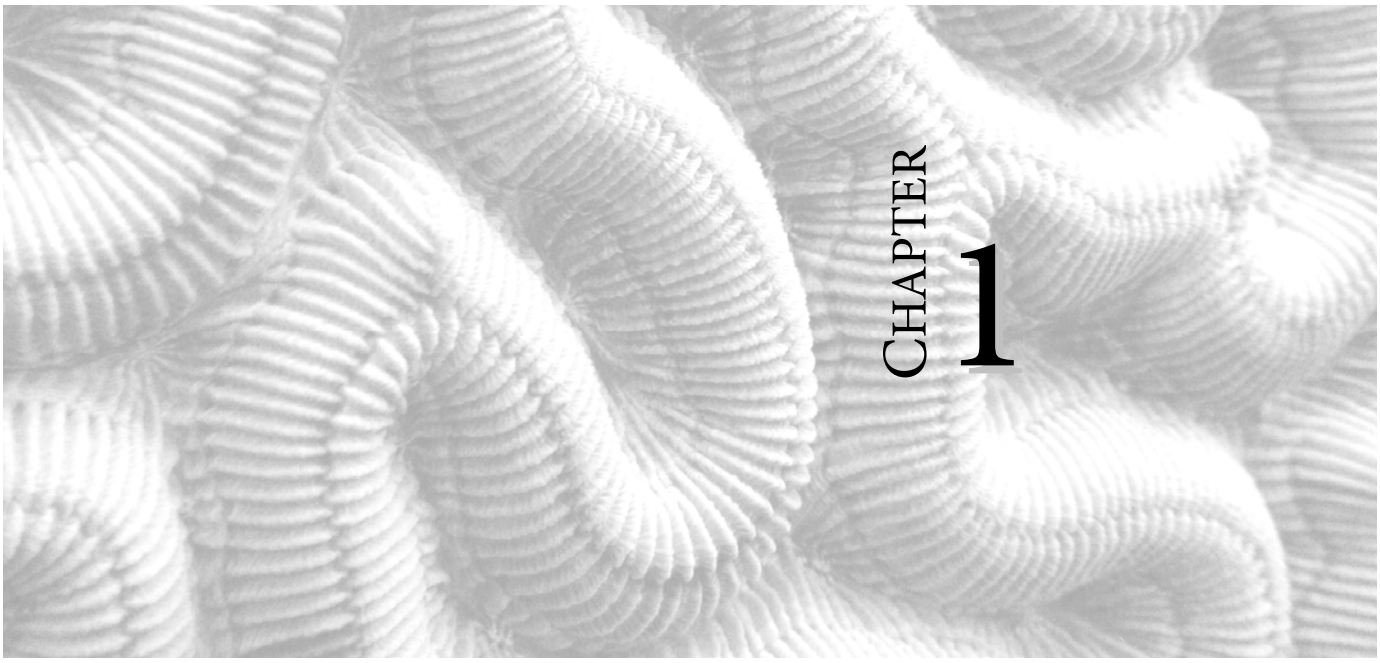

CHAPTER 1

GENERAL INTRODUCTION

1.1 White matter and its disorders – concepts and definitions

The white matter beneath the cortex in the central nervous system (CNS) is composed of nerve fibres (axons), which connect neurons in different regions with each other. The nerve fibres are wrapped in a proteinaceous-fatty sheath called myelin. The most important function of myelin is to accelerate the propagation of electrical impulses. It is responsible for the colour of the white matter. Besides myelinated axons, the white matter contains specialised supporting cells, the glial cells: astrocytes, oligodendrocytes, ependymal cells and microglia (1, 2). The star-shaped astrocytes are the most diverse of the glial cells. They perform many functions, including the transport of nutrients to the neurons, regulation of water and ionic homeostasis, a principal role in the repair and scarring process following damage, a supportive framework for other cells, and part of the blood-brain barrier (3, 4). Oligodendrocytes are the most numerous cells whose most important function is to produce and maintain the myelin membrane (2, 5). Ependymal cells are the epithelial cells that line the ventricular system of the brain (6). Microglia belong to the macrophage / monocyte system of phagocytic cells. They are the resident macrophages of the brain and thus act as the first and main form of active immune defences in the CNS. They normally exist in small numbers, but they multiply and become activated in reactive states such as inflammatory and demyelinating disorders (7).

"White matter disorders" or "leukoencepalopathies" are disorders of the brain that predominantly or exclusively affect the white matter. They constitute a heterogeneous group of diseases. Some disorders are progressive, whereas others are not, depending on the cause. White matter disorders may either be inherited or acquired. Most reserve the word "leukodystrophy" for the inherited and progressive disorders, whereas others use the word "leukoencephalopathy" for all genetically determined white matter disorders. "Demyelination" literally means loss of myelin, which can be seen in both inherited and acquired disorders. Some prefer to speak of "dysmyelination" if the loss of myelin occurs in the context of an inherited defect that affects myelin metabolism or oligodendrocytes; others prefer to avoid the word dysmyelination, because the literal translation of the word indicates that myelination is abnormal, which is often not the case in these disorders (8-13). Enzymatic defects leading to substrate accumulation figure prominently in the pathogenesis of the inherited progressive disorders affecting white matter. X-linked adrenoleukodystrophy (X-ALD), globoid cell leukodystrophy (GLD) and metachromatic leukodystrophy (MLD) are among the classic leukodystrophies. Two other major categories in the pathologic classification of the leukoencephalopathies are hypomyelinating disorders: Pelizaeus-Merzbacher disease (PMD), Pelizaeus-Merzbacher-like disease, Cockayne syndrome, infantile-onset neuronal storage disorders, such as GM1 and GM2 gangliosidoses; and vacuolating myelinopathies (or spongiform leukoencephalopathies), such as megalencephalic leukoencephalopathy with subcortical cysts (MLC) and Canavan disease (8-13). For other hereditary disorders, like Alexander disease and Vanishing White Matter disease (VWM), the pathologic classification into a specific group is not as clear (14, 15).

There are also white matter disorders, which are not inherited. The most common acquired inflammatory white matter disorder in the western world is multiple sclerosis (MS). Also, some infections acquired through the mother before birth, may cause white matter damage, such as congenital Cytomegalovirus (CMV) infection (16). Periventricular leukomalacia (PVL) is the most common acquired condition with static white matter lesions, caused by hypoxic-ischemic damage of the periventricular white matter in the perinatal period (17).

In addition, approximately half of the children with a white matter disorder and discernable abnormalities of the white matter on MRI remain without a classifying diagnosis and are referred to as "the unclassified white matter disorders". The cause of the diseases is not known. By collecting as many unclassified cases as possible and searching for comparable cases multi-institutionally, several “new” (i.e., the disease has not been described previously) leukoencephalopathy syndromes have been defined on the basis of homogeneous and distinct patterns of MRI abnormalities or on the basis of specific MRS findings, together with medical history and laboratory data (18-21). Once a number of patients are identified with apparently the same disease, a search for the common cause can begin. In inherited disorders, a search for the responsible gene can be initiated, as soon as a sufficient number of patients and informative families have been identified. For acquired leukoencephalopathies the common cause can sometimes also be found, such as a congenital CMV infection (22).

1.2 Histopathology of white matter disorders

In many classified white matter disorders the pathology is known, because of sufficient autopsy and biopsy material. In other disorders there is little or no information on the underlying pathology, because the disease is unclassified or no autopsy reports are available. In general, there are several gross neuropathologic features that are common to most of the leukoencephalopathies, such as reduced brain weight (except for the megalencephalic disorders, i.e. Canavan disease, Alexander disease and MLC), ventriculomegaly, atrophy of the corpus callosum, optic atrophy, and bilateral extensive loss or lack of cerebral and cerebellar white matter, replaced by firm gray tissue consisting of fibrillary astrogliosis (sclerosis). In some disorders sparing of the subcortical U-fibers is characteristic. Rarefied white matter with formation of gross cysts due to massive axonal and myelin losses and an inadequate astrocytic reparative response is rare in most leukoencephalopathies, but is characteristic of VWM (23, 24). Also in Alexander disease extensive cystic degeneration of the cerebral white matter may occur (25).

Likewise, light-microscopically most leukoencephalopathies display common neuropathologic features: reduced myelin staining, loss of oligodendrocytes, axonal loss, but often with a relative sparing of axons despite marked myelin loss, increase in extracellular space, reactive astrocytosis in the early stages to fibrillary astrogliosis in later stages. Macrophages with myelin debris are typical of the classical leukodystrophies, but tend to be sparse in the hypomyelinating and spongiform leukoencephalopathies. Also, the macrophages in the white matter are not restricted to the classic leukodystrophies, but may

be seen in a variety of lesions, such as infarcts of the brain. Other than macrophages, traditional inflammatory cells (i.e. lymphocytes) are usually inconspicuous, except in X-ALD (26).

In addition, there are more distinctive neuropathologic features in the leukodystrophies that can be seen by light microscopy. For example, Rosenthal fibers are the hallmark of Alexander disease (25). The appearance of myelin breakdown products in the macrophages can be distinctive in the classic leukodystrophies (i.e., metachromatic – a blue to red shift in staining when using a basic dye- in MLD and, orthochromatic in GLD with the formation of large globoid cells) (27). Apparent increase in the number of oligodendrocytes in the early lesions and foamy oligodendrocytes in later stages have been reported in VWM (23, 24, 28), but can also be seen in some hypomyelinative disorders (own observations). The spongiform leukoencephalopathies share a common light-microscopic feature of spongy to vacuolated myelin. The vacuoles are due to the accumulation of fluid within myelin sheaths and astrocytes. It is difficult to determine the exact site of vacuoles in human biopsy or autopsy specimens due to poor tissue preservation. In addition, axonal and oligodendroglial sparing is seen in spongiform leukoencephalopathies, together with reactive astrocytes. These features are not specific for the genetically transmitted disorders (like MLC and Canavan disease) (29-30) and can also be seen in acquired vacuolating myelinopathies (like hexachlorophene and cuprizone toxicity) (31).

In PMD cases of hypomyelination with delayed and decreased production of myelin, the central white matter appears irregular and patchy (tigroid pattern of myelin deposition), related to the perivascular presence of myelin (30). This typifies PMD, but can also be seen in Cockayne syndrome (30).

Small foci of mineralization of the abnormal white matter have been noted in several leukoencephalopathies, but are prominent and more widespread in Cockayne syndrome (perivascular distribution), Aicardi-Goutierès syndrome (especially in the basal ganglia) and congenital CMV infection (30, 22).

Light-microscopic pathologic features, which can be seen and scored in biopsy or post-mortem specimens of white matter disorders, include reduced myelin and axonal stainings, the number of oligodendrocytes (recognized morphologically by their perinuclear halo), hyperplasia and hypertrophy of astrocytes (identified using immunohistochemical staining for glial fibrillary acidic protein (GFAP)), and the number of macrophages (which stain positive for the antibody against CD68).

1.3 Magnetic resonance in white matter disorders

For all types of white matter disorders, T1 and T2 relaxation times become longer, leading to decreased signal intensity on T1-weighted MR images and increased signal intensity on T2-weighted images. Conventional T1- and T2-weighted MR images have a high sensitivity for identifying white matter abnormalities and can also be used for following changes in extent over time. The pattern of the distribution of abnormalities on conventional MR

images often has a high diagnostic specificity for a particular entity or a group of disorders. However, the specificity with regard to the pathologic substrate is very low (8, 9, 32, 33). For information on the type of white matter lesions, quantitative MR techniques may be more useful.

Quantitative MR encompasses several imaging techniques that yield quantitative information about brain tissue, three of which are used in this thesis.

Magnetization-transfer imaging (MTI) is based on the interactions between protons in free fluid and protons bound to macromolecules. MTI gives information about the tissue integrity. The magnetization transfer ratio (MTR) value reflects the capacity of the protons bound to macromolecules (in white matter mainly in myelin and axonal membranes) to exchange magnetization with the surrounding protons in free water (34, 35). In normal development, the amount of magnetization transfer in the white matter increases during myelination (35). A low MTR is interpreted as an indication of damage to myelin and to other cellular structures, such as the axonal membranes. Evidence for this concept comes from correlative post-mortem studies showing strong correlations of MTR values in MS lesions and normal-appearing white matter (NAWM) with the percentage of residual axons and myelin (36-37). Guidelines for using MTR in monitoring treatment of MS (e.g. the degree of remyelination) have been developed (38). In a study of X-ALD patients, two zones of MTR-change have been reported: one zone reflecting the central, entirely demyelinated area of the lesion and a second, outer zone reflecting the ongoing but still incomplete demyelination (39).

Diffusion-weighted imaging (DWI) uses differences in diffusion of free water molecules in tissues as a source of contrast. Diffusion-tensor imaging (DTI) is a quantitative technique that allows the production of maps which show the value and the principal direction of diffusion in each voxel. Due to the presence of cells and membranes, which act as a barrier for diffusion, the diffusion coefficient of water within biological tissues is lower than the diffusion coefficient in free water (34, 40). It is not possible to measure the "pure" diffusion coefficient D in vivo. The diffusion coefficient measured, the apparent diffusion coefficient (ADC), averages different tissues with different diffusion coefficients present in one voxel; it is also influenced by other parameters, such as presence of micro-particles of iron. The ADC is a measure for the mobility of water molecules in a voxel, averaged over all directions. Fractional anisotropy (FA) is a measure of the differences in diffusion of water molecules in different directions. In other words, it measures the degree of diffusion anisotropy (40, 41). Diffusion in brain can be isotropic, such as in cerebrospinal fluid, in which the water can diffuse equally in each direction, or it can be anisotropic, such as in the white matter tracts where diffusion is less restricted along the long axis of the white matter tract than it is perpendicular to the tract. FA is low in cerebrospinal fluid (CSF) and high in compact white matter tracts. The greatest degree of anisotropy is found in the corpus callosum, internal capsules, brainstem and cerebellar peduncles (41). DTI may be used to create a three-dimensional (3D) map by connecting the principal vector orientation per voxel. The result is fibre tracking, which is a means to visualize white matter tracts (42). In the white matter, as the brain matures, the amount of motion of the water molecules (as

measured by the ADC) decreases as a result of increased complexity of white matter pathways and increasing myelination. During myelination the FA increases (43-45).
In most white matter disorders damage to the tissue, which results in a loss or increased permeability of restricting barriers, are characterized by an enhanced mobility of water molecules (measured as increased ADC) (46-47).

DWI and DTI have been applied extensively in MS. In MS lesions, visible on T2-weighted images, ADC is consistently higher than in NAWM. Similarly, FA has been shown to be lower in MS lesions than in NAWM. Post-mortem studies have shown that more severe loss of myelin is associated with a more pronounced increase in water diffusion (48). In the white matter in patients with X-ALD, DTI has shown a gradual increase in diffusivity and reduction in diffusion anisotropy towards the centre of the MRI-visible lesions (49). In early lesions in MLD, ADC values are low, whereas in later stages of the disease ADC increases and FA decreases; similar findings are reported in GLD (50).

MR spectroscopy (MRS) provides information about metabolites that are present in the brain. MRS may be performed using a single voxel technique (single voxel spectroscopy) or multiple voxels (known as magnetic resonance spectroscopic imaging or chemical shift imaging). Rather than images, MRS data are usually presented as line spectra. The peaks of the spectra correspond with various metabolites, normal and abnormal, which can be identified precisely (51-53). The area under the peak reflects the concentration of the metabolite. The metabolites that can be identified with proton MRS include total N-acetylaspartate and N-acetyl-aspartyl-glutamate (tNAA), choline-containing compounds (Cho), total creatine and phosphocreatine (tCr), lactate, myo-inositol (Ins), glutamate, glutamine and some macromolecular proteins and lipids (54).

Concentrations of these metabolites vary with age (55). In the first few years of life, the NAA peak increases, related to neuronal maturation and axonal outgrowth. The high Cho peak in the immature brain decreases with age as myelin synthesis slows down. The large Ins peak in the spectra of newborns diminishes substantially during the first year of life. Final values of Cr concentrations are reached after a few months. Generally, by the time a child is 2 years old, the spectra are similar to those of an adult.

NAA is present in relatively high concentrations in the brain and is the highest peak in the normal spectrum. In the mature brain NAA is almost entirely confined to neurons and their axons. The utility of NAA as an axonal marker is supported by the loss of NAA in the abnormal white matter in many white matter diseases, including the classic leukodystrophies and MS (with the exception of Canavan disease, in which NAA accumulates) (54, 56).

Cho is a metabolic marker of membrane density and integrity, i.e. phospholipids synthesis and degradation. Changes are generally associated with alterations in membrane density and metabolism. Increases in Cho relative to NAA and Cr are noted in white matter lesions with active demyelination, related to enhanced membrane lipid synthesis and turnover. In hypomyelination, Cho tends to be low, indicative of decreased (myelin) membrane synthesis and turnover (54, 56).

Cr is a marker of energy metabolism. This peak remains fairly stable and for this reason Cr has often been used as an internal reference in semi-quantitative MRS studies. Cr has been considered a marker for cellular density (54, 56).

Under normal circumstances, lactate is present in minute amounts in the brain. However, under conditions where aerobic oxidation fails and anaerobic glycolysis takes over, such as in hypoxia and with macrophage accumulation in metabolic disorders, lactate levels increase significantly (54, 56, 57).

In the brain, Ins is synthesized primarily in glial cells. It is found almost exclusively in astrocytes. An increase in Ins content is believed to represent hyperplasia or hypertrophy of astrocytes. It is typically elevated in the setting of gliosis in white matter disorders (54, 56, 58).

Studies in white matter disorders, including MS, have shown that MRS can provide information on the density of axons (NAA), the presence of gliotic tissue (Ins) and the presence of active breakdown of myelin (Cho) and inflammation (lactate). In addition, it sometimes shows changes specifically related to the basic biochemical defect.

1.4 Aims and outline of the thesis

In the previous paragraphs it has become clear that conventional MR imaging is highly sensitive in the detection of white matter abnormalities, but lacks specificity when it comes to differentiation of different pathologic substrates of white matter lesions. Therefore, there is need for the application of additional, quantitative MR techniques, such as MRS, MTI and DTI, to characterize these lesions.

The following three chapters of this thesis describe the research that was initiated to further correlate MRI findings with histopathology and clarify the role of quantitative MR techniques in determining the nature of underlying pathology in white matter disorders.
In Chapter 2, the purpose is to understand a characteristic MR imaging pattern seen in three white matter disorders (MLD, GLD and infantile GM1 gangliosidosis) by correlating post-mortem MRI with histopathology.

In Chapter 3, two quantitative MR studies of white matter disorders with a known pathology are described. The purpose of the first study is to investigate whether quantitative MR parameters allow discrimination between different types of well known pathologic conditions that underlie signal intensity abnormalities in white matter. The second study aims at characterizing the degree of specificity of quantitative MR parameters with regard to histopathologic parameters in vivo and in fresh and formalin-fixed post-mortem brain tissue of patients with X-ALD.

In Chapter 4, three quantitative MR studies of white matter disorders in which the nature of the pathology related to the MR imaging findings is not or not completely clear, will be described. These studies aim at increasing the understanding of the pathologic substrates of the MR findings in patients with Leukoencephalopathy with brainstem and spinal cord involvement and high lactate (LBSL), Alexander disease and congenital CMV infection.

The results of these chapters will be summarized and discussed in Chapter 5, and suggestions for future research will be made.

REFERENCES

1. Barkovich AJ. Concepts of Myelin and Myelination in Neuroradiology. AJNR 2000;21:1099-1109

2. Baumann N and Pham-Dinh D. Biology of oligodendrocyte and myelin in the mammalian central nervous system. Physiol Rev 2001;81(2): 871-927

3. Ransom B, Behar T and Nedergaard M. New roles for astrocytes (stars at last). Trends Neurosci 2003;26(10):520-522

4. Sofroniew MV and Vinters HV. Astrocytes: biology and pathology. Acta Neuropathol 2010;119 (1):7-35

5. Bradl M and Lassmann H. Oligodendrocytes: biology and pathology. Acta Neuropathol 2010;119 (1): 37-53

6. Del Bigio MR. Ependymal cells: biology and pathology. Acta Neuropathol 2010;119 (1):55-73

7. Graeber MB and Streit WJ. Microglia: biology and pathology. Acta Neuropathol 2010;119 (1):89-105

8. van der Knaap MS and Valk J (editors). "Magnetic resonance of myelination, and myelin disorders". 3rd edition. 2005. Springer, Berlin.

9. Van der Knaap MS. Magnetic resonance in childhood white-matter disorders. Dev Med Child Neurol 2001;43:705-712

10. Costello DJ, Eichler AF and Eichler FS. Leukodystrophies: Classification, diagnosis and treatment. The Neurologist 2009;15:319-328

11. Phelan JA, Lowe LH and Glasier CM. Pediatric neurodegenerative white matter processes: leukodystrophies and beyond. Pediatr Radiol 2008;38:729-749

12. Lyon G, Fattal-Valeski A and Kolodny. Leukodystrophies, clinical and genetic aspects. Top Magn Reson Imaging 2006;17:219-242

13. Schiffmann R, van der Knaap MS. The latest on leukodystrophies. Curr Opin Neurol 2004;17:187-192

14. Sawaishi Y. Review of Alexander disease: beyond the classical concept of leukodystrophy. Brain and Development 2009;31:493-498

15. van der Knaap MS, Pronk JC and Scheper GC. Vanishing white matter disease. Lancet Neurology 2006; 5(5):413-423

16. Mejaski-Bosnjak. Congenital CMV infection: a common cause of childhood disability. Dev Med and Child Neurol 2008;50:403

17. Deng W, Pleasure J, Pleasure D. Progress in periventricular leukomalacia. Arch Neurol 2008;65:1291-1295

18. Leegwater PA, Pronk JC and van der Knaap MS. Leukoencephalopathy with vanishing white matter: from magnetic resonance imaging pattern to five genes. J Child Neurol 2003;18:639-645

19. Leegwater PA, Yuan BQ, van der Steen J, et al. Mutations of MLC1 (KIAA0027), encoding a putative membrane protein, cause megalencephalic leukoencephalopathy with subcortical cysts. Am J Human Genet 2001;68 (4):831-8

20. van der Knaap MS, Naidu S, Pouwels PJ, et al. New syndrome characterized by hypomyelination with atrophy of the basal ganglia and cerebellum. AJNR 2002; 23(9):1466-74

21. Huck JH, Verhoeven NM, Struys EA, et al. Ribose-5-phosphate isomerase deficiency: new inborn error in the pentose phosphate pathway associated with a slowly progressive leukoencephalopathy. Am J Human Genet 2004;74(4):745-51

22. Van der Knaap MS, Vermeulen G, Barkhof F, et al. Pattern of white matter abnormalities at MR imaging: use of polymerase chain reaction testing of guthrie cards to link pattern with congenital cytomegalovirus infection. Radiology 2004;230:529-536

23. van der Knaap MS, Barth PG, Gabreels FJ, et al. A new leukoencephalopathy with vanishing white matter. Neurology 1997;48(4):845-55

24. van der Knaap MS, Kamphorst W, Barth PG, et al. Phenotypic variation in leukoencephalopathy with vanishing white matter. Neurology. 1998;51(2):540-7

25. van der Knaap MS, Naidu S, Breiter SN, et al. Alexander disease: diagnosis with MR imaging. AJNR 2001;22(3):541-52

26. Eichler F and Van Haren K. Immune response in leukodystrophies. Pediatr Neurol 2007;37(4):235-44

27. Suzuki K and Suzuki K. Lysosomal diseases, In "Greenfield's Neuropathology" (Graham DI and Lantos PL), 7th Edition, Vol 1, pp.653-735. Arnold, London

28. Van Haren K, van der Voorn JP, Peterson DR, et al. The life and death of oligodendrocytes in vanishing white matter disease. J Neuropathol Exp Neurol. 2004;63(6):618-30

29. van der Knaap MS, Barth PG, Vrensen GF, Valk J. Histopathology of an infantile-onset spongiform leukoencephalopathy with a discrepantly mild clinical course. Acta Neuropathol. 1996;92(2):206-12

30. Harding BN and Surtees R. Metabolic and neurodegenerative diseases of childhood, In "Greenfield's Neuropathology" (Graham DI and Lantos PL), 7th Edition, Vol 1, pp.485-517. Arnold, London

31. Graeber MB, Blakemore WF and Kreutzberg GW. Cellular pathology of the central nervous system, In "Greenfield's Neuropathology" (Graham DI and Lantos PL), 7th Edition, Vol 1, pp.123-191. Arnold, London

32. Schiffmann R and van der Knaap MS. An MRI-based approach to the diagnosis of white matter disorders. Neurology 2009;72:750-759

33. Barker PB, Horská A. Neuroimaging in leukodystrophies. J Child Neurol. 2004;19(8):559-70

34. Symms M, Jäger HR, Schmierer K, Yousry TA. A review of structural magnetic resonance neuroimaging. J Neurol Neurosurg Psychiatry. 2004;75(9):1235-44

35. Barkovich AJ. Magnetic resonance techniques in the assessment of myelin and myelination. J inherit Metab Dis 2005;28:311-343

36. Schmierer K, Tozer DJ, Scaravilli F, et al. Quantitative magnetization transfer imaging in postmortem multiple sclerosis brain. J Magn Reson Imaging. 2007; 26:41-51

37. van Waesberghe JH, Kamphorst W, De Groot CJ, et al. Axonal loss in multiple sclerosis lesions: magnetic resonance imaging insights into substrates of disability. Ann Neurol. 1999; 46:747-54

38. Horsfield MA, Barker GJ, Barkhof F, et al. Guidelines for using quantitative magnetization transfer magnetic resonance imaging for monitoring treatment of multiple sclerosis. J Magn Reson Imaging 2003;17(4):389-97

39. Engelbrecht V, Rassek M, Gärtner J, et al. The value of new MRI techniques in adrenoleukodystrophy. Pediatr Radiol 1997;27(3):207-15

40. Le Bihan D and van Zijl P. From the diffusion coefficient to the diffusion tensor. NMR Biomed 2002;15:431434

41. Beaulieu C. The basis of anisotropic water diffusion in the nervous system - a technical review. NMR Biomed 2002;15:435-455

42. Yamada K, Sakai K, Akazawa K, Yuen S, Nishimura T. MR tractography: a review of its clinical applications. Magn Reson Med Sci. 2009;8(4):165-74

43. Engelbrecht V, Scherer A, Rassek M, et al. Diffusion-weighted MR imaging in the brain in children: findings in the normal brain and in the brain with white matter diseases. Radiology 2002;222(2):410-8

44. Hermoye L, Saint-Martin C, Cosnard G, et al. Pediatric diffusion tensor imaging: normal database and observation of the white matter maturation in early childhood. Neuroimage 2006;29(2):493-504

45. Ben Bashat D, Ben Sira L, Graif M, et al. Cohen Y, Assaf YNormal white matter development from infancy to adulthood: comparing diffusion tensor and high b value diffusion weighted MR images. J Magn Reson Imaging. 2005;21(5):503-11

46. Horsfield MA and Jones DK. Applications of diffusion-weighted and diffusion tensor MRI to white matter diseases - a review. NMR Biomed. 2002;15(7-8):570-7

47. Kim MJ, Provenzale JM and Law M. Magnetic Resonance and diffusion tensor imaging in pediatric white matter diseases. Top Magn Reson Imaging 2006;17:265-274

48. Schmierer K, Wheeler-Kingshott CA, Boulby PA, et al. Diffusion tensor imaging of post mortem multiple sclerosis brain. NeuroImage 2007, 35:467-477

49. Ito R, Melhem ER, Mori S, et al. Diffusion tensor brain MR imaging in X-linked cerebral adrenoleukodystrophy. Neurology 2001;56(4):544-7

50. Oguz KK, Anlar B, Senbil N, Cila A. Diffusion-weighted imaging findings in juvenile metachromatic leukodystrophy. Neuropediatrics. 2004;35(5):279-82

51. Govindaraju V, Young K, Maudsley AA. Proton NMR chemical shifts and coupling constants for brain metabolites. NMR Biomed. 2000;13(3):129-53

52. Provencher SW. Estimation of metabolite concentrations from localized in vivo proton MR spectra. Magn Reson Med 1993; 30: 672-679

53. Ernst T, Kreis R, Ross BD. Absolute quantification of water and metabolites in the human brain. I. Compartments and water. J Magn Reson 1993; 102:1-8

54. Soares DP and Law M. Magnetic resonance spectroscopy of the brain: review of metabolites and clinical applications. Clin Radiol. 2009;64(1):12-21

55. Pouwels PJ, Brockmann K, Kruse B, et al. Regional age dependence of human brain metabolites from infancy to adulthood as detected by quantitative localized proton MRS. Pediatr Res. 1999;46(4):474-85

56. Cecil KM and Savcic Kos R. Magnetic resonance spectroscopy and metabolic imaging in white matter diseases and pediatric disorders. Top Magn Reson Imaging 2006; 17: 275-293

57. Kang PB, Hunter JV, Kaye EM. Lactic acid elevation in extramitochondrial childhood neurodegenerative diseases. J Child Neurol. 2001;16(9):657-60

58. Hattingen E, Raab P, Franz K, et al. Myo-inositol: a marker of reactive astrogliosis in glial tumors? NMR Biomed 2008; 21:233-41