We recommend ITB early in the disease course for patients in whom oral baclofen no longer sufficiently reduces painful spasms and when spasticity hinders daily care.

In **chapter 5**, we compared our transplanted patients with patients no longer eligible for HCT, diagnosed in the same time period. We showed that HCT is a safe procedure, with no treatment related mortality (TRM) in this study.[3] HCT has proven to be able to stop the disease once performed in pre- or early symptomatic patients with the juvenile or adult onset type. For more advanced and late-infantile patients, HCT at best delays disease progression. Together with our study, two other large studies compared the effect of HCT in MLD patients to non-transplanted patients.[4,5] Boucher et al report a TRM of 23%, which they partially explain by the fact that part of their cohort was transplanted years ago when HCT was riskier due to less advanced protocols and techniques. In consensus with our results they report efficacy for early, pre-symptomatic transplantation in later onset MLD types, but their results also show benefit from HCT for long-term survival across all MLD subtypes. Gröschel et al report a TRM of 17% in a group of juvenile MLD patients. They recommend HCT in juvenile patients with an age of onset older than 4 years, pre- or early symptomatic (GMFC-MLD 0 or 1 and IQ ≥ 85), MRI score less than 17 (with a temporal or parietooccipital white matter subscore ≤4) and no involvement of U-fibers.

The difference in TRM between the studies of Boucher and Gröschel and our own study without TRM is remarkable. Boucher et al already point out the influence of older, less advanced protocols. In line with this, different conditioning regimens used are likely of influence on TRM. Another difference is that our cohort consisted of a large percentage (46%) of adult patients, whilst the other studies did not include adult patients or a smaller number of patients. How the subtype would influence TRM however is not evident, since the same conditioning regimens are used for children and adults. Moreover, the incidence of GvHD tends to be lower in children than in adults.[6] Due to the slower disease progression, adults are usually less affected at time of transplantation, which might make them less vulnerable to the intensive treatment.

Unfortunately, some of our patients did deteriorate cognitively after HCT, despite a pre- or early symptomatic clinical condition prior to HCT. The disease manifestations in these

patients are not only evidently slower compared to the natural course, but also progress despite stable white matter changes on MRI. This suggests neuroaxonal involvement less amenable to HCT. Why this decline is seen in some, but not all patients after HCT remains unclear. Both late-infantile, juvenile and adult patients were affected, so the subtype and pace of disease progression are not the only explanation. Longer follow-up time also for adult patients in whom the disease evolves slower will clarify how the disease fully unfolds after HCT and how often this cognitive deterioration occurs.

Pre- and early symptomatic juvenile and adult MLD patients are good candidates for HCT. In these patients, HCT can result in disease stabilization or even some improvement. Patients that are no longer able to walk without support and whose cognitive function is clearly affected (IQ below 75) will have no benefit from HCT. However, for patients with early juvenile onset IQ should preferably be higher, whereas in adult patients the slow disease progression may allow less stringent criteria. Additionally, brain abnormalities (rated with the MLD-Loes score) are predictive for outcome: patients with an MRI score above 15 at diagnosis are likely to have an unsuccessful outcome. Quantitative MRS can further be of aid in determining eligibility for HCT in ambiguous cases: severely reduced concentrations of (NAA) indicate low probability of a successful outcome.

In **chapter 6** we compared brain tissue of transplanted and non-transplanted patients to compare the inflammatory response and oligodendrocyte numbers between these two groups to gain further insight into the exact mechanism by which HCT halts further demyelination or even improves myelination. We found that in transplanted patients, there is presence of metabolically competent macrophages that are able to digest sulfatides, with a polarization of these macrophages towards an M2-like phenotype. There was a higher number of oligodendrocyte precursors and mature myelin forming oligodendrocytes in transplanted than untreated patients. These data suggest additional beneficial effects of HCT beyond cross-correction of enzyme deficiency that could be further exploited in order to

improve outcome. That these changes could be demonstrated in spite of the fact that transplantation was not successful, underlines the robustness of these findings.

Quantitative MRI techniques

Quantitative MRI techniques such as proton MRS and DTI broaden our knowledge about the pathomechanisms involved in the disease. In **chapter 7** we described that MRS at diagnosis is predictive for clinical outcome.[7] Patients with abnormal concentrations (severely reduced NAA, Glu and Glx and increased Lac and Ins) had poor outcome, whilst patients with concentrations closer to normal had moderate outcome. NAA was the main explanatory variable. Notably, in some patients in whom we observed a normalization in the ratio of Cho/NAA, this improvement did not coincide with an improvement in MRI score or reduction in lesion volume. The MRI score also includes other WM regions and cerebral and cerebellar atrophy, which forms a partial explanation. MRS concentrations at diagnosis are of aid in deciding whether HCT will be beneficial, especially for patients with a borderline neurological and cognitive examination. If baseline metabolite concentrations are severely abnormal, there is low probability of a good outcome. In **chapter 8** we studied DTI parameters at diagnosis and follow-up in MLD patients and found decreased FA and increased MD and RD in NAWM, corpus callosum, and pyramidal tracts in patients compared to controls. In the thalamus no differences in FA were observed, but all diffusivities were increased in both patient groups. We found increased AD in the thalamus but decreased AD in the corpus callosum and NAWM of patients. These changes are most likely reflective of different pathological processes occurring in MLD, reflecting a balance between neuro-axonal loss and intracellular storage accumulation, depending on region and disease stage.

Extra-neurological involvement

Despite the fact that ASA activity has been shown to return to normal reference values after HCT,[5,8] both sulfatide excretion in urine (own unpublished findings) as sulfatide accumulation in visceral organs and in the peripheral nervous system are not affected by HCT.[4,8,9] In our transplanted patients, we saw remarkably large intrasubject fluctuations of sulfatide excretion in urine after HCT (unpublished data). All values remained above normal references values. We do not understand the reason for this large intrasubject fluctuations. Measurements were corrected for dilution of urine, and a catabolic versus anabolic state would not be expected to be of influence. For the increased sulfatide excretion itself after HCT we hypothesize that damage to kidney tissue, not repaired by the transplantation, or clearance of sulfatides in a tissue dependent pace (possibly slower in the kidney than in the central nervous system), results in this increased excretion. An alternative hypothesis is that the degradation of stored sulfatides requires higher amounts of ASA enzyme than the prevention of storage,[10]implying that HCT does prevent further deterioration but does not restore ASA activity enough to ameliorate stored sulfatides. Another explanation might be that the donor macrophages do not reach the visceral organs (including the gallbladder and kidney) and the peripheral nervous system. The peripheral neuropathy can severely hamper motor function, especially in patients with an earlier onset, after transplantation.[11,12]

All in all, the precise mechanism for the ongoing accumulation after HCT is not yet understood but definitely requires further attention since it will help us to optimize treatment options for MLD. It is evident that with the limitations of HCT, other therapy strategies are needed, perhaps even in combination with HCT, certainly for patients with early onset and for those in more advanced stages of the disease. This will be discussed under future perspectives

In **chapter 9** we reported a high incidence of gallbladder abnormalities found in our MLD patients, suggestive of a causal relationship between MLD and the development of

gallbladder polyps and eventual carcinoma. [13] The difficulty with gallbladder carcinoma is the extremely fast evolution, implying that usually, once it is symptomatic, curative treatment is no longer possible. Due to the various pathological abnormalities we found in our patients, including hyperplastic polyps, a known precancerous condition, we believe screening of the gallbladder by abdominal ultrasound should be added to the standard clinical care of MLD patients. If the ultrasound shows no abnormalities we recommend a follow-up ultrasound every 2 years. A cholecystectomy is advised for polyps exceeding 5 mm in HCT treated patients and untreated patients in good clinical condition in order to prevent untimely death from a preventable cause and improve quality of life. It is important to bear in mind that, due to the thickness of the gallbladder wall and a small collapsed gallbladder often found in MLD patients, there is a substantial risk of missing a polyp on ultrasound, with an increased risk of evolving into carcinoma. To minimalize this risk, we therefore advice considering cholecystectomy when polyps cannot be ruled out on the ultrasound. Kim et al[9] also report a large MLD patient cohort with a high incidence of gallbladder abnormalities. Remarkably, they do not recommend to add an ultrasound to the standard clinical care of patients, but only for patients who present with abdominal pain. Additionally, they only advise cholecystectomy for patients with gallbladder abnormalities in the setting of relevant clinical symptoms, and not a prophylactic cholecystectomy in the case of asymptomatic polyps found on ultrasound.

Follow-up of transplanted patients

A standard, uniform treatment and follow-up protocol would be beneficial for patient care. Our current protocol contains a first assessment for transplanted patients 6 months after HCT, including neurological examination with scoring of gross motor function (GMFC-MLD), brain MRI (rated by the MLD-Loes score) including MRS, measurement of ASA activity, sulfatide excretion in urine and assessment of nerve conduction velocity. These

assessments are repeated a year after HCT and consequently each year until 5 years after HCT. Cognitive function is evaluated one year after HCT, depending on age of the patient through the Bayley Scales of Infant Development-II, the Wechsler Intelligence Scale for Children-III or the Wechsler Adult Intelligence Scale-III. Chimerism analysis is usually performed at day 60 after HCT and subsequently every year after HCT, at least during the first 5 years. After that time, follow-up is adapted per patient and clinical status.

As previously stated, screening of the gallbladder by abdominal ultrasound should be added to the standard clinical care of MLD patients and included in the general evaluation prior to HCT; follow-up depends on the findings, but even in patients with normal ultrasound it should be repeated every 2 or 3 years.

We see ovarian dysfunction after chemotherapy in a substantial number of our female HCT-treated patients. Hormonal substitution is advised to prevent or treat symptoms related to estrogen deficiency such as osteoporosis and climacteric symptoms. Bone density should be followed as well.

Palliative care

Unfortunately, many patients are diagnosed when the disease has already progressed to a point where HCT or gene therapy would no longer be beneficial. For these patients, it is important that they receive the best possible care for their inevitably progressing symptoms, to maintain good quality of life.

ITB as potent treatment of spasticity has been described above. Another treatment option for spasticity aside from ITB is a selective dorsal rhizotomy (SDR), in which the posterior lumbosacral rootlets from the spinal cord are partially transected in order to reduce the excitatory sensory input.[14] The advantage of SDR is that it requires only one surgical intervention, whereas ITB requires multiple hospital visits for adjustment and refill of the

pump. We have little experience with SDR for our MLD patients, but it has been shown that it can have a positive effect on comfort in non-walking children with spasticity, but pain is not always completely alleviated and daily care problems often persist.[14] Additionally, patients are at increased risk for developing dystonia, which should closely be evaluated when considering SDR.

Epilepsy is a frequent symptom, especially in more advanced patients. Frequently occurring seizures should be treated in order to prevent possible co-morbidities as trauma, encephalopathy, aspiration and hospitalization.[15] Seizures are usually well under control with medication.

With disease progression, drooling and dysphagia usually occur, which ultimately makes feeding via gastrostomy necessary. Timely placement of a gastrostomy, when first signs of swallowing dysfunction develop, is important to reduce the risk of aspirations and malnutrition. Another frequent problem is sialorrhoea, which is often treated with anticholinergics, of which glycopyrronium is the first choice. In advanced cases glycopyrronium may no longer be sufficient. Botulinum toxin injections in salivary glands can also be used to suppress salivation. It should be used with caution since a possible side effect is deterioration of the dysphagia and thickening of secretions.[16]

Chronic pain and irritability are unfortunately not uncommon, especially in later stages of the disease. One should be aware of possible underlying causes such as neuropathic pain, spasticity, joint dislocation, bone fractures, constipation, bowel obstruction, appendicitis, gastroesophageal reflux and dental injury.[15] Gallbladder colics and urinary retention, the latter due to neuropathy, are other possible causes of pain in MLD patients. It is also important to distinguish pain from discomfort as a response to strong stimuli or overstimulation. The response to analgesics or sedation can help discriminating between these causes. Sleep can be affected by the irritability, pain and discomfort. Melatonine is recommend as first step, other options are alimemazine and gabapentine. Gabapentine is a

calcium channel modulator and is typically used to treat chronic pain or epilepsy.[15] Apart from its regular indication, we used gabapentine in three patients suffering from irritability accompanied by increased muscle tone not sufficiently treated by ITB, with positive effect.

Future perspectives

Novel treatments

It is likely that in the future, therapy for MLD will be multimodal, including HCT-GT and enzyme replacement therapy. We learned from patients in whom HCT was successful that there are still obstacles to overcome such as the previously mentioned cognitive decline and the continuing peripheral neuropathy.

Hematopoietic Stem Cell- Gene Therapy (HSC-GT)

In HSC-GT, HSCs culture and manipulation are essential steps to achieve gene transfer. Vectors integrate into the host genome, thereby expressing the corrective gene in their progeny.[17] Preliminary results of a lentiviral mediated HSC-GT clinical trial of 9 patients with presymptomatic late-infantile and early symptomatic early juvenile patients report safety of the procedure.[18] At a median follow-up of 3 years after treatment, all patients were alive with halted disease progression or prevention of disease onset. One patient, who did have disease progression between enrollment and treatment initiation, did not benefit from the treatment. Remarkably, peripheral neuropathy (already present at diagnosis) improved in one third of patients 2 years after HSC-GT. This suggests that the above normal enzyme expression reached by HSC-GT has an advantage in correcting MLD.[18] Remyelination, by local Schwann cell precursors, is thought to take place after removal of sulfatides from nerve tissue.[18]

Intrathecal GT with viral vectors encoding *ARSA* has the advantage of more rapid and significant expression of *ARSA* in the brain over lentiviral mediated HSC-GT. Disadvantages

are the invasiveness of the procedure and the risk of an immune reaction against the transgene.[17] Intrathecal GT is thought to target mostly neurons, but the lysosomal enzyme could be secreted by transduced neurons and recaptured by other cells and thereby also correct the enzyme deficiency in oligodendrocytes. A phase 1/2 clinical trial to assess the safety of and efficacy of intrathecal GT with AAVrh.10hARSA into the white matter of both hemispheres (NCT01801709; clinicaltrials.gov) was stopped because of lack of efficacy (P. Aubourg, personal communication).

Intravenous ERT has not been efficient in controlling CNS disease manifestations, and therefore intrathecal ERT agent delivery and trials are ongoing to prove its efficacy. One such trial, using a biological recombinant of human ASA, has now completed, but results are pending (clinical trials.gov: NCT01510028).

Newborn screening

Criteria for inclusion of diseases in screening programs are broadly based on frequency, severity of the disease in the untreated population, availability of reliable testing methodology, effective treatment options and cost effectiveness.[19] Other metabolic diseases such as X-linked adrenoleukodystrophy (X-ALD) and mucopolysaccharidosis type I (MPS-I) have recently been recommended for newborn screening (NBS) in the Netherlands. Krabbe disease, another lysosomal storage disorder affecting the CNS and PNS, has a disease course comparable to MLD. NBS for Krabbe disease has been implemented in the US (in the state of New York) since 2006.[20] Early diagnosed infants can be treated with HCT. The inclusion of MLD in the NBS program is complicated by the fact that HCT is not an effective therapy for all subtypes. HSC-GT is now emerging and results are promising and suggestive of a safe and effective therapy also for the late-infantile subtype. This would make MLD a disease with (possible) effective treatment options for all subtypes, warranting therefore implementation within NBS. Still, it is essential to know what subtype a patient would develop; most of all to determine the best moment for treatment. Subtype determination

would require mutation screening after ASA activity has been found low, but is complicated by the heterogeneity of disease causing mutations. However, it is known that if a patient is homozygous for mutations predicted to lead to complete loss of ASA activity, they will develop the late-infantile form of the disease.[21] In general, the more effective ASA is produced, the later the onset of the disease.

Regarding the best moment for treatment, the question arises whether the earlier is per definition the better. For late-infantile patients this seems to be the case, but for adult patients one could question whether the benefit of treatment earlier than needed at a young age outweighs the possible risk of treatment related mortality and morbidity, risking otherwise healthy years. The best moment for treatment will therefore always remain a decision of both doctor and patient, different for each individual. More experience with the above-mentioned therapies and combinations of these will broaden our knowledge and will able physicians to provide the best possible counseling,

Better understanding of the disease and its pathomechanisms will in the future optimize treatment options and will hopefully eventually make this devastating disease a treatable disorder, for all patients.

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Nederlandse samenvatting

Klinische aspecten

Om patiënten op tijd te kunnen diagnosticeren voor HCT is het van groot belang dat de verschillende symptomen waarmee patiënten zich kunnen presenteren worden herkend door artsen. In **hoofdstuk 3** beschrijven we dat in juveniele en adulte patiënten, psychiatrische symptomen vooraf kunnen gaan aan neurologische klachten. De combinatie van een initieel normaal ontwikkelend kind bij wie een duidelijke verandering van gedrag gepaard gaat met milde cognitieve achteruitgang zou moeten leiden tot diagnostische evaluatie gericht op neurometabole ziekten. Een vroege en juiste diagnose is niet alleen van essentieel belang voor de mogelijk levensreddende behandeling met HCT, maar ook voor het kunnen geven van de juiste palliatieve behandeling en genetische counseling. Daarnaast maakt alleen een correcte en vroege diagnose het mogelijk ook broers en zussen te diagnosticeren als zij nog presymptomatisch, en dus ideale kandidaten voor HCT, zijn.

Behandeling

In **hoofdstuk 4** hebben we gekeken naar de behandeling van MLD patiënten met intrathecale baclofen (ITB), om spasticiteit te verminderen. We hebben dit vergeleken met dezelfde behandeling voor patiënten met spastische cerebrale parese (SCP). We laten hier zien dat ITB een veilige en haalbare behandeling is ter verbetering van comfort en dagelijkse verzorging voor MLD patiënten. De behandeling in MLD patiënten is vergelijkbaar met SCP patiënten wat betreft baclofen dosering en complicaties. Wij adviseren ITB in een vroeg stadium voor patiënten voor wie orale baclofen de spasmen niet langer adequaat verminderd en als de spasticiteit de dagelijkse zorg hindert.

In **hoofdstuk 5** hebben we onze getransplanteerde patiënten vergeleken met patiënten die in dezelfde periode waren gediagnosticeerd maar niet langer in aanmerking kwamen voor HCT. We hebben laten zien dat HCT in deze studie een veilige behandeling is, zonder behandeling gerelateerde mortaliteit. HCT is bewezen effectief in het stoppen van de ziekte,

mits de behandeling wordt uitgevoerd in pre of vroeg symptomatische patiënten met de juveniele of adulte vorm. Voor patiënten in een verder gevorderd ziektestadium of voor patiënten met de laat infantiele vorm, vertraagt HCT in het beste geval de ziekte progressie. Pre en vroeg symptomatische juveniele en adulte MLD patiënten zijn goede kandidaten voor HCT. Voor deze patiënten kan HCT resulteren in ziekte stabilisatie of zelfs enige verbetering. Patiënten die niet meer in staat zijn om zonder steun te lopen en wiens cognitief functioneren reeds duidelijk aangedaan is (IQ onder de 75) zullen niet van HCT profiteren. Een hoger IQ is te prefereren bij patiënten met de vroeg juveniele vorm, in het geval van adulte patiënten laat de langzame ziekteprogressie wellicht ruimte voor minder strikte criteria. Hersenafwijkingen op de MRI (gescoord met de MLD-Loes score) zijn voorspellend voor de klinische uitkomst; patiënten met een MRI score boven de 15 ten tijde van diagnose hebben een grote kans op een slechte uitkomst. Kwantitatieve MRS kan, in het geval van twijfel, verdere ondersteuning bieden bij het bepalen van wie er in aanmerking komt voor HCT; duidelijk verlaagde concentraties van N-acetylaspartaat (NAA) duiden op een lage kans op een succesvolle uitkomst.

In **hoofdstuk 6** hebben we hersenweefsel van getransplanteerde en niet getransplanteerde patiënten vergeleken om de inflammatoire response en het aantal oligodendrocyten tussen deze 2 groepen te kunnen vergelijken. Het doel hiervan was meer inzicht te krijgen in het exacte mechanisme waarmee HCT de demyelinisatie stopt of myelinisatie zelfs verbetert. We hebben ontdekt dat in getransplanteerde patiënten metabool competente macrofagen voorkomen die in staat zijn om sulfatides te verteren. Deze macrofagen laten een polarisatie zien richting het M2 fenotype. Het aantal oligodendrocyt voorlopers en volgroeide myeline vormende oligodendrocyten was hoger in getransplanteerde dan in niet behandelde patiënten. Deze data suggereren dat HCT, naast cross correctie van de enzymdeficiëntie, positieve effecten heeft die verder onderzocht kunnen worden om de klinische uitkomst te verbeteren. Het feit dat deze veranderingen konden worden aangetoond ondanks dat de

transplantatie niet succesvol was in deze patiënten, onderstreept de robuustheid van deze bevindingen.

Kwantitatieve MRI

Kwantitatieve MRI technieken zoals proton MRS en DTI verbreden onze kennis over de pathomechanismen die ten grondslag liggen aan de ziekte. In **hoofdstuk 7** beschrijven we dat MRS ten tijde van diagnose voorspellend is voor de klinische uitkomst. Patiënten met afwijkende metaboliet concentraties (duidelijk verlaagd NAA, Glu en Glx en verhoogd Lac en Ins) hadden een slechte klinische uitkomst, terwijl patiënten met minder afwijkende concentraties een matige klinische uitkomst hadden. NAA was hierin de belangrijkste verklarende variabele. Metabolieten concentraties ten tijde van diagnose kunnen de beslissing voor al dan niet transplanteren ondersteunen, met name voor patiënten wiens neurologisch en cognitief functioneren zich op de grens bevindt. Als er duidelijk afwijkende metaboliet concentraties ten tijde van diagnose zijn, is de kans op een goede klinische uitkomst laag.

In **hoofdstuk 8** hebben we DTI parameters ten tijde van diagnose en follow up onderzocht in MLD patiënten. We vonden een verlaagde FA en verhoogde MD en RD in de normaal uitziende witte stof, het corpus callosum en de piramidebanen voor patiënten in vergelijking met controles. In de thalamus vonden we geen verschillen in FA maar waren alle diffusie parameters verhoogd in beide patiënten groepen. AD was verhoogd in de thalamus maar verlaagd in het corpus callosum in de normaal uitziende witte stof van patiënten. Deze verandering zijn het meest waarschijnlijk het gevolg van verschillende pathologische processen die plaats vinden in MLD, en geven een weergave van de balans tussen neuro-axonaal verlies en intracellulaire accumulatie van sulfatides, afhankelijk van de regio en staat van ziekteprogressie.

MLD buiten het zenuwstelsel

In **hoofdstuk 9** beschrijven we een hoge incidentie van galblaas afwijkingen in onze MLD patiënten, suggestief voor een causaal verband tussen MLD en de ontwikkeling van galblaas poliepen en uiteindelijk galblaas carcinoom. Vanwege de verschillende pathologische afwijkingen die we in onze patiënten vonden, inclusief hyperplastische poliepen (een bekende premaligne aandoening), zijn wij van mening dat het screenen van de galblaas door middel van een echo van de bovenbuik zou moeten worden toegevoegd aan de standaard klinische zorg voor MLD patiënten. Als de echo van de bovenbuik geen afwijkingen laat zien raden we aan om de echo elke 2 jaar te herhalen. We adviseren een verwijdering van de galblaas (cholecystectomie) als er poliepen groter dan 5 mm worden gezien bij getransplanteerde patiënten en bij niet getransplanteerde patiënten in een redelijke klinische conditie. Het doel van deze ingreep is het voorkomen van een voortijdig overlijden en het verbeteren van de kwaliteit van leven. Het is belangrijk om te realiseren dat vanwege de dikte van de galblaaswand en de smalle samengevallen galblaas zoals die vaak gevonden wordt bij MLD patiënten, poliepen kunnen worden gemist op de echo. Dit resulteert in een verhoogd risico op het ontwikkelen van galblaas carcinoom. Om dit risico te minimaliseren adviseren we daarom ook een cholecystectomie als poliepen niet kunnen worden uitgesloten op de echo.

Toekomst

Wij verwachten dat in de toekomst de behandeling van MLD een combinatie zal vormen van verschillende facetten, waaronder een combinatie van HCT en gentherapie (HCT-GT) en enzym substitutie. Er lopen op dit moment verschillende studies naar zowel HCT-GT en intrathecale enzym substitutie waarvan de resultaten binnen korte tijd bekend zullen worden.

Als er een effectieve behandeling voor alle subtypen van MLD beschikbaar komt, maakt dit de implementatie van MLD in de hielprik screening mogelijk. Vraagstukken die daarvoor van belang zijn is zekerheid over welk subtype een patiënt zal ontwikkelen en wanneer het beste

moment van behandeling precies is. De vraag is of hoe eerder per definitie ook het beste is. Voor de vroege subtypes lijkt dit het geval te zijn, maar men kan zich afvragen of in het geval van de adulte vorm een risicovolle behandeling eerder dan strikt noodzakelijk opweegt tegen het risico van verlies van anders nog gezonde jaren. Het juiste moment van behandeling zal daarom altijd een individuele beslissing blijven. Maar met meer ervaring en inzicht in de nieuwe behandelingsmogelijkheden zullen artsen in de toekomst nog beter in staat zijn om het best mogelijke advies te bieden.

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Curriculum Vitae

Diane Francisca van Rappard was born on the 19th of December 1984 in Breda, the Netherlands. After completing her high school at the Stedelijk Gymnasium Breda in 2003 she went to Groningen to study Psychology. She finished her bachelor in 2007 and then moved to Amsterdam to complete (in 2009) her selective two-year master in Neurosciences for which she did an internship in the Childhood White Matter Center under supervision of dr. Nicole Wolf. This made here realize that she really wanted to combine her research interests with clinical work and she applied for the selective four-year master Arts-Klinisch Onderzoeker (Medical Doctor–Clinical Researcher) for which she was accepted in Maastricht. She graduated in July 2013 and started her PhD regarding Metachromatic Leukodystrophy in September 2013 under supervision of professor dr. Marjo van der Knaap and dr. Nicole Wolf and dr. Petra Pouwels, back at the Childhood White Matter Center in Amsterdam.

In October 2017, she started her residency in Psychiatry at the UMC Utrecht.

She currently lives in Zeist with her husband Jasper and her 2 sons Zweder and Splinther.