MRI and histopathology in multiple sclerosis

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7

SUMMARIZING DISCUSSION

In this thesis, histopathological, clinical and MRI findings were combined with the focus on grey matter damage, diffusely abnormal white matter and atypical white matter lesions. In addition, the same methods were applied to a different field, where differences between WM hyperintensities in Alzheimer and non-Alzheimer patients were studied.

The method of combining histopathology and MRI as applied in this thesis was summarized in **Chapter 2**. This review focusses on the advances of knowledge that were gained with this method, as well as the challenges of this approach and gives recommendations for future research.

CHAPTER 3: THE GREY MATTER IN MULTIPLE SCLEROSIS

Grey matter (GM) lesions are one example of a research trend, which dramatically changed our understanding of MS within only a couple of years. While in the past, MS was seen as a demyelinating disease of the white matter, it is presently firmly acknowledged that GM damage is as least as abundant and important as WM damage. Histopathological studies have shown that grey matter demyelination can occupy up to 30% of the grey matter (1) which is twice as much as the demyelinated area in the white matter. Demyelination is not restricted to neocortical areas, paleo- and archicortical structures such as the insula and hippocampus, the basal ganglia, hypothalamus, the cerebellar cortex and spinal cord grey matter can also be affected (2, 3). However, the plethora of cortical lesions seen in pathology is in sharp contrast to the numbers detected in vivo by conventional MRI, where up to 95% of intracortical lesions are missed (4). Cortical GM lesion detection is hindered by the characteristics of the lesions itself -i.e. their small size, the sparse inflammatory cell infiltration and blood-brain barrier damage, and the low myelin density in the upper cortical layers - as well as several technical factors, including limited image resolution, and lower contrast between small cortical lesions and surrounding normal cortical GM due to intrinsically longer GM relaxation times (5).

With the introduction of a new MRI sequence, double inversion recovery (DIR), intracortical lesion detection could be dramatically improved (6-8), and subsequently, this technique was applied in numerous (imaging) studies. These studies provide important information about the accumulation of cortical lesions, and the clinical consequences thereof.

However, DIR applications have been criticized because this sequence is prone to image artefacts, which makes DIR- detected hyperintensities difficult to interpret. In

addition, DIR has regional variations in GM signal intensity (9) and an intrinsically low signal to noise ratio, which gives DIR images a "grainy" appearance (6, 8, 10). These properties might obscure the detection of cortical lesions on the one hand, but also lead to false positive ratings on the other hand (11). This, together with the fact that the number and volume of cortical lesions detected with DIR is much lower than those reported in histopathology studies, have fueled discussions about the validity of this technique.

However, for an accurate correlation with clinical deficits, and inclusion of GM lesion burden in treatment studies, a reliable quantification of grey matter lesions is essential. Therefore, the post-mortem study described in **Chapter 3.1** was performed to assess the sensitivity (percentage of lesions detected on MRI as compared to histopathology), and specificity (percentage of false positive lesions, which are seen on MRI, but lack histopathological correlation) of 3D-DIR for cortical lesions (CL). In this study, brain samples from patients with chronic MS were scanned with both 3D-DIR and 3D-FLAIR sequences at 1.5T. Cortical lesions were scored on both sequences twice, once blinded (prospective scoring), and once unblinded (retrospective scoring) to histopathology.

This study could confirm the in vivo observations of the higher CL detection rate with 3D-DIR as compared to 3D-FLAIR. In addition, 3D-DIR showed a high pathologic specificity (90%), and therewith a low number of false positive scorings.

Although the sensitivity of 3D-DIR for all cortical lesions was 1.5 -2.0-fold higher as when scored on 3D-FLAIR alone, the detection rate for purely intracortical lesions was still disappointingly low. In the best case, and with awareness of the histopathology (retrospective scoring), 29% of all intracortical lesions could be picked up, however, this number dropped to disillusioning 9% when the prospectively scored lesions only were considered. Especially, the detection of subpial type III lesions, which are the most common lesion type, was very low (7% detected in the prospective scoring).

How should in vivo DIR studies be interpreted with these results in mind?

Obviously, DIR at 1.5 Tesla detects only a small percentage of all cortical lesions. However, if lesions are detected, it can be confidently assumed that these are indeed true lesions rather than artefacts, as nearly all hyper-intensities seen on MRI corresponded to histopathologically proven lesions.

It can be assumed that in vivo DIR studies have a similar sensitivity and specificity for cortical lesions, as comparable image contrasts were used in the post-mortem 3D-DIR and 3D-FLAIR sequences. However, DIR acquisition protocols and field strengths vary considerably between centers, which makes interpretation of DIR images difficult, and hampers comparison of (multicenter) in vivo data on cortical lesions. Geurts et al could show that even when standardized scoring criteria are used for cortical lesions, an international panel of experts agrees completely only on 19%

of all scored cortical lesions (12). This underlines the necessity of a standard protocol for DIR scanning besides standardized scoring criteria. Especially “old” (multi- slab) 2D DIR sequences suffer from artefacts (incidental magnetic transfer effects and flow artefacts), whereas single-slab, 3D images are not affected by this phenomenon (13).

Several studies have used DIR in vivo, so were cortical lesions described in up to 36% in CIS and in 64 to 81% of definite MS patients (14-17). If we interpret these studies with the numbers given in **Chapter 3.1**, it seems likely that these percentages are considerably higher in reality, but on the other hand it can be safely assumed that the described hyper-intensities truly correspond to lesions.

Therefore, cortical lesions as imaged with DIR can be utilized for clinical correlations, but also to facilitate the diagnosis of MS. Filippi et al included cortical lesions as detected with DIR in the diagnostic criteria, which led to a significant improvement in the diagnostic accuracy (81% accuracy instead of 75 to 78%). Furthermore, the presence of cortical lesions are independent predictors of conversion to MS within 4 years after clinical onset (18). Most recently, CLs were incorporated in the dissemination in space criteria for MS, as an extension to the current inclusion of juxtacortical lesions (19). In addition, consideration of CLs could be helpful in the management of RIS, where cortical lesions were described in 40% (20). There, they could possibly identify patients at high risk for development of MS and could allow targeted follow up or even treatment. Furthermore, subpial cortical lesions represent a diagnostic hallmark of MS, as they are not seen in other chronic inflammatory or degenerative brain disease (21). In summary, consideration of cortical lesions improves the accuracy of diagnosis and prognosis in MS (22), but it has of yet to be established whether DIR is the MRI technique most suited to do so. Besides the described suboptimal sensitivity, the coefficient of variation between DIR detected grey matter lesion counts on a patient level is as large as 42% (12).

Apart from DIR, several other sequences have been applied with the goal to increase the visibility of cortical lesions. These included the application of MRI techniques such as 3D-T1-based techniques (23), T2* weighted imaging (24) and phase-sensitive inversion recovery (PSIR) (25) either alone or in combination, and at various field strengths (results of selected studies are presented in **Table 1**). Higher field strengths, which have higher signal to noise ratio, can provide higher spatial resolution and therefore higher sensitivity for cortical lesions as compared to standard field strengths (26-29). So could for example, cortical lesion detection with DIR be improved when moving from 1.5 to 3T (30). Interestingly, DIR loses its benefits at 7T, and sequences that were suboptimal at 1.5 and 3T (such as FLAIR and T2) seem to perform better at ultra- high field strengths (**Table 1**).

Table 1: Simplified comparison of MR sensitivities for overall cortical lesion detection (selected studies)

Author/year	Sequence (Field strength)	Histopathology
Geurts et al, 2005 (7)	**T2 (1.5T) < FLAIR (1.5T) < DIR (1.5T)**	no
Simon et al, 2010 (29)	**DIR (1.5T) < DIR (3T)** T2 (1.5T) ≥ T2 (3T) FLAIR (1.5T) ≤ FLAIR (3T) T2 (1.5T) < FLAIR (1.5T) < DIR (1.5T) T2 (3T) < FLAIR (3T) **< DIR (3T)**	no
Nelson et al, 2007 (11)	**FLAIR (3T) < PSIR (3T) + DIR (3T)**	no
Wattjes et al, 2007 (31)	[a]T2 (3T) < FLAIR (3T) < DIR (3T)	no
Sethi et al, 2012 (25)	**DIR (3T) < PSIR (3T)**	no
Tallantyre et al, 2010 (32)	FLAIR (3T) < MPRAGE (7T) < DIR (3T)	no
De Graaf et al, 2012 (26)	T1 (3T) < T1 (7T) **T2 (3T) < T2 (7T)** **FLAIR (3T) < FLAIR (7T)** T1 (3&7T) < T2 (3&7T) < FLAIR (3&7T)	no
Nielsen et al, 2012 (24)	DIR (3T) < T2* (7T)	no
Pitt et al, 2010 (33)	WHAT-TFE (7T) < T2* (7T)	yes
Kilsdonk et al, 2013 (34)	**T1 (7T) < DIR (7T)** < T2 (7T) **< FLAIR (7T)**	no
Kilsdonk et al, 2016 (28)	[b]DIR (3T) < DIR (7T) **FLAIR (3T) < FLAIR (7T)** **T2* (3T) < T2* (7T)** T1 (3T) < T1 (7T) T2 (3T) < T2 (7T) T1 = T2 = T2* = FLAIR = DIR (all 7T)	yes
Jonkman et al, 2015 (27)	T2* (7T) ≤ T2 (7T)	yes
Beck et al, 2018 (35)	**T2* (7T) < MP2RAGE (7T)**	[c]yes

Displayed results include intra-cortical lesions and mixed grey-white matter lesions (type I-IV) except stated otherwise.

[a]: mixed grey-white matter lesions only

[b]: prospective scoring

[c]: sensitivity data from in-vivo analysis, one post-mortem brain separately analysed for specificity.

bold font: statistically significant differences

So far, it is unknown which sequence or combination of sequences works best for cortical lesion detection (and various types of cortical lesions), and a comprehensive post mortem study comparing the various techniques is lacking at present. It seems that this question is of particular importance at lower field strengths, where the sensitivity of various sequences can vary significantly. At ultra high field on the other hand, differences between sequences seem to be smaller and non-significant with a smaller absolute difference. At 7T however, a combination of various techniques might help to characterize the pathologic features of MS lesions (36, 37).

For a better interpretation of studies investigating CLs it would be helpful to know why some CLs are visible on MRI and others are not. In theory, various factors could influence the visibility of CLs, including size, inflammation or destructive properties. To assess the selective visibility of CLs, the comparative post-mortem study described in **Chapter 3.2** was performed. In this study, MRI visible and invisible lesions (as determined by T2SE and FLAIR images on 1.5 Tesla), and non-lesional grey matter were compared by histopathology. In addition, cortical tissue was characterized by quantitative MRI measurements.

This study demonstrates, that visible CLs are significantly larger than their invisible counterparts. Therefore, visibility of CLs is exclusively determined by size, and neither quantitative MRI measurements, nor histopathological parameters (such as inflammation, or neuroaxonal loss) could discriminate between visible and invisible lesions. Because our histopathological assessment was not exhaustive, it is possible that other factors than those measured might have contributed to the visibility of CLs. It can however be assumed that these factors were not significant contributors to visibility as the histopathological findings were confirmed by quantitative MRI measurements which also showed no difference between visible and invisible lesions. For the same reason, it can be argued that results determining visibility would have been comparable if assessments were performed with DIR instead of T2 weighted sequences. Unfortunately, no postmortem DIR protocol existed at the time this study was performed.

None of our lesions showed macrophage or lymphocytic infiltration and it was therefore not possible to assess whether inflammation would lead to better visibility of even smaller lesions. The absence of acute inflammation in the cortex seems to be typical for autopsy studies (38, 39), as opposed to biopsies from patients with early MS (40, 41). While this has fueled the discussion whether CLs are neurodegenerative vs inflammatory, the findings from biopsy and autopsy may merely present the outer ends of a spectrum, starting with inflammation (as seen in biopsies), and ending with an “inactive” CL (42). Although CLs triple between early MS and SPMS (43), their inflammatory activity is rarely picked up by gadolinium enhanced scans (44). A milder disruption of the

BBB and a shorter inflammatory phase than in WM lesions might be possible reasons for this phenomenon and an experimental autoimmune encephalomyelitis (EAE) rat model affirms that cortical inflammation resolves relatively rapidly (45).

The second important finding of this study was that visible, and therefore larger lesions were associated with a higher cortical lesion number and higher percentage of demyelination. This indicates, that if cortical lesions are visible on MRI, they only represent the "tip of the iceberg", as many more invisible lesions may be present. With this in mind, the presence of cortical lesions on DIR images could give a sufficient idea of the extent of GM damage in clinical routine. On the other hand, studies addressing the "normal appearing grey matter" have to be interpreted with the knowledge that most of the cortical lesions will fall within MRI defined extra-lesional GM and both lesions and extra-lesional GM may contribute to the changes measured (46). This could be an explanation for the only mild to moderate correlation of CLs with disability (15, 47). In addition, disability correlations of the current DIR studies are heavily distorted towards the most readily visible type I lesions and under-represent Type II and III lesions or -in the worst case - do not represent them at all. Even at 7T, approximately 70% of all cortical lesions and approximately 80% of type III lesions are missed in prospective scoring, whereas up to 100% of type I lesions could be detected (28).

Cortical pathology extends well beyond areas of focal demyelination. Histopathological studies have demonstrated various heterogeneous processes including neuro-axonal loss, gliosis, loss of dendritic spines and various degrees of remyelination (48-51).

For this reason, quantitative MRI measurements might be better suited for the overall assessment of damage in the GM. Our studies show, that MTR, and to a lesser extent T2 measurements reflect demyelination in the cortex. MTR did not differ between non-lesional GM and GM lesions, whereas T1 and T2 relaxation times showed a significant difference. Other studies at 3, 7 and 9.4T respectively, indicate that higher field strengths with consecutive better contrast and resolution are required to detect subtle differences within the cortical grey matter (52-54). Jonkman et al looked at the capacity to distinguish normal appearing GM from (subpial) lesions with quantitative MR imaging techniques at 7T and found lower mean MTR in subpial lesions compared with myelin-density matched normal appearing grey matter (55). This observation supports the notion that lower MTR in the outer cortex as measured in vivo is at least partly related to demyelination and could reflect type III lesions which are underrepresented on conventional MRI (56). As far as our associations between T1- and T2 relaxation times and histopathology are concerned, our findings are similar to the results by Schmierer et al, who found that T2 is a predictor of demyelination and T1 is a predictor of neuronal density (53). Opposed to these findings, in a postmortem

study by Tardif et al, T1 was strongly correlated to myelin content (54). This illustrates that it can be challenging to pin (changes of) quantitative MR measurements to a single underlying pathology. To give another example, the increased fractional anisotropy (FA) of demyelinated grey matter lesions has been attributed to either local activation of microglia or neuronal, dendritic or synaptic loss (57-59). Recently, Jonkmann et al have proposed an increased cellular density due to tissue compaction as possible alternative explanation (52).

This shows that advanced MRI methods are sensitive to microstructural changes, as they reflect alterations in the physical characteristics of brain tissues, but lack specificity for pathological processes (60). This problem can be partly overcome by another technique, positron emission tomography (PET), which measures the distribution of specific ligands, therewith allowing for the highest possible specificity at cellular and tissue level (61). Only a couple of studies have explored the cortical grey matter with PET (for a comprehensive overview see **Table 2A** in addendum, a summary of findings for the cortical grey matter is presented in **Table 2**) and most studies used PET to visualize activated microglia.

Activated microglia form a central aspect of neuroinflammation and are a key element of neurodegeneration, but cannot be visualized with conventional MRI (84-86). Histopathological studies focusing on cortical GM show an increased number of activated microglia in patients with subpial lesions in early disease stages (87), and presence of rims of activated microglia is associated with younger time at death and a shorter disease course, however this is not uniformly observed (50). PET studies on activated microglia with results for cortical grey matter show a similar heterogeneous picture, but technical aspects, small sample sizes and genetic polymorphisms influencing the binding affinity of radioligands have to be taken into account when interpreting these results. Overall, it seems, that at least in a subset of patients, more cortical microglia activation is present as compared to controls (66, 69, 73). Within MS patients, microglia activation in the cortex is more prominent in progressive MS and correlates with physical and cognitive disability (66, 69). So far, only four longitudinal treatment studies with observation times between 6 months and 1 year and small participant numbers have been performed.

Table 2: Cellular processes measured in the MS cortex with PET (human in-vivo studies only)

Pathophysiologic Mechanism	Radioligand	Cortical GM findings in MS vs HC	Cortical GM findings within MS group	Clinical correlations
Inflammation	[^{11}C]PK11195	MS > HC (66,63*, 68*) MS = HC (62, 64, 65)	MS BL > MS FU (+T) (67) MS BL = MS FU (+T) (62,63)	EDSS (66)
	[^{11}C]PBR28	MS > HC (69) MS = HC (70,71)	RR < SP (69)	EDSS, cognition (69)
	[^{18}F]FEDAA1106	MS = HC (72)		
	[^{11}C]DPA713	MS > HC (73)	MS BL < MS FU (+T) (73)	
	[^{11}C]TMSX	MS = HC (74)		
Demyelination	[^{11}C]PiB	MS = HC (75)		
Astrocyte activation	[^{11}C]acetate	MS > HC (76)		
Neuronal Damage	[^{11}C]FMZ	MS < HC (77)		cognitive performance (77)
Neuronal Integrity/ Inflammation	[^{18}F]FDG	MS < HC (79, 82) MS = HC (78)	MS BL < MS FU (+T) (78) MS (+C) < MS (-C) (82)	fatigue, depression (78, 80) walking speed (79)
Cholinergic metabolic profile	[^{11}C]MP4A	MS = HC (83)		cognitive performance (83)

MS: Multiple Sclerosis, **HC:** Healthy control, **BL:** Baseline, **FU:** Follow-Up, **(+T):** with treatment, **RR:** relapsing remitting MS, **SP:** secondary progressive MS, **(+C):** with cognitive impairment, **(-C):** without cognitive impairment, ***:** data not significant

Two studies exploring the effects of natalizumab (62) and fingolimod (63) showed no difference in microglial activation between baseline and follow up, while treatment with glatiramer acetate led to a decrease (67) and a group treated with IFN or Fingolimod showed an increase (73) in cortical microglia activation. Interpretation of these results is challenging, as at the moment it is not possible to distinguish between functional states of microglial cells (pro-inflammatory vs protective) with TSPO PET

(60), but might also indicate that some medications might be more suited to influence microglial activation than others.

Besides inflammation, remyelination is an important process in the grey matter, which is even more extensive in cortical- than in white matter lesions, but is heterogeneous between patients (51, 88). MRI techniques are sensitive to alterations in myelin density, but cannot distinguish between "good" (i.e. remyelination) and "bad" (i.e. demyelination). Therefore, an imaging marker for remyelination would be of interest for measuring treatment effects. [^{11}C]PIB is a radioligand which allows observations of positive and negative changes over a follow- up period in white matter lesions, corresponding to dynamic re- and demyelination (89). So far, only one cross-sectional study has compared cortical [^{11}C]PIB uptake between MS and controls, showing no difference between the groups (75). Similarly, no difference was found when the whole grey matter (deep grey matter and cortex) was compared between MS and controls (89). The low spatial resolution of PET systems (above 4 mm for most), together with the low thickness of the cortical ribbon and the low myelin density of the cortex may explain this result (60).

PET could also contribute to our understanding of neurodegeneration by selectively targeting neuronal damage. Neurodegenerative changes within the GM range from axonal transection to synapto-dendritic pathology and neuronal loss, occur independent from GM demyelination (48, 49) and are thought to be a major cause of neurological decline. Neuronal damage can be indirectly measured by glucose metabolism with [^{18}F]FDG PET, and reduced uptake in the cortex has been correlated to cognitive disability, fatigue and depression (78, 80, 82). While [^{18}F]FDG is not a pure neuronal marker (as glucose is metabolised by other than neuronal cells as well) other radioligands which are specific for neurons have been developed. For example, [^{11}C] FMZ reflects axo-somatic and axo-dendritic synapses which have been shown to be reduced in the MS cortex and reduced uptake of this radioligand correlates with cognitive performance (77). This mirrors a histopathological study by Jürgens et al, showing pronounced loss of dendritic spines in the MS cortex independent of cortical demyelination and axon loss (48).

In summary, visualization of "hidden pathology" such as microglial activation, neurodegeneration and remyelination contributes to our understanding of the pathogenesis of cortical pathology. Further efforts should be made to validate the above mentioned techniques so they can find application in treatment studies. In addition, future attempts should be made to improve visualization of cortical lesions, especially type III lesions.

Unsolved questions and suggestions for further research:
A focus on the visualization of hidden inflammation (microglia) and remyelination and repair will help to explain pathological processes leading to cortical lesion formation and could serve as therapeutic targets. Further efforts to increase the visibility of cortical lesions will allow for more accurate correlations with MRI and clinical measures.

- What is the independent contribution of cortical lesions to cortical atrophy?
- Do all subpial lesions arise from leptomeningeal inflammation? Which role does microglia play in cortical lesion development and remyelination?
- Which (combination of) MRI sequences are best suited to image and characterize cortical lesions?
- Are diffuse changes in the NAGM predictors of cortical lesion development?

CHAPTER 4: THE WHITE MATTER IN MULTIPLE SCLEROSIS

The white matter harbors not only the most prominent feature of MS- the white matter lesion- but also hidden, diffuse damage, *outside* lesions. Damage in this so-called NAWM has been extensively studied with quantitative MRI measurements, and has been shown to be a significant contributor to disability (90-92). Invisible on conventional MR images, this damage defies the eye of the assessing clinician and contributes to the so called clinico- radiological paradox (93).

However, one aspect of diffuse damage is readily visible on conventional MRI: "Diffusely abnormal white matter", or DAWM, which presents as a subtle signal hyper-intensity with dispersed borders on T2-weighted images (94). Although extensive at times, these changes are disregarded in conventional measures of the lesion burden, because their underlying histopathology and clinical impact is unclear. Several processes have been claimed to contribute to DAWM, including inflammation, newly forming lesions, blood brain barrier disruption, demyelination and axonal loss. A clear definition of DAWM could lead to its inclusion in routine assessments and bring about a better picture of disease burden.

We therefore aimed to define DAWM in terms of histopathology and quantitative MRI measurements. In the post-mortem study presented in **Chapter 4.1**, DAWM was compared to NAWM and focal WM lesions in a region- of- interest approach. DAWM differed significantly between NAWM and WM lesions, and formed an intermediate between NAWM and lesions in the histopathological and quantitative

MRI measurements. DAWM was found to consist of extensive axonal loss, decreased myelin density, and chronic fibrillary gliosis. Acute axonal damage, acute inflammation or blood brain barrier disruption was absent in DAWM. As such, DAWM is likely to reflect chronic, axonal loss and might therefore serve as imaging marker for disease progression. Therefore, monitoring DAWM in vivo might give important information if one wants to focus on the neurodegenerative aspect of the disease. To determine whether the findings of **Chapter 4.1** can be reproduced and reliably measured *in vivo*, the study presented in **Chapter 4.2** was performed.

In this study, DAWM, NAWM and lesions were characterized by four quantitative MRI measurements, and compared between PPMS and SPMS patients. DAWM differed significantly between lesions and NAWM, and formed an intermediate between these two types of pathology. This shows that the results from **Chapter 4.1** can be reproduced *in vivo.* In addition, DAWM was found to vary in severity between SPMS and PPMS patients, suggesting a more severe tissue damage in patients with SPMS.

These findings pose two essential questions. First, is DAWM related to disability and if so, can DAWM be measured with a more practical method, not requiring sophisticated MRI techniques? And second, how does DAWM relate to white and grey matter lesions and atrophy?

If DAWM truly represents neurodegeneration, one would expect DAWM to correlate with disease severity. Therewith, DAWM could serve as an important marker for disease status and progression in addition to WM lesions, especially in patients which are defined to have “not- active” disease (95).

So far, the clinical consequences of DAWM have only been addressed in two studies, and surprisingly, both fail to show a correlation between DAWM and disability (96, 97). Neither the presence or absence of DAWM (96), nor the severity of DAWM as measured in ROI’s by quantitative MRI (97) seem to have an influence on the EDSS or the Multiple Sclerosis Severity Score (MSSS). One explanation for these findings could be the method of assessment of DAWM: The volume and extent of DAWM might be more predictive of disability than quantitative MRI measures of single ROIs, which are placed in allegedly minuscule areas of DAWM. DAWM can be extensive and extend throughout the white matter; we and others (94, 97, 98) have seen DAWM as well in periventricular as in deep white matter regions of the occipital, parietal, temporal and frontal lobes. Furthermore, the anatomical location of DAWM may be an important contributor to disability. Especially in the corticospinal tract, in which axonal loss is known to underpin the spastic paraparesis that typifies progressive MS, DAWM could be an important marker for disability.

Based on the above, the inclusion of both volume and location in the assessment of DAWM might lead to a more accurate correlation with clinical parameters. We

have therefore proposed a semi-quantitative scale to quantify the extent of DAWM, in similarity to the scales used to quantify white matter hyper-intensities in vascular disease (99). This rating scale provides three sum scores per hemisphere in a semi-quantitative way (see **figure 1 - 3** and **table 1**) and is rated on axial PD and T2-weighted images. To quantify the inter-observer agreement between four raters, intra-class correlation coefficients (ICCs) were calculated. ICCs were 0.57 between all four raters, varying between pairs from 0.43 to 0.92.

Consecutively, the DAWM rating scale was applied in a pilot study, which aimed to explore DAWM in RR and SP patients and its relation to T2 lesion load and clinical parameters. For this retrospective study, the MRI scans of 20 secondary progressive MS patients before and after their conversion were compared to those of relapsing remitting MS patients and healthy controls. Lesion counts, lesion volumes and DAWM was assessed by two raters blinded to clinical information.

DAWM scores of SP- MS patients were significantly higher after their conversion, and also significantly higher than those of RR- MS patients with a comparable disease duration. Significant correlations were found between DAWM scores and T2- and black hole counts- and loads. Expanded disability status scale (EDSS) scores positively correlated with frontal DAWM scores, but no other correlations between DAWM scores and clinical measures (including results of the multiple sclerosis functional composite and Guy's Neurological disability Scale) were found (data not shown). However, these data have to be interpreted with care, as the effects of disease duration and age were only partly accounted for in the matched groups and selection bias might have occurred.

We therefore aimed to verify these data in a bigger study of 51 MS patients who converted from RR- to SPMS. Furthermore, the development of DAWM over time and its relation with disease progression was addressed. Preliminary results show that DAWM is extremely variable between patients and that a certain score is not tied to the relapsing or remitting phase of the disease. DAWM does increase over time in the majority of patients, but unexpectedly, also the opposite was observed in a subset of patients. This phenomenon could be a result of the increasing difficulty to detect DAWM at later stages of the disease, due to the accumulation of lesions and signal related changes, as well as brain atrophy. In other words, DAWM might well be present in those progressive patients, but may be hidden by the higher lesion load and the reduced brain volume, which would translate in lower DAWM scores. Also, the differentiation between peritrigonal zones and DAWM can be challenging (100). Unfortunately, this finding defers DAWM as a tool to distinguish between progressing and relapsing patients.

Most recently, Vertisky et al published similar findings with regards to DAWM progression and EDSS. While DAWM could either decrease, increase or stay the same

over an eight year period, DAWM increase did not predict EDSS progression in a group of RR patients but did correlate with brain atrophy (101).
It has yet to be determined if the presence of DAWM at disease onset is a predictor for long term disability. Furthermore, it might be worthwhile to determine which factors contribute to a decrease in DAWM and whether DAWM correlates better with other than physical measures of disability, for example cognitive decline.

The second question that emerges from the studies in **Chapter 4**, is the relationship between DAWM and other MS related pathology. Upon consideration of the relationship between WM lesion load and DAWM, it was observed that the severity of DAWM measurements did not differ between patients with many and few WM lesions (97). This is not surprising, as it is well known that there is only a marginal correlation between focal white matter lesions and diffuse white matter injury in the brain and spinal cord in general (102-104), although variable results exist when an influence of focal white matter lesions on certain tracts is sought (105-108). This supports the argument that DAWM is not exclusively due to Wallerian degeneration from WM lesions, but develops by mechanisms at least partly independent from WM demyelination.

Cortical lesions are functionally connected to remote white matter just as WM lesions are, and their contribution to DAWM could probably be even more important than those of WM lesions. A recent study has shown that cortical lesion volumes and counts strongly correlate with changes in NAWM (109). The contribution of unseen cortical lesions to the development of DAWM, and more destructive WM lesions in SPMS, could explain the observed differences in DAWM between SP- and PPMS patients in **Chapter 4.2.**

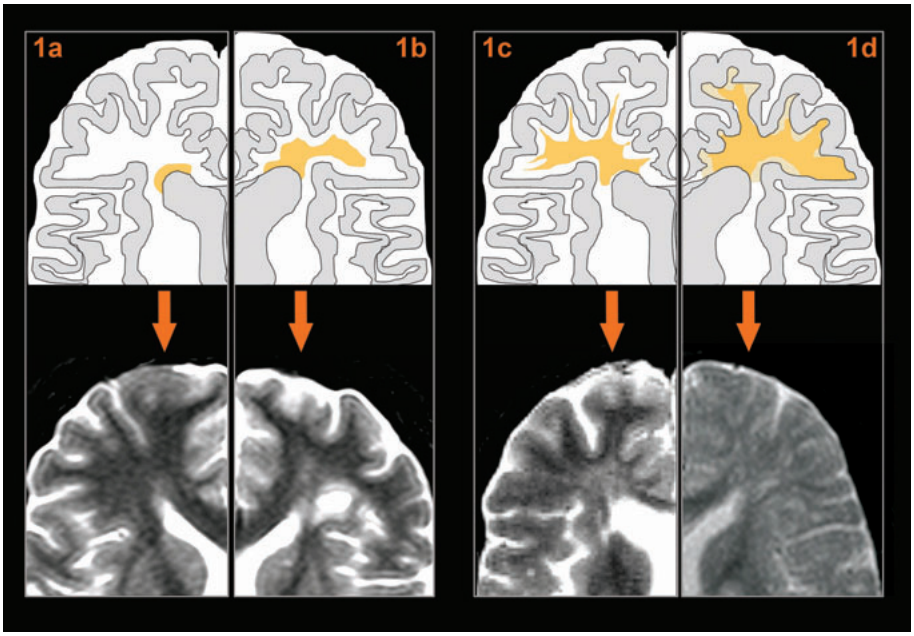
However, the temporal and causal relationship between inflammation (white and grey matter lesions) and neurodegeneration (DAWM and atrophy) is complex and only partly understood (110). Neuropathological observations indicate a close association between inflammation and ongoing axo-glial degeneration, also in late progressive disease (111). The very close relationship between inflammation and neurodegeneration at all stages of the disease makes answering the question whether inflammation or neurodegeneration comes first impossible. So far, most authors interpret this relation as evidence that inflammation drives neuro-degeneration throughout the stages of MS. However, also the opposite could be true: Ongoing axo-glial degeneration which is driven by an unknown factor, could elicit a continuous inflammatory response by degenerating cellular elements. Laule et al argues that DAWM reflects a primary lipid degeneration in the myelin- and axonal bilipid membranes (112). Progression in MS may then occur independently of radiologically visible lesion load, but as a consequence of microglial mediated axonal destruction that may in part be triggered by diffuse white matter lipid abnormalities and modified

by Wallerian degeneration caused by lesions. As such, DAWM could represent the "most pure" form of MS pathology and the visible "core" problem of the disease (113). Therefore, studying the relationship of DAWM to grey and white matter atrophy and lesions might provide an important piece of information in the puzzle of MS pathology.

Unsolved questions and suggestions for further research:

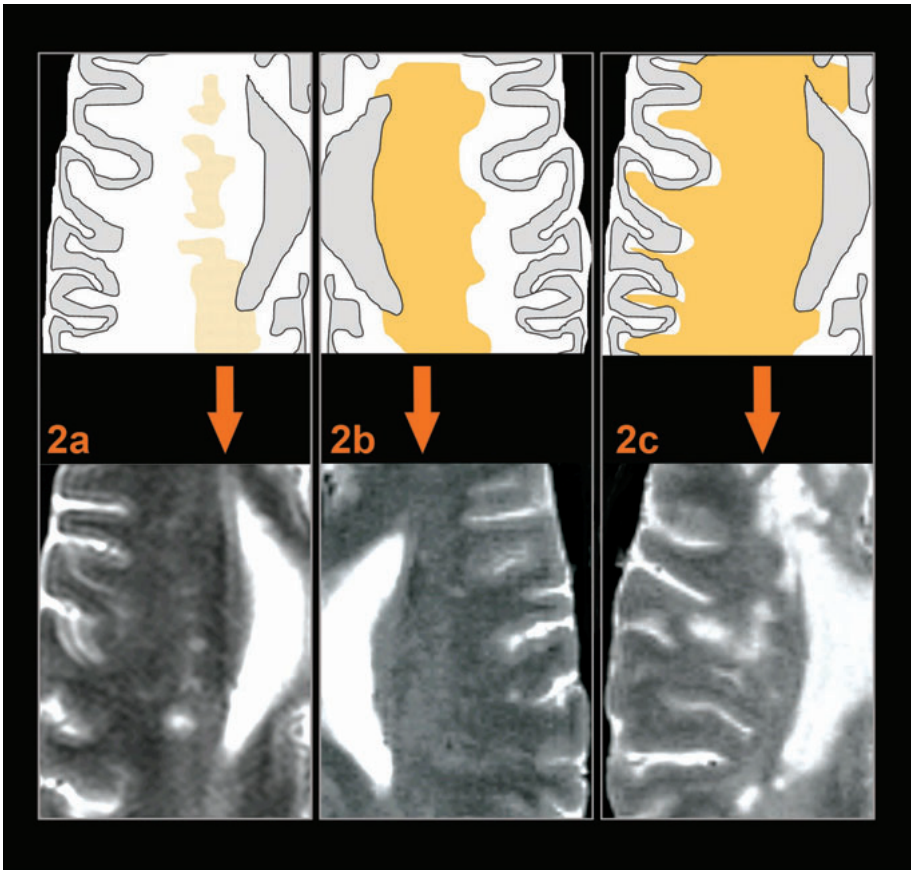
- The temporo-spatial relationship between the development of cortical lesions, cortical/deep grey matter atrophy and DAWM is unclear. In this respect it would be interesting to assess the relationship between the development of (tract related) DAWM, cortical atrophy and cortical lesion development in a longitudinal study.
- Can DAWM mirror unseen GM damage and is it the counterpart of cortical atrophy?

Figure 1: Rating of frontal DAWM



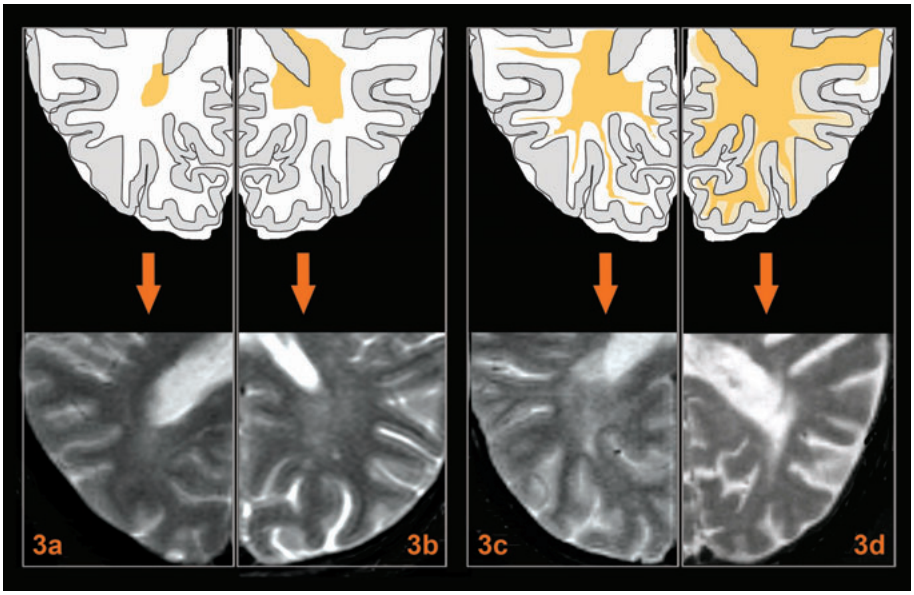
1a: frontal DAWM grade 1 (F1): diffuse abnormalities are seen around the ventricles, not exceeding 5mm Ø.
1b: frontal DAWM grade 2 (F2): Diffuse abnormalities stay confined to the deep white matter, reaching to the basis of the frontal gyri. The U-fibers and NAWM can be clearly distinguished from DAWM.
1c: frontal DAWM grade 3 (F3): DAWM extends in frontal gyri, not affecting the U fibers.
1d: frontal DAWM grade 4 (F4): Diffuse abnormalities affect the deep white matter and frontal gyri including U-fibers.

Figure 2: Rating of periventricular DAWM



2a: periventricular DAWM grade 1 (PV1): Diffuse incoherent abnormalities periventricular and in the deep white matter, not extending to the gyri. NAWM and U-fibers are visible.
2b: periventricular DAWM grade 2 (PV2): DAWM reaches from periventricular to the basis of the gyri. U fibers and gyri are free of DAWM.
2c: periventricular DAWM grade 3 (PV3): Diffuse abnormalities affect the entire white matter including U-fibers.

Figure 3: Rating of parietooccipital DAWM



3a: parietoocipital DAWM grade 1 (PO1): diffuse abnormalities around the ventricles, not exceeding 5mm Ø.
3b: parietoocipital DAWM grade 2 (PO2): Diffuse abnormalities stay confined to the deep white matter, spreading to the basis of the parietooccipital gyri. The U-fibers and NAWM can be clearly distinguished from DAWM.
3c: parietoocipital DAWM grade 3 (PO3): DAWM extends in the gyri, not affecting the U-fibers.
3d: parietoocipital DAWM grade 4 (PO4): Diffuse abnormalities affect the deep white matter and parietoocipital gyri including U-fibers.

Table 1: Visual rating of DAWM

Frontal (F 0-8)*	0/1/2/3/4	0= absent 1= periventricular, ≤ 5mm 2= deep white matter, 5mm, U-fibers visible 3= reaching in frontal gyri: “fingers”, U-fibers visible 4= deep white matter and gyri, U-fibers not or barely visible X= not applicable
Periventricular (PV 0-6)*	0/1/2/3	0= absent 1= present, incoherent areas periventricular 2= periventricular and deep white matter, extending to basis of gyri, U fibers visible 3 deep white matter and frontoparietal gyri, also involving U-fibers X= not applicable
Parietooccipital (PO 0-8)*	0/1/2/3/4	0= absent 1= periventricular, ≤ 5mm 2= deep white matter, 5mm, U-fibers visible 3= reaching in frontal gyri: “fingers”, U-fibers visible 4= deep white matter and gyri, U-fibers not or barely visible X= not applicable

Semiquantitative rating of DAWM is performed on T2 and Pd weighted images in three brain regions on each side. * The range of the scale for both hemispheres.

The periventricular DAWM is rated on an axial section through the corpus callosum/ cella media of the lateral ventricle. The frontal and parietooccipital DAWM is rated on an axial section through the basal ganglia/ insular cortex.

CHAPTER 5: ATYPICAL LESIONS IN MULTIPLE SCLEROSIS

The big bulk of MS lesions present as described in **Chapter 1,** but rarely (approximately 0.3/100000 people or in 1-2/ 1000 MS patients (114, 115)), lesions can deviate from their normal characteristics and present as large (>2 cm), isolated masses. Referred to as “atypical”, “tumefactive”, or atypical IIDLs (idiopathic inflammatory demyelinating lesions), their diagnosis is challenging because they can mimic tumors and abscesses, and because they can be found in a heterogeneous group of demyelinating disorders (116). Once a lesion is diagnosed as atypical IIDL, the further disease course is undefined and warrants a “wait and see” strategy in most cases. While some atypical IIDLs occur only once and remain the sole clinical event, some can recur. Furthermore, they can

present at the beginning of, or even during a relapsing remitting disease, suggesting a relation with "classical" MS. The frequency of this relation is unclear and it is unknown which patients are finally affected from relapses. Due to their various morphological presentations and various overlap with other demyelinating diseases (117, 118), an internationally approved classification of atypical demyelinating lesions is lacking.

In **Chapter 5.1**, we attempted to classify atypical IIDLs by MRI morphological patterns while bearing in mind that these patterns should be suited for a prospective registry of atypical IIDL cases. After a literature review, 69 cases were included in the study and five MRI patterns of atypical lesions were identified: A megacystic type, a Balo-like type, a ring like type, and an infiltrative type. The fifth "unclassified" group consisted of imaging patterns which were not representative of either of the groups. The inter-observer agreement varied for the defined groups, and ranged from substantial (for the Balo- and megacystic groups) to almost no agreement (for the unclassified group). Subsequently, we linked the identified patterns to demographical, clinical and para-clinical data. Although consisting of relatively small numbers, the five groups seemed to differentiate in terms of relapses and recurrence, death, and of the development of lesions typical for MS.

But how reliable and applicable are these data in a real- life clinical setting? As already mentioned in the discussion of **Chapter 5.1.**, clinical information provided has to be interpreted with care, as data were insufficient and likelihood of bias is big. This might lead to the concern whether the proposed classification of atypical IIDLs is reproducible and clinically meaningful.

It is therefore of interest to compare the present work to the findings of longitudinal studies of atypical IIDLs with long term clinical and radiological evaluation. To date, the largest case series comes from a study from Lucchinetti et al, which reports the clinical course and radiological features of 168 biopsy confirmed cases of atypical IIDLs (119). In this study, and in subsequently published smaller case series, MRI enhancement patterns were used to describe the radiological features of IIDLs. Specifically, the presence of open- and closed rings, homogeneous, heterogeneous, or nodular enhancement was used to classify atypical lesions, in addition to the presence or absence of T2W hypointense rims (120-123). While these studies give an impression about the various morphological aspects of atypical IIDLs and their overall clinical course, none could attribute MRI characteristics to outcome parameters.

The second largest published case series, which includes 90 patients with a mean follow up of 4 years, has used the classification proposed in **Chapter 5.1** to investigate MRI-clinical relationships in patients with atypical IIDLs. This study confirms not only the occurrence of the in **Chapter 5.1.** proposed lesion types throughout eight centers, but also validates their clinical correlations (124).

In the study by Wallner et al, infiltrative lesions were described as the most frequent lesion type (49%), which is higher than one would expect from the data in **chapter 5.1.** The inclusion of different populations, especially Asian cases in the review data of **Chapter 5.1**, and the relative exclusion of ring- like lesions in the follow up study, which are presently accepted as part of the classical MS spectrum, could be a possible explanation for this difference (125-132). Other studies also suggest that ring like enhancement patterns are the most common presentations of IIDLs (119, 120, 122, 123), but these most likely include both "ring-like" and "megacystic" lesion types, which typically present with ring enhancement. The mean age and age range of patients presenting with atypical IIDLs was similar in both review and follow up study (124, 133).

The data presented in **Chapter 5.1** and the follow- up study by Wallner-Blazek et al (124) contradict the historical notion that AIIDLs are associated with a malignant disease course. This seems to be true both in terms of recovery of the atypical attack, and in terms of the further disease course. Other recent studies have reported similar observations (119, 122, 134, 135).

With regards to recovery from atypical demyelination, half of the patients of Kuan et al achieved almost total recovery or only mild residual impairment (EDSS less than 1) after 2 years (122). Lesion size and location were not associated with the clinical course (76) as in other reports (119, 121). However, a more detailed prognosis might be possible: Both the data in **Chapter 5.1**, and the study by Wallner et al suggest, that the clinical outcome is lesion type dependent. Overall, outcome seems to be worst for patients with infiltrative lesions (between 10% and 20% good recovery), whereas the majority (>80%) of Balo- like cases show marked improvement during the follow up period. In contrast, half of the patients with megacystic- and ring- like lesions recover well from the attack (124).

With regards to the further disease course, Luchinetti et al. and Altintas et al. found similar rates of conversion to clinically definite MS of 66-70% (119, 120). However, this differs from both the review data and the results of Wallner et al, where only roughly one third of patients experienced a second attack (124), although the average follow up was shorter than in the other two studies. Here also, lesion type seems to be of importance: Patients with ring like and infiltrative atypical IIDLs show further attacks in 62% and 35% respectively. Therefore, the differences in reported conversion rates might be based on the different distribution of lesion types within the studied case series, in most of which ring like patterns dominate.

In addition, most patients who relapse will have lesions typical of MS, with only a minority relapsing in a tumefactive lesion (119, 124). In this line, Wallner et al could show that new MS lesions at follow up develop in more than half of patients with lesions at baseline, vs in only 28% of patients without MS typical lesions.

The presence of MS typical lesions is reported in up to 70% (119), and could be a helpful hint in the differential diagnosis of atypical lesions, especially given the fact that atypical IIDLs are associated with a first clinical attack in most instances (119, 122, 124). Interestingly, the presence of grey matter lesions has been described in nearly 40% in association with atypical IIDLs (40). As this finding is solely based on histopathological analysis of cortical tissue obtained in passing during biopsy sampling of white matter lesions, cortical lesions might be found even more frequently on imaging and should be actively looked for. Infiltrative- and megacystic lesions most likely present as solitary lesions, and awareness of these IIDL variants in addition to the presence of MS typical lesions could be helpful in the diagnostic process. However, one has to be aware that the presence of MS typical lesions (or a pre-existing diagnosis of MS) does not exclude the possibility of a coexisting tumor or other pathology (136-138). Gliomas in patients with MS may have a different appearance than gliomas in other patients, with a higher incidence of a diffusely infiltrative or multi-centric appearance (30% compared to 2.5-5%) (138).

In retrospect, some of the described cases might well fall in the spectrum of neuromyelitis spectrum disorder (NMOSD), which is accepted as a separate entity of demyelinating disease. The articles used for the review were published before 2004, i.e. before the aquaporin-4 (AQP4) antibody era. During this time, a relative paucity of brain involvement was considered characteristic for NMOSD (139). With the availability of AQP4-IgG assays however, it became clear that brain abnormalities are common (up to 89% in patients with NMOSD), and that these are not only located in areas with high AQP4 expression but also occur in brain areas where AQP4 expression is not particularly high (140-142). Furthermore, brain abnormalities are present at onset in 43%-70% in patients with NMOSD (141, 143, 144), and might be even responsible for the presenting symptom (145).

im et al have grouped the MRI characteristics of 78 AQP4+ NMOSD patients into five patterns (146), of which two are of specific interest in the light of the similarity to the described atypical MS lesions. The first are lesions in the hemispheric white matter, which are described as extensive and confluent and were found in 29%. Interestingly, most of these lesions are tumefactive (>3cm in diameter) or configured as long spindle-like or radial-shaped signal changes and are following white matter tracts. Some tended to shrink or disappear over time, whereas others revealed cystic-like or cavitary changes. It is quite likely that some of the described atypical MS lesions which we have classified as “infiltrative” might fall into this category of NMOSD- lesions. This suspicion is supported by the behaviour of gadolinium uptake, which was described as “patchy” or “cloud- like” in NMOSD and matches our descriptions of “inhomogeneous” uptake for the “infiltrative” group.

The second group described by Kim et al is characterised by peri-ependymal lesions which surround the lateral ventricles and were found in 40%. These can involve the entire thickness of the corpus callosum and may also extent in cerebral hemispheres forming an extensive and confluent white matter lesion. Again, some of our "infiltrative" cases, especially those which expand throughout the splenium and to the occipital lobes might be part of this group. Whether infiltrative lesions per se could be a feature of a separate subtype in the demyelinating diseases spectrum has yet to be determined, as well as the degree of overlap of this radiological feature with other syndromes. The use of biomarkers, for example anti myelin-oligodendrocyte glycoprotein, which is found in a subset of patients with NMOSD (147), might render helpful.

Turning to CSF, provided data are limited in the literature and firm conclusions about the frequency of oligoclonal bands in atypical IIDLs as compared to prototypic CIS and their relation to risk of conversion to MS cannot be drawn. It seems however, that oligoclonal bands are overall less common than in classical MS (119-121). In our review material, oligoclonal bands were most frequently found in the infiltrative (54%) and ring-like (30%) types and interestingly, these types are overall most common to lead to relapses on follow up (124).

The histopathologic findings in our review data did not differ between the lesion types, apart from Balo-like lesions. This is not surprising as the prime reason for histopathology was the exclusion of pathology other than demyelination and therefore, selection of staining techniques and interpretation of results might have served only this purpose. The pathology of atypical IIDLs is similar to that of typical MS lesions with areas of confluent demyelination and relative axonal sparing, although areas of widespread axonal damage might be sometimes seen. Additionally, inflammatory infiltrates of foamy macrophages are admixed with reactive astrocytes, and perivascular and parenchymal lymphocytic infiltrates are common. Previous studies have described four immunopathological patterns of demyelination in early multiple sclerosis lesions (128). Of those, the antibody/complement-mediated pattern II has gained most attention as it seems responsive to B-cell directed therapy (148, 149). Therefore, evaluation of the underlying immunopathology of atypical IIDLs could have major implications for their treatment and could offer perspectives for pathology directed immunotherapy in the future. The antibody- mediated pattern of MS has also been tentatively linked with ring enhancement patterns (128, 150). Further evidence for a possible correlation between radiological and pathological features comes from a more recent study, which suggests that atypical IIDLs with different contrast enhancement patterns on MRI have different underlying histopathology (151).

In conclusion, characterization of atypical IIDLs by MRI patterns might render helpful for prognosis, diagnosis and treatment decisions. In the future, disease registries with

documentation of clinical, radiological and pathological data should further advance our understanding of atypical IIDLs and hopefully lead to a standardized classification and common nomenclature.

Unsolved questions and suggestions for further research:

- Do the described lesion types represent distinct subtypes of demyelinating diseases? Under which circumstances are they part of the continuum of MS?
- The use of disease registries and documentation of clinical, histopathological and radiological data and long term follow up is necessary to advance our understanding of these presentations.
- The development of new biomarkers and the use of advanced MRI techniques might allow further classification of atypical presentations.

CHAPER 6: NEURODEGENERATIVE AND VASCULAR DISEASE

Similar to the clinico-radiological dissociation observed in MS, the clinical expression of MRI defined small vessel disease is generally moderate and heterogeneous. This can be partly explained by the pluriformity of underlying pathological changes and the relative inability of clinically applied MRI sequences to differentiate between these changes. The review presented in **Chapter 6.1** summarizes studies that directly correlate post-mortem MRI and histopathology of white matter hyper-intensities (WMH), lacunes and microbleeds with the aim to better characterize the pathological substrates of small vessel disease and to better explain their clinical manifestations.

More specifically, the study in **Chapter 6.2** addresses WMH in patients with Alzheimer's disease and in controls without dementia. WMH can be found in a significant proportion of Alzheimer patients, but whether or not WMH have an effect on cognitive decline in dementia is unclear (152-155). The heterogeneity of the neuropathological substrates underlying WMH and the lack of specificity of T2 weighted images to differentiate between these substrates might be the reason for inconsistent results in studies correlating cognitive decline with WMH. Quantitative MRI is claimed to be more specific to the presence of structural brain damage in vivo, however, the neuropathological substrates that define the changes in quantitative MRI parameters in WMH are not well defined (156-159).

Consequently, the study presented in **Chapter 6.2** was designed to explore differences between WMH of Alzheimer patients and controls without dementia and to assess whether quantitative MRI can reflect these differences. In this post-