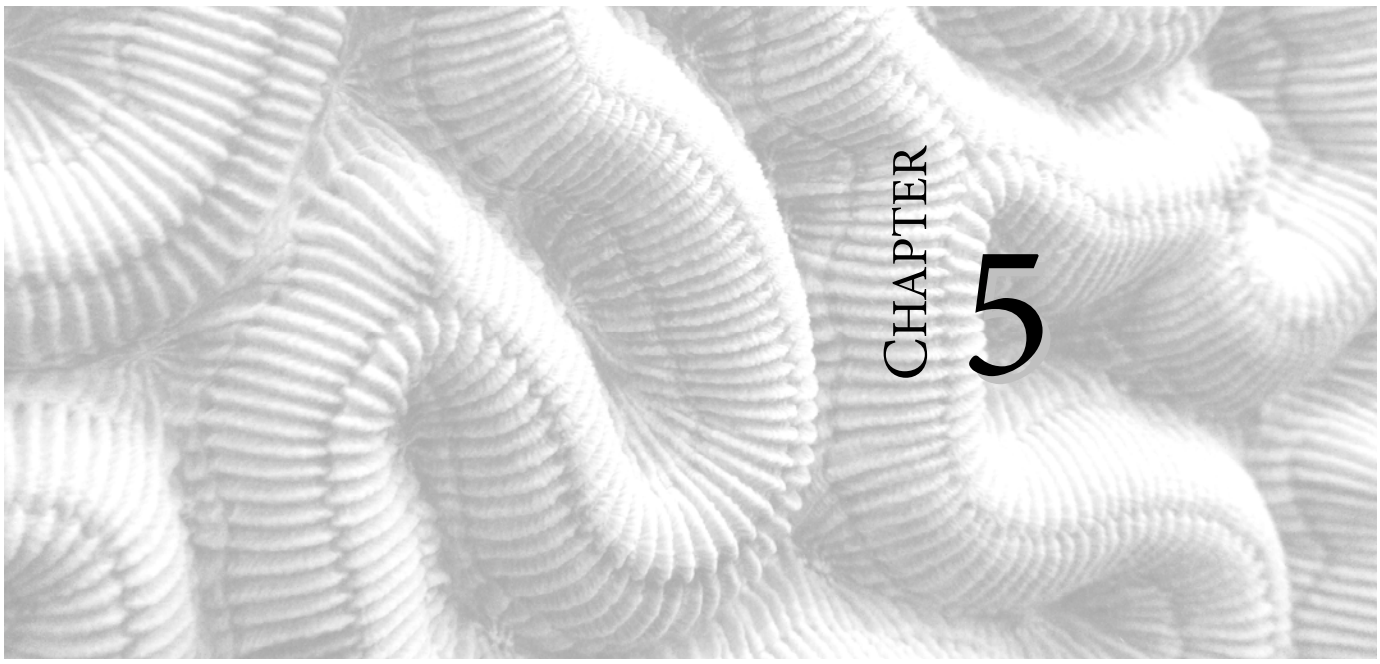

CHAPTER 5

SUMMARY, GENERAL DISCUSSION AND FUTURE PERSPECTIVES

The studies presented in this thesis have focused on the application of quantitative MR techniques, such as MRS, MTI and DTI, to study the cerebral white matter in patients with a white matter disorder.

The main goal of this thesis was to determine the correlation between MR and pathology. Two different sub-goals were set: (I) to determine the histopathologic correlate of specific MRI features; and (II) to determine the specificity of quantitative MR parameters with regard to histopathologic substrate. Within the second goal the first studies concerned white matter disorders with known histopathology, whereas subsequent studies concerned white matter disorders in which the histopathologic substrate of MRI findings is not known or not well known.

5.1 *Histopathologic correlate of a specific MRI feature in WM disorders*

In order to understand a characteristic MR imaging pattern seen in specific lysosomal storage disorders (MLD, GLD and infantile GM1 and GM2 gangliosidosis) a post-mortem MR imaging and histopathologic correlative study was performed (**chapter 2**). A peculiar MR imaging appearance consisting of radially oriented stripes of apparently normal signal intensity within otherwise diffusely abnormal cerebral white matter is typically seen in these diseases and its histopathologic correlate is unknown. We included three patients with MLD, GLD and infantile GM1 gangliosidosis in this study and performed both in vivo and post-mortem MRI and histopathology. Histopathologic studies in GM1 revealed that the stripes were related to a relative high amount of myelin in perivenular regions, corresponding to the stripes on MR imaging. In MLD, in addition to relative sparing of myelin in perivenular regions, there was also an accumulation of glial cells and macrophages containing lipids in these areas. In GLD, many globoid cells containing lipid material in the perivenular regions, in the absence of any myelin, corresponded to the stripes on MR imaging.

5.2 *Quantitative MRI in white matter disorders with known pathology*

In **chapter 3.1**, various white matter disorders in which the histopathology was known from the literature (disorders characterized by demyelination, hypomyelination, myelin vacuolation and white matter rarefaction and cystic degeneration) were studied with multiple MR techniques to investigate whether quantitative MR parameters allowed discrimination between different types of white matter pathology. Clear differences in quantitative MR parameters were found between the different types of disorders. For the hypomyelination group all MR parameters were close to normal with the exception of elevated tCr and Ins levels and decreased MTR values. The most striking findings for the demyelination group were the highly elevated Cho and Ins levels. The findings for the myelin vacuolation and cystic degeneration groups tended to be similar with high ADC values and variable decreases of most MRS metabolites, but they were more pronounced in the cystic degeneration group. Especially the reduction in MTR was markedly more severe

in the cystic degeneration group than in the myelin vacuolation group. Using these MR parameters, white matter abnormalities with a different underlying pathology could be discriminated: LDA showed that the combination of tCr, Cho, Ins, MTR and ADC resulted in a correct classification in 95% of all patients.

A direct post-mortem MR imaging and histopathologic correlative study (**chapter 3.2**) was performed to characterize the degree of histologic specificity of quantitative MR parameters with regard to histopathologic parameters *in vivo* and in fresh and formalin-fixed postmortem brain tissue of patients with X-ALD.

In brain tissue of the controls we found that in unfixed postmortem white matter, MTR values were essentially unchanged, ADC values were greatly reduced and FA was reduced by ~50% as compared to in vivo values. Formalin fixation led to an approximately 20% reduction in MTR and a further decrease in ADC, whereas FA rose to values close to in vivo values.

We demonstrated that differences in quantitative MR parameters, present in living X-ALD patients and related to severity of white matter pathology, were retained in postmortem brain tissue, with a very low MTR, high ADC and low FA in the center of the lesion and similar but less pronounced changes at the edge of a lesion. This progression in abnormality of ADC, FA and MTR from NAWM to lesion periphery to lesion core indicated primarily a correlation between these MR parameters and severity of the general tissue damage. In contrast, the MRS alterations seen in the edge of the lesion were essentially different from those seen in the center of the lesion, reflecting different histopathologic processes. We found decreased NAA, low tCr, elevated mIns, and elevated lactate in the center of lesions. MRS at the edge of the lesion showed an increase in Cho, some increase in mIns, a more pronounced lactate elevation and less severe decreases in NAA and tCr. Furthermore, we showed that MR parameters correlated with white matter histopathologic parameters. The prediction of histopathologic parameters in the white matter abnormality was reliable using DTI parameters (ADC and FA) only; adding MTR did not improve this prediction significantly.

From the results of these two studies we learned that the three quantitative imaging techniques we used yield important quantitative information about brain tissue. Increased ADC, decreased FA and decreased MTR indicate damage to the tissue matrix: They reflect damage and loss of myelin sheaths and axonal membranes in the white matter, reduced cellular density and increased water-filled spaces. At the extreme end all the tissue structures are lost in the white matter of patients with VWM; at autopsy these patients revealed diffusely rarefied to cavitated white matter with profound losses of oligodendrocytes, myelin sheaths and axons. Likewise, compatible with the loss of brain tissue, MRS of the white matter in patients may show a reduction of all normal signals.

In contrast, the changes in concentrations of MRS metabolites can give additional information on the nature of the pathology related to the MR imaging findings. A decrease in tNAA concentration can be ascribed to axonal damage and loss. The increase in Cho is related to enhanced membrane turnover associated with active demyelination, and possibly

also the accumulation of myelin breakdown products. The high mIns reflects astrogliosis. Elevated lactate is seen in areas of inflammation with infiltrating macrophages.

We used the above interpretations to predict pathology in some white matter disorders with unknown pathology, described in the next chapter.

5.3 *Quantitative MRI in white matter disorders with unknown pathology*

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, were presented.

Chapter 4.1 presents the results of a serial quantitative MRI study in several patients with Alexander disease. MR spectroscopy showed highly elevated Ins, Lac and Cho levels and decreased tNAA levels in our patients. In addition, we found high ADC and decreased MTR an FA values in the abnormal white matter. Using the discriminant functions described in **chapter 3**, the white matter pathology was predicted to be demyelination rather than hypomyelination. However, the sequential MR imaging findings in Alexander disease provided strong evidence against active demyelination as underlying pathology: the conventional MRI showed remarkably stable white matter abnormalities, even at prolonged follow-up, despite clinical progression of the disease. An alternative explanation for our spectroscopic, DTI and MTI findings could be hyperplasia and hypertrophy of astrocytes with Rosenthal fiber deposition.

Chapter 4.2 corresponds to a study in which we wanted to determine whether the nature of the white matter pathology in congenital CMV infection could be similar to the known pathology of PVL, by comparing changes in quantitative MR parameters and MRS metabolite concentrations. In both groups ADC values were increased, FA and MTR values were reduced, concentrations of NAA and Cho were reduced; and mIns concentrations were slightly increased. No differences were found between the two groups, suggesting that the pathology of the white matter lesions in congenital CMV infections is similar to that of PVL and also characterized by axonal losses, lack of myelin deposition due to oligodendrocytic losses, and astrogliosis.

Chapter 4.3 corresponds to a MRI study in eight patients with LBSL, with a distinct magnetic resonance imaging pattern of inhomogeneous cerebral white matter abnormalities and selective involvement of brainstem and spinal tracts. MR spectroscopy showed increased lactate, normal to mildly elevated Cho, decreased NAA and increased mIns concentrations in the abnormal white matter. In addition, ADC was increased, FA was decreased and the MTR of the abnormal white matter was decreased. Using the discriminant functions described in **chapter 3.1**, the white matter pathology was predicted to be demyelination. At present, no pathology is available to confirm this.

5.4 *Future perspectives*

The results presented in this thesis substantiate that the use of quantitative MR techniques measuring diffusion, MTR and metabolite concentrations within the white matter abnormalities yields important information about the underlying pathology of these lesions. Our post mortem MRI and histopathology correlation studies have shown the effects of tissue decay and formalin fixation of brain tissue on changes of quantitative MR parameters and have provided important insights into the pathology reflected by MRI.

A limitation of our correlative studies in living patients was the relatively large volume of interest (VOI), determined by the size of the single voxel proton MRS VOI. Data obtained had to be averaged for this VOI for all parameters, although histopathology and all other MR parameters would have allowed a much better spatial resolution. In chemical shift imaging (CSI), also called spectroscopic imaging, spectra are generated from many smaller VOIs at the same time, covering more or less an entire brain slice (1). The smaller VOIs of CSI make it easier to compare metabolite concentrations with data from other quantitative MR techniques and histopathology. Apart from CSI, higher field strength would allow a better spatial resolution and may improve the characterization of the white matter lesions and the correlation with histopathology.

For future studies, it would also be interesting to image brain material from patients with a leukodystrophy post mortem at higher field strengths, and to compare the results to histopathologic characteristics. With the higher spatial resolution it should be possible to improve the characterization of MR parameters of small areas and to facilitate a more direct comparison with histopathology, increasing our insight into the histopathologic substrate of different white matter lesions.

A problem for correlating MR parameters with individual histopathologic parameters is that the pathologic changes are interdependent. In demyelinating diseases, the myelin loss also leads to loss of axons and the resulting tissue damage is associated with inflammation, macrophage activation and astrogliosis. The simultaneous histopathologic changes hampered our assessment of the specificity of the MR parameters for individual histolopathologic parameters. There are two approaches conceivable to gain more knowledge on the specificity of the MR parameters for individual histolopathologic parameters. One is to use animal models, in which only one of the histopathologic parameters is abnormal or deficient, like in a myelin-deficient rat (2). The other approach is to study specific changes of MR parameters in well-controlled in vitro model systems of neural tissue, and to measure how, for instance, the process of demyelination (3) or inflammation (4) alone affects changes in quantitative MR parameters.

Correlating post mortem MRI examination directly after death in fetuses, neonates and children, with histopathology findings at autopsy will improve the diagnostic accuracy of post-mortem MRI to be used as an alternative for conventional autopsy in the future. Examination after death can provide important information about why a fetus did not survive. At the moment, MRI is particularly useful for assessing the brain post mortem, because it is very sensitive in detecting gross structural abnormalities (i.e., developmental

abnormalities in fetuses). However, its specificity for tissue pathology is low, because of the lack of histological information provided by microscopic examination (5, 6).

It is essential that future work will continue to focus on the improvement of the characterization of white matter lesions by quantitative MR techniques, both in vivo and post mortem. At present many patients with white matter abnormalities still remain without a specific diagnosis. Better understanding of what is happening in the abnormal white matter of such patients may help to determine the disease or type of disease. This thesis demonstrates that application of the results obtained in MR studies on patients with white matter abnormalities of known origin helps to predict the histopathologic basis in patients with white matter abnormalities of unknown origin. Quantitative MR techniques may also prove valuable in the search for homogeneous groups of patients among the patients with a white matter disorder of unknown origin and help define novel disorders. Finally, quantitative MR parameters will be essential in the monitoring of disease progression and the effects of treatment, when more and better therapies become available.

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