CHAPTER 1

General Introduction

Partly based on:
“Gray matter damage in multiple sclerosis: impact on clinical symptoms”


Neuroscience. 2015 Sep 10;303:446-61.
CHAPTER 1

**Multiple Sclerosis**

**Epidemiology**
Between 1829 and 1842, Carshwell and Cruveilhier described and illustrated lesions and atrophy in the spinal cord and pons varolii. However, it was not until 1868 that the French neurologist Charcot published on “sclérose en plaques”, that multiple sclerosis (MS) was recognized as a distinct neurological disease. Nowadays, MS is seen as an inflammatory, demyelinating and neurodegenerative disorder, and the most common central nervous system (CNS) disease in young adults. MS has an estimated 2.5 million affected individuals around the world and affects woman twice as often as men.¹

**Physical and Cognitive Features**
Clinical manifestations of MS are extremely variable and may occur in isolation or in various combinations. The first symptom is often loss of vision due to optic neuritis.² A long held belief was that only the white matter (WM) was affected, but gray matter (GM) pathology has received a more prominent role in (clinical) MS research during the last two decades.³⁻⁵ Lesions of the brain stem, cerebellar pathways and spinal cord produce disruptions in motor and sensory functions, which are nearly always present in MS.¹

Physical disability can be assessed in various ways. A frequently used rating scale in MS is the Kurtzke Expanded Disability Status Scale (EDSS).⁶ This rating scale categorizes physical symptoms into eight functional clinico-anatomical systems (e.g. brainstem functions, motor function). Based on these functional scores, the severity of patients’ physical disability is rated on an ordinal scale between 0 and 10. Another commonly used scale, which also includes some cognitive function testing, is the Multiple Sclerosis Functional Composite (MSFC).⁷ This composite includes functional measures of three key clinical dimensions of MS: leg function/ambulation (timed 25-foot walk), arm/hand function (9-hole peg test), and assessment of the cognitive domains “information processing speed” and “working memory” (by means of the Paced Auditory Serial Addition Test (PASAT)). Cognitive impairment, measured through neuropsychological testing, is reported in 40-65% of MS patients and mostly affects processing speed, visual learning and memory.⁸ Furthermore, patients often experience (episodes of) fatigue and depression throughout their disease course.

**Clinical Course**
The clinical course of MS may follow a variable pattern but can usually be divided into a few clinical subtypes (see figure 1): relapse-remitting (RR), secondary-progressive (SP), primary-progressive (PP) and progressive-relapsing (PR).⁹ Patients who have had a single episode of neurologic symptoms are referred to as clinical isolated syndromes (CIS).¹⁰ Approximately 80-85% of patients have RRMS, characterized by symptoms of neurological dysfunction followed by complete or partial remission.¹¹ Approximately ten years after disease onset, an estimated 50% of patients develop persistent signs of dysfunction, which progresses the disease into SPMS.¹² 15-20% of patients have PPMS, which is characterized by a gradual progressive course from disease onset.¹¹ Noteworthy is that this type of MS typically has a
higher age of onset (around 40 years) and men and women are equally susceptible. A rare form (5%) of MS is progressive-relapsing MS, characterized by a steady increase in disability from the beginning, including acute relapses without remission or recovery. Recently, an update on MS phenotypic classification has been published, in this new classification CIS has been added as a subtype and PRMS has been removed. Furthermore, subcategories “active” and “non-active” have been suggested; active MS is characterized by the occurrence of a clinical relapse or presence of new T2 or gadolinium-enhancing lesions. Non-active is characterized by an absence of these signs. Lastly, it is recommended to differentiate progressive patients who show signs of disability progression from patients who have remained stable over a specified period of time.

![Figure 1](https://via.placeholder.com/150)

**Figure 1** | Types and courses of multiple sclerosis (MS). From Lublin FD & Reingold SC. Defining the clinical course of multiple sclerosis: Results of an international survey. Neurology 1996: 46(64); 907–911.

**Diagnosis and Treatment**

MRI has greatly aided the diagnostic process; gadolinium-enhancing lesions and T2 hyperintense lesions in the periventricular WM, brain stem, cerebellum and spinal cord can be visualized to support clinical findings. For diagnosis, dissimilation in space (more than one region of the CNS affected) and dissimilation in time (more than one disease event) are required and can be assessed with MRI. CSF analysis can be a helpful indicator of intra-CNS inflammation as CSF often shows an increased intrathecal synthesis of immunoglobulins in which oligoclonal bands may be present.

Even though MS is still incurable, some advances have been made in treating the disease, mostly in the RRMS phase. Conventional therapeutics, such as interferon beta or glatiramer acetate, are aimed at modulating the immune response. They reduce the occurrence of relapses by 30%. Newer therapeutics, such as Natalizumab or Fingolimod, are aimed at stopping the immune response all together. They reduce relapses by approximately 70%, but come with a higher risk of serious side effects.
CHAPTER 1

UNDERLYING MECHANISMS

Genetics
Multiple Sclerosis is seen as a complex polygenic disease; an interaction of multiple genes with small effects, influenced by gene-environment interactions.\(^{19}\) The absolute risk of MS in a first degree relative of a patient is less than five percent. However, this risk is still 20 to 40 times higher than in the general population.\(^{20}\) Among monozygotic twins the concordance rate is approximately 30 percent, 6 times higher than in dizygotic twins.\(^{20}\) Nevertheless, family members can be strikingly different with respect to disease course or severity, indicating that these aspects are also influenced by non-genetic mechanisms.\(^{21}\) With the introduction of improved statistical methods and extensive international collaborations to create larger datasets for study, the hunt for “MS genes” has tremendously improved over the past few years.\(^{22}\) Various researchers have found an association between the HLA-DR2 haplotype on chromosome 6p21, and it was found that there is an increased risk for MS in northern Europeans.\(^{23}\) The HLADRB1*1501 gene has come forward as the main susceptibility gene from three candidate risk genes of this haplotype (HLADRB1*1501, HLADRB5*0101, and HLADQB1*0602).\(^{22}\) The HLA-DRB1*1501 allele seems to be associated with an earlier age of disease onset,\(^{24}\) with gender (female),\(^{25}\) and with disease severity.\(^{26,27}\)

Lesion Pathology
MS received its name from the sclerotic plaques that are a result of acute focal inflammatory demyelination, axonal loss and gliotic scar formation (sclerosis). Oligondendrocytes synthesize myelin sheaths for nerve axons in the CNS. This myelin sheath is a membrane that spirals around the axon for insulation of axonal electrical signal conduction. Action potentials travel down the myelinated nerve segment to an unmyelinated node of Ranvier where voltage-gated sodium channels cluster and transmit another action potential to the next node of Ranvier. Due to demyelination, this process gets disrupted and the axon can no longer effectively transfer signals.\(^{1}\) Pathologically, a distinction can be made between different types or stages of WM lesions, depending on their degree of demyelination and the pattern of microglia/macrophage activation.\(^{28}\) These stages may be characterized as preactive, active, chronic active and chronic inactive.

Demyelinated lesions at the border of the (cortical) GM, or even entirely within the GM, were also found and described early on.\(^{29-32}\) These preliminary studies on describing the involvement of the GM in MS were, however, initially largely ignored due to suboptimal histological staining techniques. Development of more advanced immunohistochemical techniques improved the visualization of GM pathology; it appeared that GM pathology was often extensive, involving both demyelination (lesions) and tissue destruction (neurodegeneration). Cortical pathology was found in up to 70-80% of MS patients.\(^{33}\)

Using the newer immunohistochemical staining methods, it was now possible to categorize cortical demyelination into four distinct lesion types according to their location (see figure 2).\(^{34}\) Type I lesions involve the deeper layers of the GM as well as the adjacent WM (and are thus
“mixed lesions”). The other lesion types are purely intracortical: type II lesions are often small and confined within the cortex; type III lesions account for the majority of all cortical lesions and extend from the pial surface into the cortex, most often reaching to cortical layers 3 or 4 (and are referred to as “subpial lesions”). When these lesions involve the entire width of the cortex (without entering the subcortical WM), they are sometimes defined as type IV lesions. Cortical demyelination increases dramatically with disease progression and is generally widespread in chronic MS, although the frontal and temporal lobes are often slightly more affected than the occipital and parietal lobes.

MS lesions also show varying degrees of remyelination, a process of repair or replacement of myelin around the denuded axon. Nevertheless, repeated attacks lead to less effective remyelination over time, and lesional scarring and disease progression is often inevitable.

**Figure 2** | Different types of GM lesions

---

**Imaging Multiple Sclerosis**

**White and Gray Matter Lesion Visualization at Standard MRI Field Strength (1.5T)**

Since the introduction of MRI, WM lesions have been visualized easily and accurately. In contrast, cortical GM lesions were more difficult to visualize. In a post-mortem study at standard (1.5 Tesla (T)) field strength, only 5% of intracortical lesions were prospectively detected. Various reasons have been given for this low detection rate. Firstly, because normal cortical GM contains little myelin, loss of myelin in cortical lesions provides little contrast change on MRI. Secondly, cortical lesions may be small and thus potentially undetectable with insufficient spatial MRI resolution. Lastly, (partial) volume effects from nearby CSF may disrupt the ability to distinguish (especially superficial) lesions from the surrounding normal tissue.
Various attempts have been made to improve the detection of cortical lesions. One such attempt was the application of a fluid-attenuated inversion recovery (FLAIR) sequence. Although approximately 60% more (sub)cortical lesions were detected with this technique ex-vivo, most of the lesions were still missed, in particular the purely intracortical lesions.\(^{37}\)

A greater improvement was achieved with the development of the double inversion recovery (DIR) sequence. This technique allowed better visualization of the cortex, by suppressing the signal of the surrounding WM and CSF.\(^{41}\) Consequently, more cortical lesions were detected, in particular the purely intracortical lesions could now be visualized more often. Later, phase-sensitive inversion recovery (PSIR) at 1.5T also showed better GM-WM distinction than conventional sequences.\(^{42}\) Unfortunately, despite these improvements, most of the histopathologically confirmed cortical lesions were still missed: approximately 82% of histopathologically verified cortical lesions remained undetected with DIR at 1.5T.\(^{41}\)

**Lesion Visualization: from 1.5T to 3T to 7T**

Increasing the MRI field strength proved fruitful in improving cortical lesion detection. This is likely explained by an increased signal-to-noise ratio, a better spatial resolution and better image contrast-to-noise. Compared to DIR at 1.5T, DIR at 3T provided a 192% increase in intracortical lesion detection.\(^{43}\) Furthermore, both PSIR and 3-dimensional magnetization-prepared rapid acquisition with gradient echo (3D-MPRAGE) at 3T were investigated. Cortical lesion detection with PSIR appeared to be comparable to DIR, but the combination of DIR and PSIR improved detection rates further.\(^{44}\) Compared to DIR and PSIR, 3D-MPRAGE at 3T achieved more accurate classification of cortical lesions into the lesion types described above.\(^{45}\)

Techniques at even higher field-strengths brought further improvements. Three-dimensional susceptibility-weighted imaging (3D-SWI) and two-dimensional T2-weighted fast spin echo (2D-T2WFSE) at 4.7T were investigated.\(^{46}\) The overall (WM and GM) lesion detection rate was increased when using 3D-SWI in addition to 2D-T2WFSE. 3D-SWI found additional lesions to 2D-T2WFSE. However, detection of cortical lesions appeared to remain low at 4.7T (<10%) when compared to immunohistochemical techniques on post-mortem material.\(^{47}\) Furthermore, visualization varied for different subtypes of cortical lesions: mixed lesions were fairly well detectable, but many subpial lesions remained concealed.\(^{47}\) This was improved in-vivo with 7T 3D-MPRAGE, fast low-angle shot (FLASH)-T2*-weighted imaging and 3D-FLAIR.\(^{48,49}\) More cortical lesions were detected with these techniques,\(^{50}\) and a better classification of purely intracortical lesions was possible.\(^{48,49}\) The improvement that came with 7T was further reflected in a better inter-rater agreement than at lower field strengths.\(^{49}\)

**Lesion Characterization; the Role of Quantitative MRI**

The pathological substrate of cortical lesions is predominantly demyelination, but also axonal degeneration, microglial activation and (minor) neuronal, glial and synaptic loss.\(^{51-53}\) Combined post-mortem MRI and histopathology studies have shown that quantitative MRI (qMRI) techniques are more sensitive and pathologically specific than conventional techniques to detect small changes at the pathological level. For instance, at 1.5 Tesla, magnetization transfer
(MT) imaging can detect focal abnormalities in normal-appearing white matter (NAWM) before the appearance of lesions on conventional MRI. MT ratio (MTR) has also revealed abnormalities in the GM of MS patients; previous studies showed lower MTR in cortical lesions compared to normal appearing gray matter (NAGM), and average lesion MTR showed predictive value for patients’ worsening disability. Diffusion tensor imaging (DTI) has also been used to characterize GM tissue. In MS patients, cortical normal-appearing GM (NAGM) fractional anisotropy (FA) either decreased or increased, while mean diffusivity (MD) either increased or showed no difference compared to healthy controls. The reason for these contradictory results in MS NAGM could be the missed cortical lesions that are still in these measurements. Comparing lesional to non-lesional tissue, FA of cortical GM lesions increased, while MD measures either decreased or showed no difference. Quantitative R2* (=1/T2*) also revealed lower values in GM lesions than in nearby non-lesional cortex. Furthermore, T1 relaxation time (RT) correlated with myelin content and axonal count in the WM, which are both decreased in lesions compared to NAWM. Only very little research has been done regarding lesional differentiation, one study used MTR to differentiate type I from type III lesions, but was unsuccessful to do so, likely due to insufficient power.

Aims of this Thesis and Thesis Outline

The general objective of this thesis was to better visualize and characterize GM and WM tissue abnormalities with MRI. This was done by means of post-mortem MR imaging, a unique way to directly visualize histopathologically confirmed abnormalities with MRI. Additionally, we aimed to find genetic or clinical correlates of changes observed histopathologically or with advanced MRI. The main research questions at onset of this research were:

- Do specific sequences and/or ultra-high field strength (7T) improve lesion detection?
- Can different stages of WM lesions and different types of GM lesions be distinguished using advanced MRI sequences? At standard and (ultra-)high field strength?
- Can histological variations in demyelination and inflammation in MS patients be explained by carriage of HLA-DRB1*1501?
- Can MRI distinguish clinical deficits between MS phenotypes?

Answering these questions would dramatically improve our understanding of the potential of MRI as a tool for (early) pathology detection and phenotyping in MS. Initially, perhaps only in the post-mortem setting, but with interesting leads to follow up in the clinic. Leads such as how (improved) cortical lesion visualization and characterization can be applied to provide a better prognosis for MS patients, who currently face a highly variable disease course and a lot of insecurities. Also, a better understanding of ‘what MRI reflects’ will increase its usefulness in monitoring potential treatment effects.

In chapter 2, visualization of cortical MS lesions at (ultra-)high field strength will be central. Chapter 2.1 focuses on visualizing cortical lesions at 3T and 7T with five different sequences to
(i) assess if higher field strength detects more cortical lesions *per se* and (ii) to assess if there are specific sequences that are better at visualizing cortical lesions at a certain field strength. Chapter 2.2 will take this one step further by focusing on two sequences (T2 and T2*) and compare their sensitivities at ultra-high (7T) field strength.

**Chapter 3** deals with *characterization* of lesions at standard field strength. In *chapter 3.1* T1 relaxation time mapping is used in an attempt to distinguish different types of WM lesions, namely preactive, active, chronic active and chronic inactive lesions. A similar exploration is undertaken in *chapter 3.2*. The same WM lesion types were investigated, but this time the research question was whether they might be better distinguished by means of a T2-w texture analysis using a local spatial frequency-based approach.

*Characterization* at ultra-high field strength is explored in **Chapter 4**. In *chapter 4.1* magnetization transfer ratio and qR2* are used in an attempt to differentiate cortical GM lesions types (I-IV and normal appearing GM (NAGM)). In *chapter 4.2* the influence of GM lesions, in relation to NAGM, on diffusion tensor imaging measures such as fractional anisotropy (FA) and mean diffusivity (MD) is studied. An increase in FA was found and a further histopathological study was undertaken to explore the possible underlying pathological substrates that could explain this increase.

**Chapter 5** is about delving deeper, from histopathology to possible genetic underpinnings. Associations are explored between the main susceptibility gene in MS, namely HLA-DRB1*1501, and histopathological characteristics of GM lesions that have shown to correlate with clinical outcome measures.

The opposite is being done in **Chapter 6**, where translation to the clinic is the aim. Advanced MRI measures were correlated to subtle GM damage and measures of cognition. Furthermore, the differential relation between MRI-cognition for relapsing-remitting and primary-progressive MS is explored.

These chapters are brought together in **chapter 7**, which summarizes and discusses the previous chapters and concludes with recommendations for future research.
Magnetic resonance imaging (MRI) works by detecting small magnetic moments of protons (nucleus of hydrogen). The MRI techniques used in this thesis will be described below.

**Box A: Qualitative sequences - DIR, FLAIR, T1, T2 and T2**

Magnetic resonance imaging (MRI) works by detecting small magnetic moments of protons (nucleus of hydrogen). The MRI techniques used in this thesis will be described below.

**Figure 3** | From “Increased cortical grey matter lesion detection in MS with 7 Tesla MRI: a post-mortem verification study” by Kilsdonk, Jonkman, et al. (submitted, 2015).

- **T1 and T2-weighted imaging**

The basic MRI pulse sequences are the longitudinal (T1) and transverse (T2) relaxation time sequences. In these sequences, a combination of T2 relaxation (decay of the detectable signal) and T1 relaxation (return to equilibrium), give rise to tissue specific contrasts. Important are the repetition time (TR) and echo time (TE). T1-w images have a short TR and short TE, T2-w images have a long TR and long TE. T1-w images are useful for anatomically viewing of the brain; in MS, T1-w images can be used to detect black holes (chronic persistent lesions) and, when contrast such as gadolinium is administered, acute inflammatory lesions. T2-w images can be used to detect various types of lesions. They will appear as hyperintensities due to a lack of myelin (fat) which is normally gray on a T2-w sequence.

- **Fluid Attenuated Inversion Recovery (FLAIR) and Double Inversion Recovery (DIR)**

Inversion recovery techniques are used to suppress certain tissue types and enhance the contrast with the tissue type(s) of interest. FLAIR has a single inversion recovery which suppresses the cerebrospinal fluid (CSF) and therefore improves detection of periventricular lesions. DIR has a double inversion recovery, suppressing both the cerebrospinal fluid (CSF) and white matter (WM). It is a sequence frequently used for cortical lesion detection.

- **T2* imaging**

A slightly different sequence is T2*. This sequence is sensitive to (e.g. ferromagnetic) compounds that distort the local magnetic field which results in loss of signal. As previously mentioned, T2 is the decay of transverse magnetization, arising from natural anatomical or molecular interactions. However, the transverse magnetization tends to decay much faster than would be predicted. Therefore, aside from “T2 decay”, there is “actual observed T2 decay”, which is referred to as T2*. A T2* sequence usually uses a low flip angle, long TE and long TR.42
Conventional, qualitative, MR measurements have a limited sensitivity. Therefore other (semi-)quantitative techniques have been developed and investigated. These techniques measure tissue properties in a variety of ways and are described below.

-T1-RT mapping

There are various ways to create T1-RT maps; by varying inversion times, repetition times or flip angles. In this thesis, the latter method is used. By varying the flip angles and fitting the signal intensity function to the data, the T1-RT can be determined for each pixel in the image set. Histopathological analysis in MS patients has shown that T1-RT correlates with myelin content and axonal count, which are both decreased in lesions compared to NAWM.\textsuperscript{62,63}

-T2-w texture analysis

Another quantitative method is T2 texture analysis, which is a T2-w image postprocessing approach based on mathematical analysis. It evaluates the organizational pattern of image pixels which represents tissue specific substrates.\textsuperscript{65} This way the structure of an image is analogous to the homo- or heterogeneity of the underlying tissue.
- **Magnetization Transfer Ratio (MTR)**

Magnetization transfer (MT) is the transfer between freely moving protons (found in water) and bound protons (closely associated with macromolecules such as myelin). An MT pulse applies energy exclusively to the bound pool after which some of this energy is then transferred to the free water pool. This decreases the signal as an effect. The magnitude of this effect can be quantified by obtaining two images; one with an MT pulse ($S_{MT}$) and one without an MT pulse ($S_0$). From this the ratio (MTR) can be calculated ($MTR = (S_0 - S_{MT})/S_0$). Histopathological analysis in MS patients have shown that MTR correlates with myelin content and axonal damage.\(^{62,63}\)

- **Quantitative R2\(^*\)**

In Box A we have mention T2\(^*\) as the “actual observed T2”; reflecting true transverse decay and decay due to magnetic field imhomogeneities. Interaction between iron complexes and water lead to a faster dephasing of transferse magnetization (reduced T2 and T2\(^*\)), which is reflected by a loss of signal. Therefore, tissue high in iron concentration will show reduced signal intensities and appear darker on MR images.\(^{66}\) The relaxation rate R2\(^*\) is the inverse of T2\(^*\) (1/T2\(^*\)). By postprocessing acquired images at different TEs, quantitative R2\(^*\) (qR2\(^*\)) maps can be produced and is a good imaging technique to detect iron-mediated pathology.

- **Diffusion Tensor Imaging (DTI)**

DTI is a measure of diffusion properties in tissue; of water molecules in the brain. Eigenvalues are the lengths of the eigenvectors (directions). The average eigenvalue is called the mean diffusivity (MD). A measure for amount of diffusion asymmetry is fractional anisotrophy (FA). The value of FA is between 0 and 1. When all eigenvalues are equal, FA = 0, which is perfect isotrophic diffusion. When the eigenvalues become more unequal, there is more anisotrophy and FA gets closer to 1. In the brain, white matter is more anisotropic than gray matter. In the white matter of MS patients, MD values go up while FA values go down.\(^{67}\) In the gray matter FA values appear to go up.

![Figure 6](T2-w, qR2*, MTR and DTI (MD and FA) sequence.)
REFERENCES


