Diplomarbeit

Magnetic resonance imaging assessment of cortical pathology and its relation to periventricular lesions in multiple sclerosis

eingereicht von

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zur Erlangung des akademischen Grades

Doktor der gesamten Heilkunde

(Dr. med. univ.)

an der

Medizinischen Universität Graz

ausgeführt an der

Universitätsklinik für Neurologie

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Graz, am 06. Juli 2017

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Zusammenfassung

Einleitung:

Multiple Sklerose ist eine chronisch entzündliche, demyelinisierende Erkrankung des zentralen Nervensystems und die häufigste nicht-traumatische Ursache für physische Behinderung im frühen Erwachsenenalter. Wie in histopathologischen Studien gezeigt, werden besonders liquornahe Strukturen wie der Cortex oder die periventrikuläre weiße Substanz im Verlauf der Erkrankung geschädigt. Mit der technischen Weiterentwicklung der Magnetresonanztomographie (MRT) und der Implementierung neuer Methoden wie Magnetisierungstransfer-Imaging (MTI) ist es möglich, mikrostrukturelle Veränderung in verschiedenen Läsionen nachzuweisen, die mit konventionellen Sequenzen verborgen blieben. Das Ziel dieser Studie ist, einen möglichen Zusammenhang zwischen periventrikulären Läsionen, deren Schweregrad und der kortikalen Dicke als Ausdruck subpialer Demyelinisierung zu untersuchen. Im Speziellen wird getestet, ob Unterschiede zwischen periventrikulären und nicht-periventrikulären Läsionen, und zwischen verschieden MS-Subtypen existieren.

Methodik:

Wir berichten von einer kranialen MRT-Daten Analyse von 160 PatientInnen (87 *klinisch isoliertes Syndrom* (CIS), 73 *klinisch gesicherte Multiple Sklerose (*CDMS)). Darunter waren 105 Frauen (65,5%), das Durchschnittsalter bei Erkrankungsbeginn betrug 29,1 Jahre mit einem medianen EDSS von 1,5 (0 bis 7,5). Die MRT-Aufnahmen wurden mittels FreeSurfer© analysiert – zudem wurden nach Segmentation periventrikuläre von nicht-periventrikulären Läsionen anhand eines eigenen Algorithmus unterschieden. Danach wurden die gemittelten MTR-Werte der einzelnen Läsionen berechnet und sowohl miteinander als auch mit verschiedenen klinischen Parametern verglichen.

Ergebnisse:

Der Mittelwert der MTR-Werte in periventrikulären Läsionen war signifikant niedriger ($p<0,001$) als jener nicht-periventrikulärer Läsionen. Die MTR-Werte der Läsionen zeigten keinen Zusammenhang mit der kortikalen Dicke ($p>0,05$). Eine Unterscheidung der einzelnen MS-Phänotypen anhand der MTR-Werte war nicht möglich. Die Änderung der kortikalen Dicke zeigte keine Korrelation mit den MTR-Werten oder dem Prozentsatz

periventrikulärer Läsionen, jedoch mit der Progression der einzelnen EDSS-Werte (p=0,032).
Ebenso zeigte kein Parameter einen Zusammenhang mit der Konversion von CIS zu CDMS.

Diskussion:

Periventrikuläre Läsionen zeigen eine größere mikrostrukturelle Schädigung als nicht-periventrikuläre Läsionen. Dies bestätigt die Hypothese, dass liquorabhängige Faktoren, die nicht nur ventrikelnahe Strukturen, sondern auch kortikal schädigen könnten, eine wichtige Rolle in der Pathogenese der MS einnehmen. Zudem hat das Ausmaß der kortikalen Atrophie einen Einfluss auf die weitere Behinderung der PatientInnen. Eine Möglichkeit, den weiteren Verlauf einzelner PatientInnen anhand der getesteten Parameter vorherzusagen, ergibt sich allerdings nicht.

Abstract

Introduction:

Multiple sclerosis, a chronic inflammatory demyelinating disease of the central nervous system, is the most common cause for non-traumatic disability in adolescence. As shown in histopathological studies, especially periventricular and cortical regions are damaged throughout the disease course – both representing regions in direct contact with cerebrospinal fluid (CSF). With the technical advances in magnetic-resonance imaging (MRI) and the implementation of non-conventional MRI sequences like magnetisation transfer imaging (MTI), it has become possible to detect microstructural changes in lesions that cannot be seen on conventional sequences. The goal of this study was to investigate a possible correlation between periventricular lesions, their microstructural changes and the cortical thickness. Also, we investigated differences between the degree of damage in periventricular and non-periventricular lesions and whether differences between the phenotypes of MS exist.

Methods:

We conducted a study in 160 patients (87 *clinically isolated syndrome* (CIS), 73 *clinical definite multiple sclerosis* (CDMS)), 105 patients were female (65.5%), the mean age at onset was 29.1 years with a median EDSS of 1.5 (0-7.5). Magnetic resonance images were mapped using FreeSurfer© and further lesions were categorized into periventricular and non-periventricular using a specific algorithm. Further, mean MTR of all lesions were calculated and compared between lesion categories and certain clinical features.

Results:

The mean MTR of periventricular lesions were significantly lower than those of non-periventricular lesions ($p<0.001$). The MTR showed no correlation with the cortical thickness. A distinction between disease types could not be made using these parameters. The change of cortical thickness did not correlate with MTR or periventricular lesion load, but with the progression of EDSS scores ($p=0.032$)

Furthermore, the parameters did not show differences between converters from CIS to CDMS and non-converters.

Conclusion:

Periventricular lesions show a larger degree of microstructural changes than non-periventricular lesions, confirming the hypothesis that CSF-factors may damage brain regions in direct contact with CSF and thus play an important role in the pathogenesis of MS. The change of cortical thickness correlates with disability in patients with CDMS. However, a valid prediction of disease progression on an individual level cannot be made using these parameters.

Abbreviations

MS = Multiple sclerosis

CDMS = Clinically definite multiple sclerosis

CNS = Central nervous system

MRI = Magnetic resonance imaging

CSF = Cerebrospinal fluid

EDSS = Extended disability status scale

FS = Functional systems

CIS = Clinically isolated syndrome

RRMS = Relapsing-remitting multiple sclerosis

PPMS = Primary progressive multiple sclerosis

SPMS = Secondary progressive multiple sclerosis

WML = White matter lesions

FLAIR = Fluid attenuated inversion recovery

GM = Grey matter

MTI = Magnetization transfer imaging

RF = Radio frequency

MTR = Magnetization transfer ratio

NAWM = Normal-apperaing white matter

MD = Mean diffusivity

PVL = Periventricular lesion

nPVL = Non-periventricular lesion

CMT = cortical mean thickness

PVLL% = Periventricular lesion load

DMT = Disease modifying therapy

1. Introduction

1.1. Multiple Sclerosis

Multiple sclerosis (MS) is a chronic inflammatory demyelinating and neurodegenerative disease of the central nervous system (CNS), considered the most common cause for non-traumatic chronic disability among young adults with an average age of onset of 25 to 35 years[1,2]. There is a wide range of prevalence of MS observed: from 30 per 100.000 persons in more southern regions to more than 200 per 100.000 inhabitants in Scotland and Northern Ireland[2]. The estimated prevalence in Austria is approximately 148 per 100.000 inhabitants[3]. MS is affecting twice to three times as many female as male individuals[1]. Although the aetiology and pathogenesis of this autoimmune disease remains unknown, several risk factors such as viral infections (especially Epstein-Barr virus), cigarette smoking[1] as well as obesity in childhood and adolescence[4] or vitamin D deficiency[5] are under discussion. Especially the latter could in part explain the differences of incidences and prevalences in regions on higher latitude in the northern as well as the southern hemisphere[6].

Multiple sclerosis has long been considered as a disease predominantly affecting the white matter of the brain[7]. However, as shown recently in histopathological studies, the grey matter may be heavily affected as well[8]. Due to the continuous advances in conventional and non-conventional magnetic resonance imaging (MRI) techniques, grey matter pathology can also be assessed in vivo nowadays[9]. While a correlation between the percentage of periventricular lesions and cortical thickness was found using T1-weighted and FLAIR magnetic resonance imaging[10], conventional MRI-sequences have limited value for uncovering subtle changes in the microstructure of the so-called normal-appearing white matter[11]. The concurrent presence of periventricular lesions and grey matter pathology, both regions adjacent to spaces filled with cerebrospinal fluid (CSF), may represent a clue for the existence of soluble CSF factors like cytokines which diffuse into the brain tissue and trigger an inflammatory reaction[10].

Using non-conventional MRI sequences (here: magnetisation transfer imaging), the objectives of this study thus were to:

- investigate if periventricular lesions have a different MTR compared to non-periventricular lesions, indicating a different degree of microstructural change

- assess how MTR within the lesions correlates with cortical thickness as a marker for subpial grey matter pathology and how these changes evolve over the long-term and
- test if there are any correlations of these imaging parameters with clinical variables like disability or disease phenotype.

To give an overview of this complex and heterogeneous disease the following chapter describes the main aspects of MS with a special focus on the role of MRI in diagnosis and research and lastly cortical pathology.

1.1.1. Symptoms

The heterogeneity of symptoms patients with MS present with may partly be explained by the location of the demyelinating process[12]. The onset of disease can also vary depending on the phenotype of MS: a more acute beginning of the neurological symptoms may be an indication for the relapsing-remitting type while progressive forms usually begin with subtle undetected symptoms[12], although in clinical practice this differentiation is rarely seen. The most common symptoms at the beginning of the disease are shown in *Table 01*.

Common symptoms in Multiple Sclerosis
Optic Neuritis
Hypaesthesia and Paraesthesia
Paresis
Nystagmus
Ataxia
Bladder and sexual dysfunction
Cognitive dysfunction, e.g. impaired attention and fatigue

Tab. 01 List of common symptoms occurring in multiple sclerosis[13–15]

Almost one third of patients initially present with optic neuritis causing painful eye movement, partial or complete loss of vision and double vision, beginning in the course of a few days and remitting after a few weeks[13]. Additionally, paraesthesia, a feeling of ants

crawling on one's limbs, or hypaesthesia, the reduced sensation or numbness, occurs as well as motor weakness of one or more limbs[15]. During the progression of disease, the majority of patients reports bladder dysfunction, starting with higher urgency and frequency and ending in incontinence[13]. Further along the disease course, patients may also present with dysarthria and sexual dysfunction[13]. Symptoms that are less common like seizures and hyperkinesia do not occur solely; nystagmus, intention tremor and scanning speech (also called the Charcot-Triad) are rarely observed on their own[13]. Symptoms of cognitive dysfunction such as impaired attention, fatigue, and psychiatric symptoms like depression are common in MS and are reported either alone or in combination in up to 70% of patients[14].

1.1.2. Extended Disability Status Scale

A widely used scale for evaluating the functionality of CNS systems in patients with MS, used to describe progression of the disease and the effectiveness of the therapy, is the Extended Disability Status Scale (EDSS), introduced by Kurtzke in 1983 to objectify the disease severity[16]. The score ranges from 0, which represents a normal neurological status, to 10, meaning death due to MS, increasing in 0.5 steps except the interval 0 to 1[16] (*Table 02)*. For lower EDSS (up to 4.0) Kurtzke described eight different functional systems (FS): Pyramidal, Cerebellar, Brain Stem, Sensory, Bowel & Bladder, Cerebral, Visual, and Other, with every functional system being assigned 5 grades of disability (e.g. uncoordinated movement due to ataxia, grade 5 Cerebellar)[17]. The walking distance and mobility of patients is the deciding factor in higher EDSS scores[17].

Score	Criteria
0	Normal neurologic exam, all FS grade 0, cerebral grade 1 acceptable
1.0	No disability, minimal disability in 1 FS (2 FS if cerebral grade 1)
1.5	No disability, minimal disability in more than 1 FS
2.0	Minimal disability in one FS (1 FS grade 2, others 0 or 1)
2.5	Minimal disability in two FS (2 FS grade 2, others 0 or 1)
3.0	Moderate disability in one FS (1 DS grade 3, others 0 or 1); or mild disability in three or four FS (3/4 FS grade 2, others 0 or 1) though fully ambulatory

Score	Criteria
3.5	Moderate disability in one FS (grade 3) and one or two FS grade 2; or two FS grade 3; or five FS grade 2 (others 0 or 1) though fully ambulatory
4.0	Fully ambulatory without aid, self-sufficient, up and about some twelve hours a day despite relatively severe disability consisting of one FS grade 4 (others 0 or 1), or combinations of lesser grades exceeding limits of previous steps. Able to walk without aid or rest for some 500 meters.
4.5	Fully ambulatory without aid, up and about much of the day, able to work a full day, may otherwise have some limitation of full activity or require minimal assistance, characterised by relatively severe disability, usually consisting of one FS grade 4 (others grade 0 or 1), or combinations of lesser grades exceeding limits of previous steps. Able to walk without aid or rest for some 300 meters.
5.0	Ambulatory without aid or rest for some 200 meters; disability severe enough to impair full daily activities (e.g. to work a full day). (Usual FS equivalents are one grade 5 alone, others 0 or 1; or combinations of lesser grades usually exceeding specifications for step 4.0)
5.5	Ambulatory without aid or rest for about 100 meters; disability severe enough to preclude full daily activities. (Usual FS equivalents are one grade 5 alone, others 0 or 1; or combinations of lesser grades usually exceeding specifications for step 4.0).
6.0	Intermittent or unilateral constant assistance (cane, crutch, or brace) required to walk about 100 meters with or without resting. (Usual FS equivalents are combinations with more than two FS grade 3+).
6.5	Constant bilateral assistance (cane, crutch, or brace) required to walk about 20 meters without resting. (Usual FS equivalents are combinations with more than two FS grade 3+).
7.0	Unable to walk beyond 5 meters even with aid, essentially restricted to wheelchair; wheels self in standard wheelchair and transfers alone; up and about in wheelchair some 12 hours a day. (Usual FS equivalents are combinations with more than one FS grade 4+; very rarely, pyramidal grade 5 alone).
7.5	Unable to take more than a few steps; restricted to wheelchair; may need aid in transfer; wheels self but cannot carry on in standard wheelchair a full day; may require motorised wheelchair. (Usual FS equivalents are combinations with more than one FS grade 4+).
8.0	Essentially restricted to bed or chair or perambulated in wheelchair; but may be out of bed itself much of the day; retains many self-care functions; generally has effective use of arms. (Usual FS equivalents are combinations, generally grade 4+ in several systems).
8.5	Essentially restricted to bed much of the day; has some effective use of the arm(s); retains some self-care functions. (Usual FS equivalents are combinations, generally grade 4+ in several systems).
9.0	Helpless bed patient; can communicate and eat. (Usual FS equivalents are combinations, mostly grade 4+ in several systems).
9.5	Totally helpless bed patient; unable to communicate effectively or eat/swallow. (Usual FS equivalents are combinations, almost all grade 4+).

Score	Criteria
10	Death due to MS

Tab. 02 The Extended Disability Status Scale by Kurtzke (1983) [17]

1.1.3. Classification of Multiple Sclerosis

Since the underlying pathogenesis is not completely understood as of today, to classify the various subtypes of multiple sclerosis the focus lies on the phenotype of the disease, including not only the symptoms themselves but also the progression of disability[12]. Previous classifications (e.g. the definition of clinical subtypes by the US National Multiple Sclerosis Society of 1996) relied on the dominance of the inflammatory aspect in relapsing-remitting MS while the progressive forms seemed to have been dominated by the neurodegenerative aspect[12]. This point of view has since been replaced by a new revised classification scheme in 2013 after the discovery of axonal and neural loss beginning in early stages of the disease and increased understanding of MS[18]. Accurate classification of a patient's disease is a cornerstone of effective decision-making in treatment, prognostication and the design of clinical trials[18].

1.1.3.1. Clinically Isolated Syndrome

Patients presenting with clinical evidence of a first demyelinating event who do not fulfil the Mc Donald criteria, which will be described in chapter 1.1.4, are classified as clinically isolated syndrome (CIS)[12].

By definition the attack, also called relapse, has to be present for at least 24 hours without signs for infection or fever or signs for encephalopathy[19]. In about 85% of all patients with MS the disease begins with a relapse[19]. Within five years about 45% of the patients with CIS meet the McDonald criteria and are diagnosed with clinically definite MS[19] (CDMS), 60 to 70% of patients develop a second relapse within 20 years[20].

The conversion from CIS to MS is not entirely understood, the presence of oligoclonal bands, the number of lesions as well as other CSF biomarkers seem to be prognostic relevant[12,20].

1.1.3.2. Relapsing-remitting Multiple Sclerosis

The majority (80 to 85 percent) of patients has a relapsing-remitting course of MS (RRMS) of the disease. This is defined as an exacerbation of symptoms and/or disability followed by complete or incomplete recovery (*Fig. 01*) which after a period of clinical stability is again relapsing[12,21]. A relapse has to last at least 24 hours without signs of fever or infection[22].

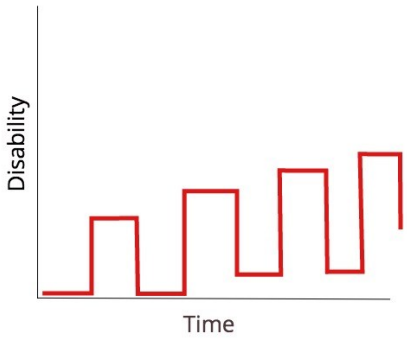

Fig. 01 Disease course over time for RRMS[23]

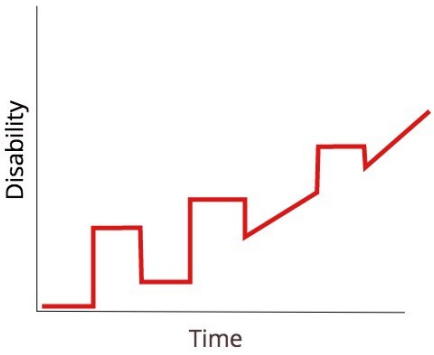

Fig. 02 Disease course over time for SPMS[23]

1.1.3.3. Secondary-progressive Multiple Sclerosis

In comparison to primary-progressive MS the progression of disability occurring in secondary-progressive multiple sclerosis (SPMS) is gradual after an initial relapsing-remitting course (*Fig. 02*). Since the progression is more subtle than in primary-progressive MS the exact transition from relapsing-remitting to SPMS is hard to pinpoint in retrospect but 40% of untreated patients develop SPMS ten years after the initial event[12].

1.1.3.4. Primary-progressive Multiple Sclerosis

The progressive decline of neurological function, the increasing disability from the time of disease onset and the absence of definite relapses (*Fig. 03*) are characteristics for primary-progressive multiple sclerosis (PPMS) which affects 10% of MS patients[12]. Further diagnostic criteria are discussed in chapter 1.1.4.1 *Diagnosis of Multiple Sclerosis*.

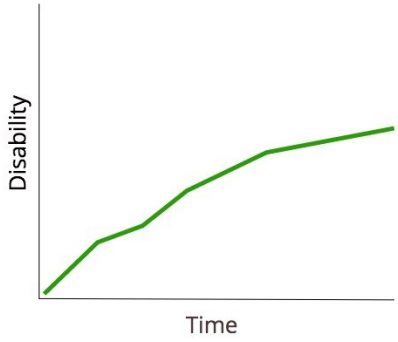

Fig. 03 Disease course over time for PPMS[23]

1.1.3.5. New form of classification: accounting for disease activity

Further, all disease types can be divided into active and non-active forms[24]. The term *active* is ascribed to either a relapse or to active lesions on MRI scans, while for progressive forms, the disease activity does not necessarily coincide with the progression of disability[24].

1.1.4. Diagnosis of Multiple Sclerosis

Before MRI became an important tool in the diagnosis of MS, patients had to have at least two clinical relapses disseminated in time in two distinct anatomical regions[25]. Until the correct diagnosis was found, several months, in some cases also several years, passed[13]. With the continuous advance in MRI, the diagnosis of MS can nowadays be made very often after the first relapse with one MRI scan[22].

For the objective diagnosis of MS the McDonald criteria, which were revised in 2010 (*Table 03*), are used worldwide[12]. These criteria, which are detailed in *Tab. 03*, are based on clinical findings and information provided by MRI[22]. The lesions found on T2-weighted MRI scans have to meet certain criteria to be included: dissemination in space and dissemination in time[12]. The dissemination in space can be demonstrated if at least one lesion is shown in two of four MS-typical areas (juxtacortical, periventricular, infratentorial or the spinal cord); the dissemination in time can be demonstrated by a

simultaneous presence of non-enhancing lesions and asymptomatic Gadolinium-enhanced T1-lesions in one scan or new lesions on follow-up MRI scan compared to a baseline scan[22].

Nevertheless, a second relapse is still sufficient for the diagnosis if there is objective clinical evidence of at least two lesions[22].

Clinical presentation	**Additional data needed for MS diagnosis**
At least 2 attacks; objective clinical evidence of at least 2 lesions or objective clinical evidence for 1 lesion with reasonable historical evidence of prior attack	None
At least 2 attacks; objective clinical evidence for 1 lesion	Dissemination in space: At least 1 T2-lesion in at least two of four typical regions; or await further attack
1 attack, objective clinical evidence for at least 2 lesions	Dissemination in space; Dissemination in time: simultaneous presence of asymptomatic Gd-enhancing and nonenhancing lesions at any time; or new T2-lesion at follow-up scan; or await second attack
1 attack, objective clinical evidence for at least 1 lesion (clinically isolated syndrome)	Dissemination in time Dissemination in space; or await second attack

Tab. 03 The revised McDonald Criteria for the diagnosis of MS[22]

The analysis of the cerebrospinal fluid is not formally required for the diagnosis of relapsing remitting MS under the McDonald criteria although it adds valuable information

for the evaluation in patients with unclear symptoms[12], when showing oligoclonal bands in isoelectric focusing[26].

For the diagnosis of PPMS, at least one year of clinical disease progression is required for the 2010 McDonald criteria as well as two of the following three criteria[22]:

- Evidence of dissemination in space in the brain (at least one T2 lesion in at least one characteristic area)
- Evidence of dissemination in space in the spinal cord (at least two T2 lesions)
- Positive CSF (oligoclonal bands and/or elevated immunoglobulin G index)

1.2. Therapy of Multiple Sclerosis

Although there is no cure for MS, the symptoms presenting during a relapse can be attenuated and the duration of the relapse itself can be reduced. The first-line therapy for an acute relapse is methylprednisolone, a glucocorticoid, suppressing the inflammation and reducing the resulting oedema as well as restoring the damaged blood-brain barrier[13]. The restoration of the blood-brain barrier happens shortly after the high-dose steroid-therapy and can lead to false negative MRI results for up to 60 days, which is why MRI scans should be secured before the intravenous application of methylprednisolone[27].

Treatment in MS over the long-term is based on immunotherapy and disease-modifying drugs: the first-line therapy for patients with MS or clinically isolated syndrome are the immunomodulating interferons-β1a and b and glatirameracetate[28]. For patients who show disease activity despite being treated with on of the basic therapies, the monoclonal antibodies natalizumab or fingolimod are indicated as an escalation therapy[13].

Since MS is not a curable disease, patients usually remain on lifelong medication. Most therapeutics used for MS are for parenteral use only, thus reducing the patients' compliance; the new oral disease-modifying substances like fingolimod, dimethyl fumarate, which are also considered basic therapeutics in some countries, and teriflunomide increase the adherence of patients[29] with similar effectiveness[30], with fingolimod and dimethyl fumarate showing a superior effectiveness compared to teriflunomide and glatirameracetate[30].

1.3. The Role of Magnetic Resonance Imaging

The use of magnetic resonance imaging (MRI) as a diagnostic tool can be explained by its sensitivity for focal white matter lesions (WML). However, due to a lack of specificity to the pathological processes conventional MRI-sequences (e.g. T2-weighted imaging) are of limited value for the research on MS pathogenesis[11]. Since the lesions found using MRI do not have a strong correlation with the clinical symptoms and neurological disability, new methods had to be found that allow better individual prediction of disease development[11].

1.3.1. Conventional MRI-Sequences

Conventional MRI-sequences mark the cornerstone of current diagnostic criteria as described above, but are also used to check for subclinical disease and as a surrogate marker of lesion burden in treatment trials. The most important sequence to visualise MS lesions are T2-sequences[31]. To improve visibility of especially periventricular lesions Fluid-Attenuated Inversion Recovery (FLAIR) sequences based on a T2 sequence are commonly used[31]. FLAIR is suppressing the signal of the CSF, allowing a better visualisation of periventricular and juxtacortical lesions while decreasing the visibility in the posterior fossa[31]. Lesions in T2-weighted and FLAIR images appear bright (*hyperintense*), while in T1-weighted images they may appear darker than the surrounding brain (*hypointense, Fig. 04*)[31].

Using intravenous contrast agents (usually Gadolinium (Gd)-based) acute lesions can be differentiated from inactive lesions using postcontrast T1-weighted imaging because of the inflammation and the resulting breakdown of the blood-brain barrier[32] – these lesions then turn hyperintense on T1-weighted scans (“enhacing lesions”).

Generally, lesions are typically ovoid and are located juxtacortically, periventricularly or infratentorially presenting in an asymmetrical and random pattern, while spinal cord lesions are cigar shaped, lie eccentrically and typically do not extend longitudinally over two vertebrae[31].

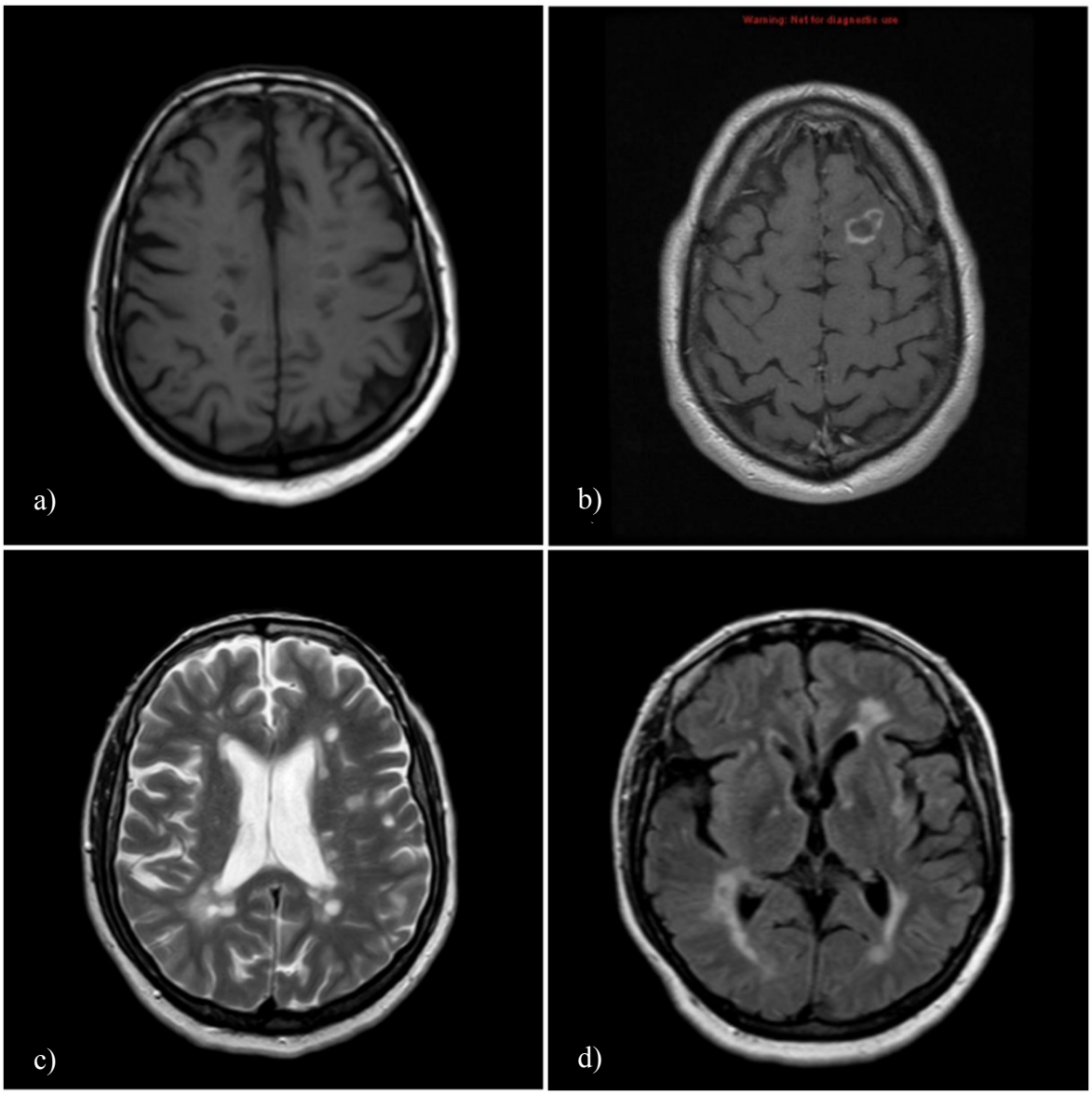

Fig. 04 Example for conventional MRI sequences[33,34]: a proportion of lesions on T1-weighted (a) sequences may appear hypointense, with added contrast-agents some show enhancement (here ring-enhancement) (b); on T2-weighted (c) and FLAIR (d) sequences lesions appear hyperintense

As opposed to T2-hyperintense lesions, the hypointensity of T1-lesions depends on the degree of pathological severity, believed to represent irreversible axonal loss and persistent demyelination ("black holes")[35].

Besides its use in the diagnosis of MS, conventional MRI has also some prognostic value for the prediction of disease progression and disability, however a valid prognosis on an individual level is still not possible[36].

The measurement of brain atrophy, which also occurs in MS and varies between the different disease courses[37], was thought to be a more reliable prognostic marker because

studies have shown a stronger correlation between disability with atrophy than with lesion load[38,39]. Since it lacks prognostic value on the individual level, it is so far only used in research and therapeutic trials, but not in clinical every day practice.

1.3.2. Non-conventional MRI-Sequences

Non-conventional MRI-sequences have been developed as a result of the technical advances and progression in MRI technology to improve the contrast and to investigate the brain, with increased pathological specificity trying to assess microstructural changes and thus explaining the pathophysiology of MS in a more refined matter[11]. Though not yet having entered individual patient care settings, non-conventional MRI has become an important research tool[11].

1.3.2.1. Magnetisation Transfer Imaging (MTI)

While the signal from conventional MRI-sequences depends almost exclusively on protons of relatively free hydrogen atoms and water molecules, the signal from bound protons that are connected to macromolecules (e.g. myelin) cannot be detected by conventional MRI because of their fast T2 relaxation time (<100µsec), which results in signal decay before detection by MRI[11]. However, this does not mean that these protons bound to macromolecules are undetectable: their interaction with free water molecules and their spins can be examined by selectively saturating bound protons with radio frequency (RF) pulses that do not resonate with free water (e.g., they are *off-resonance)*[40]. The transfer of magnetisation between free water protons and macro-molecular protons (hence the name magnetisation transfer imaging) can be measured by a reduction in signal intensity[40]. To assess the effects of magnetisation transfer, two different sets of images have to be taken: one with an off-resonance pulse saturating bound protons and one without an off-resonance pulse[11]. The magnetisation transfer ratio (MTR) then describes the relative signal difference between these images[40]. It is higher in tissues with a high proportion of protons connected to macromolecules[40]; using MTI the density of myelin in the brain can thus be determined indirectly- the lower the MTR, the more demyelination has taken place[40].

Not only does MTR reduction of variable degree correlate in MS lesions visible on conventional MR sequences (especially T1 black holes), a ratio reduction can also be

discovered in normal-appearing white matter (NAWM) days or even weeks before new lesions are formed and become detectable on T1- or T2-weighted images[11]. For the occurring processes in the grey matter that are associated with cognitive impairment or disability and occur in early stages of the disease MTR seems to be a valuable predictive measurement for patients on the group level[11].

Other non-conventional MRI-sequences are for example diffusion-weighted imaging, susceptibility-weighted imaging or iron mapping, all of which can also, like MTI, detect microstructural changes in normal-appearing white matter and grey matter[11,31].

1.4. White Matter and Grey Matter Pathology

White matter pathology in MS is found predominantly in four specific CNS regions: periventricular, juxtacortical, infratentorial and spinal cord[22]. Many lesions are found to be located in regions that are in direct contact to CSF[10]. Also, NAWM may show signs of microstructural damage early in the disease course which can be detected using non-conventional MRI techniques[41]. Most recent studies suggest that the highest degree of microstructural damage in NAWM is found directly near the lateral ventricles and decreases further into the brain parenchyma[41], where the MTR are almost the same as in healthy controls[42].

As described earlier, MS has long been considered for a long time a disease of only the white matter, but the full range of symptoms occurring in patients with MS cannot be explained just by pathologies and irregularities in the white matter[8]. Therefore, the grey matter and its abnormalities have been increasingly explored in MS research, benefitting from the advancement of immunohistochemistry and MRI. Histo-pathological studies as well as MRI studies using higher field strengts (e.g. 7 Tesla) were able to demonstrate lesions that affect the cortex[7,43].

Demyelinating lesions in the cortex can be classified into three types[44]: Type I lesions on the border of white and grey matter affect both structure and show inflammation to some degree[37]. Type II lesions lie intracortically around blood vessels, while type III lesions are found subpially[44]. These type III lesions, which are also the most common ones in MS, are found in the basal ganglia, the cerebral cortex as well as the grey matter of the spinal cord[44]. Some publications also define a fourth type of grey matter lesions as “that extend throughout the whole width of the cerebral cortex”[45].

This classification of cortical pathology, which originates in pathological studies[45], can in vivo only be made using high-field MRI (e.g. 7T) which are not used in clinical routine[46]. Similar to the periventricular regions, MTR of the cortex increase from the outer surface towards the white matter, suggesting a larger degree of damage in the outmost regions of the cortex which are also in direct contact with CSF[47].

Whole brain atrophy increases during the course of the disease, suggesting on increasing the impact of cortical abnormalities[48]. The cortical pathology may cause the cognitive impairment[48] as well as epileptic seizures[49] which patients with MS are more likely to experience. Cortical pathology also seems to play an important role in physical disability[7].

While previous work of our group has suggested links between periventricular lesion load and cortical thickness[10] and others have suggested a correlation between cortical lesions and meningeal inflammation[50] what remains unknown is the underlying pathogenesis of the cortical pathologies.

1.4.1. Periventricular lesion load

In previous work of our group, the correlation of the percentage of lesions located in the periventricular white matter and the cortical thickness was suggested as an indirect indicator for the association of white and grey matter pathology[10] – regions close to the CSF. Patients with a higher percentage of lesion in periventricular lesions show a thinner cortex compared to patients whose lesions were distributed more evenly[10]. This correlation was only found in RRMS, not in CIS[10]. Also, the volume of periventricular lesions correlated with cortical thinning, while non-periventricular lesions do not[10].

These results support the hypothesis of CSF factors that mediate the inflammation leading to typical lesions[9]. Based on this hypothesis, we here expected lower MTR for periventricular lesions than in non-periventricular lesions, suggesting a larger degree of microstructural damage. Also, a correlation between the reduction of cortical thickness and MTR of periventricular lesions but not with non-periventricular lesions would have to be expected. Together, this would further support the hypothesis of soluble CSF factors.

1.5. Objective

The purpose of this study therefore was to:

- investigate if periventricular lesions have a lower MTR compared to non-periventricular lesions, thus indicating more microstructural change
- assess how the MTR within lesions correlates with cortical thickness as a marker for subpial grey matter pathology and how these changes evolve over the long-term and
- test if there are any correlations of these imaging parameters with clinical variables like disability or disease type.

2 Methods

2.1. Patients

We report data from a follow-up study stimulated by previous work of our group published by Jehna and colleagues[10] conducted in 160 patients, 105 of which were female (65.63%) and 55 male (*Fig. 05*). The inclusion criteria were as follows:

a) Patients with CIS or RRMS
b) At least two MRI scans at the same 3T research scanner using identical protocols separated by at least 12 months
c) A complete clinical history of the disease course including age at disease onset, age at baseline, sex, EDSS at baseline, follow-up and last documented visit, number of disease modifying therapy.

All patients underwent two separate MRI-examinations, with a mean interval of 34.5 months. We analysed all baseline scans. For the analysis of the follow-up scans, we excluded patients whose scans were obtained within 12 months, leaving 142 patients. This was done to minimise technical variability and allow for sufficient time for biological changes (e.g. regarding brain atrophy) to occur. Also, patients were also excluded, if the developed SPMS - resulting in a final number of 132 patients for the analysis between the baseline and the follow-up scans.

In this thesis, the first scan conducted will be called "*baseline*", the second scan will be called "*follow-up*".

2.1.1. Informed consent and Ethics Committee

The patients gave written informed consent to be part of this study after full disclosure of data usage and the possibility to leave the study at any time without presenting a reason.

The study was approved by the local ethics committee of the Medical University of Graz.

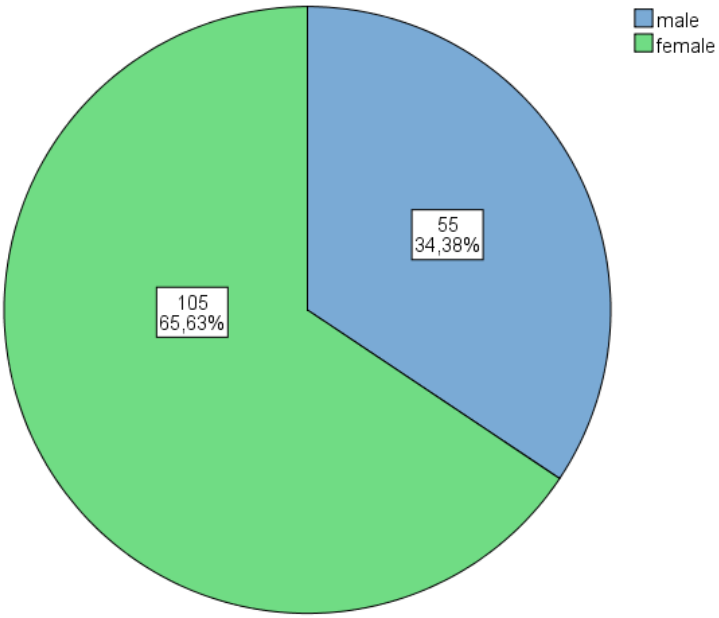

Fig. 05 Distribution of patients by gender and proportions in percentage

2.2. Magnetic Resonance Imaging

2.2.1. Image Acquisition

The magnetic resonance images were acquired at the Department of Radiology at the Medical University of Graz, the MRI machine used was a dedicated 3T Tim Trio Research System by Siemens Medical Systems (Erlangen, Germany).

2.2.2. Assessment of Magnetic Resonance Images

The brain was processed in two different steps: First, the ventricles were segmented using the open source software FreeSurfer© image analysis suite (available at http://surfer.nmr.mgh.harvard.edu) in T1-sequences (slice-thickness: 1mm)[10]. These ventricle-masks were dilated in 1-voxel increments five times – the regions were coded in different shades of green (*Fig. 06*). Then, the lesions were identified by an experienced rater and segmented semiautomatically using the images from the FLAIR-sequence (slice-thickness: 3mm) – lesions smaller than 3mm were not considered[10]. After the lesion mask was registered on the T1-images, the lesions that touched the dilated ventricle masks and shared at least 1 voxel were marked as periventricular – all others were classified as non-periventricular[10].

The percentage of periventricular lesions was calculated by dividing the amount of periventricular lesions by the total amount of lesions[10].

This procedure was repeated until every image was processed to create 3D-masks – now a white matter-mask (by FreeSurfer©) was applied, so that solely white matter was considered in further analysis[10].

The mapping of the cortex and the calculation of its thickness was also performed using FreeSurfer©[10]. To compensate for eventual confounding factors like skull and brain volume, we divided the cortical thickness with the third root of the estimated intracranial volume. All statistical analyses have been performed using the normalised cortical mean thickness, while the values provided in tables are not normalised and therefore differ from the values displayed in scatterplots. For the calculation of the volumetric change of cortical thickness, we subtracted the cortical thickness of the second scan from the thickness of the baseline scan.

In every voxel of a T2-FLAIR hyperintense focal lesion, the MTR was measured. The mean and median MTR was calculated for every lesion and later combined to a single parameter (mean and median MTR in all lesions, of periventricular and non-periventricular lesions).

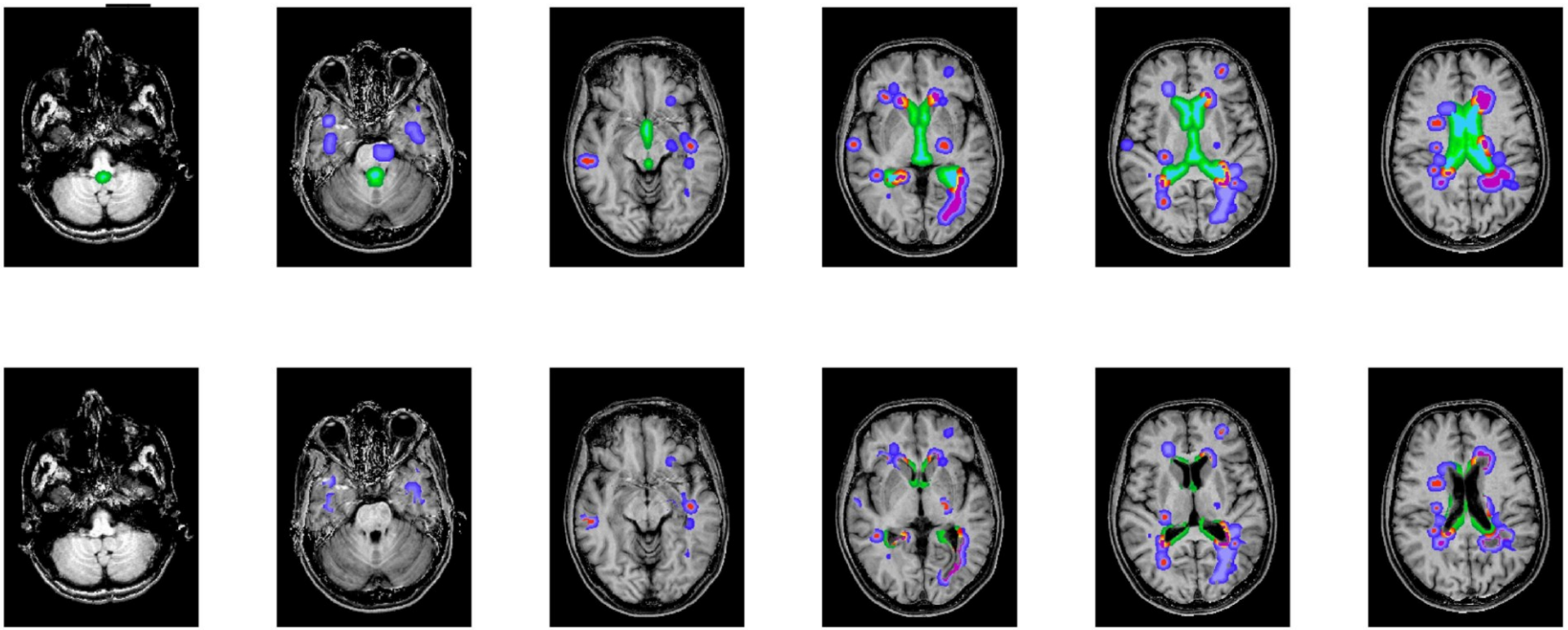

Fig. 06 Visual check for MTR: blue represents the ventricle, the different shades of green represent the dilated ventricle mask; lilac codes lesions, purple represents the dilated lesion masks; red, orange and yellow indicate shared voxels by both ventricle and lesion masks

2.3. Data Retrieval and Statistical Evaluation

Data like age of onset or last EDSS was retrieved retrospectively from medical charts using the programmes openMEDOCS© by SAP (Walldorf, Germany) and Archimed, a scientific database implemented at the MS outpatient department of the University Clinic of Neurology in Graz.

For the statistical evaluation of the data we used the programme Statistical Package for Social Sciences Statistics (SPSS) 23© by IBM (Armonk, NY, USA; http://www.spss.com). The tests applied were Pearson correlation, Mann-Whitney-U-test for nonparametric data, and t-test for parametric data. To differentiate these two types of data, the Kolmogorv-Smirnov-Test was utilised with a significance level of $p=0.05$: for p values smaller than 0.05 the data was defined parametric and vice versa.

For all above described tests, the threshold of significance was set at 0.05.

3 Results

3.1. Demographics

The median age of the patients at onset of disease was 28.0 years with a range from 8 to 51 years, while the age at baseline was 32.0 years with a range from 14 to 61 years. The median disease duration at baseline was 10.0 months with a range from 0 to 313 months (*Tab. 04*).

Further, the patients were classified according to the McDonald criteria into the groups CIS (*n=87,* 61 female*)* and CDMS *(n=73*, 44 female*)* for baseline (*Fig. 07*) and into the groups CIS (*n=41*), and CDMS (*n*=91) for follow-up (*Fig. 08*). This shows that between the scans 32 patients (36.7%, 10 male and 22 female, *Tab. 05*) first classified as CIS during the course of this study converted to MS. The lower number of patients in the follow-up scan results from exclusion of patients whose two MRI-scans were within a year and of patients who developed SPMS as described in chapter 2.1.

		Overall	***CIS***	***CDMS***
Number of patients		160	87	73
Sex	*Male*	55	26	29
	Female	105	61	44
Age at baseline	*Median*	32.0 years	31.0 years	37.0 years
	Range	14.0-61.0 years	16.0-50.0 years	14.0-61.0 years
EDSS	*Median*	1.5	1.0	2.0
	Range	0-7.5	0-3.5	0-7.5
Age at onset	*Median*	28.0 years	27.0 years	29.0 years
	Range	8.0-51.0 years	8.0 - 50.0 years	13.0-51.0 years
Disease duration	*Median*	10.0 months	5.0 months	50.0 months
	Range	0-313 months	0-216 months	0-320 months
Disease modifying therapy	*Yes*	45.7%	27.6%	64.4%
	No	54.3%	72.4%	35.6%

Tab. 04 Description of patients at baseline

		Overall	*CIS*	*CDMS*	*Converter*
Number of patients		132	41	91	35
Sex	*Male*	45	12	33	11
	Female	87	29	44	24
Age at follow-up	*Median*	35.0 years	34.0 years	36.0 years	30.0 years
	Range	17-64 years	23-53 years	17-64 years	17-50 years
EDSS	*Median*	1.5	1.0	2.0	2.0
	Range	0-8.0	0-3.0	0-8.0	0-5.0
Age of onset	*Median*	27.0 years	27.0 years	29.0 years	24.0 years
	Range	8.0-51.0 years	8.0 - 50.0 years	13.0-51.0 years	8.0-43.0 years
Disease modifying therapy	*Yes*	33.1%	48.8%	64.4%	22.9%
	No	66.9%	51.2%	35.6%	77.1%

Tab. 05 Description of follow-up cohort with both scans

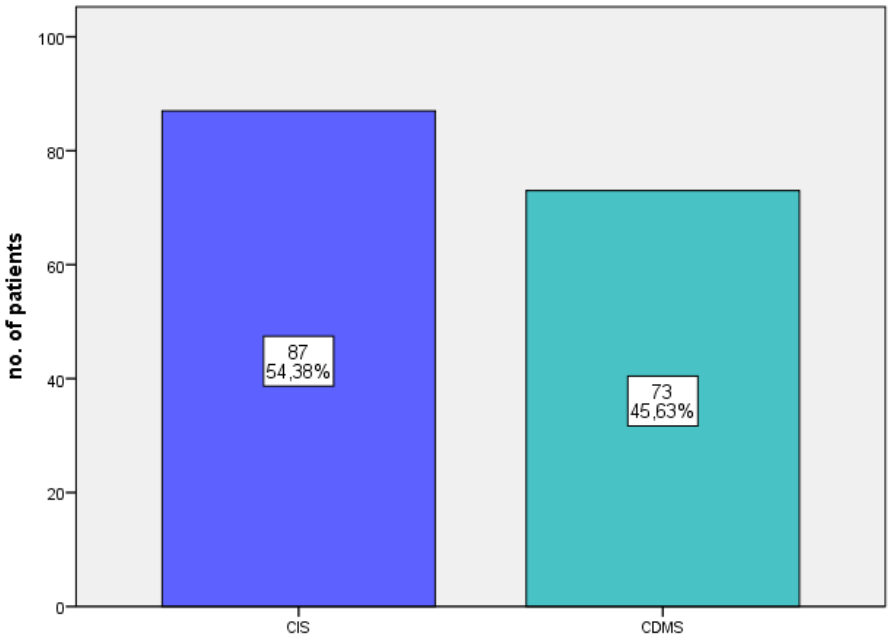

Fig. 07 Patients' classification of disease at baseline

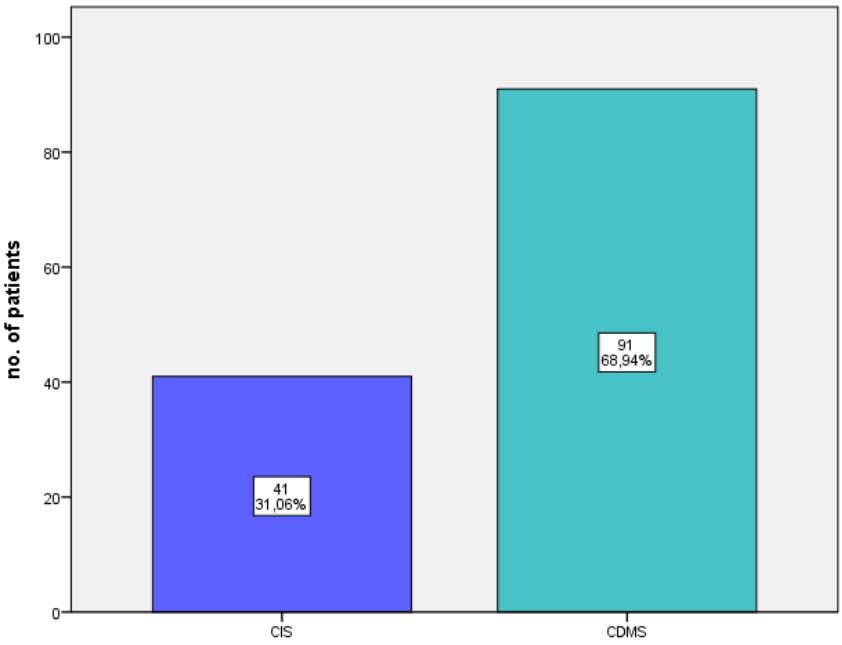

Fig. 08 Distribution of disease classification at follow-up

3.1.1. EDSS

The median EDSS at baseline was 1.5 (range from 0 to 7.5, *Fig. 09*), at follow-up 1.5 (range from 0 to 8.0, *Fig. 10*). During check-ups following after the second scan, the EDSS was determined once again (1.5, range from 0 to 8.0) in a period of 44.56 ± 24.49 months. 21 patients were not assessed clinically within this period due to unknown reasons.

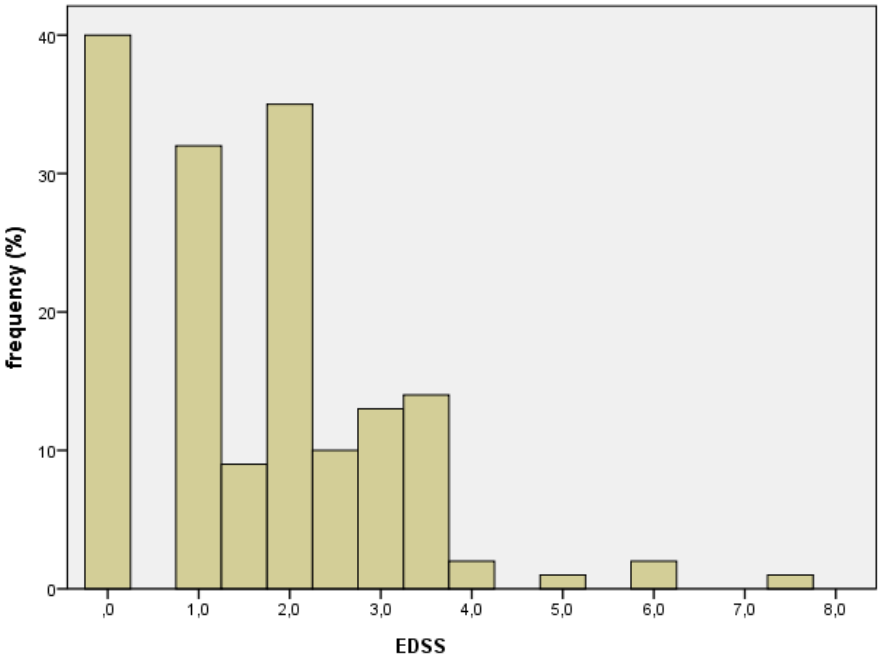

Fig. 09 EDSS of patients at baseline

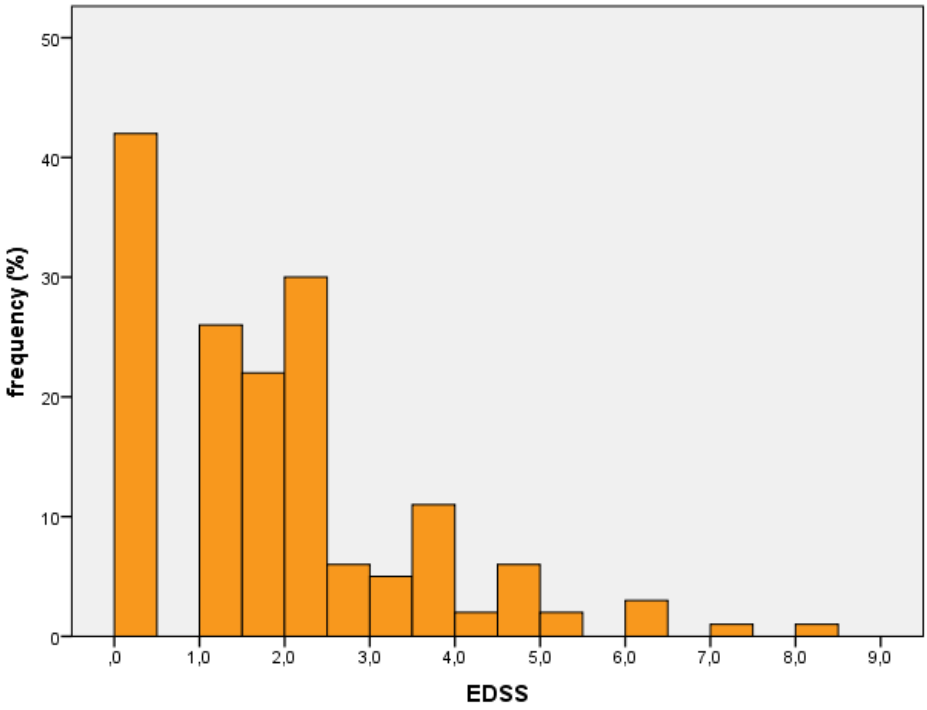

Fig. 10 EDSS of patients at follow-up

3.1.2. Disease modifying therapy

In this study, we did not divide the patients into different groups depending on the individual treatment they were receiving at the time; we just differentiated between generally having received a treatment or not. 54.3 % (n=88; CIS: 72.4%, CDMS: 35.6%) did not receive any disease modifying treatment at the baseline (*Tab. 04*), at follow-up 34% (n=55; CIS: 51.2%, CDMS: 35.6%) did not receive disease modifying therapy.

In the group of the 69 patients who received DMT at baseline, there were 12 converters from CIS to MS and 12 non-converters and 45 patients already had been classified as MS at baseline. The group of patients who did not receive DMT (n=88), can be divided in 26 converters, 36 non-converters and 26 patients with MS already at baseline.

3.2. Results for Cross-sectional Cohort

We first ran statistical analyses on scans of the baseline cohort, comparing MTR between periventricular with non-periventricular lesions and the relation of the periventricular MTR to the cortical thickness.

3.2.1. Difference of MTR in periventricular and non-periventricular lesions

The mean MTRs in periventricular lesions (PVL, 0.317 ± 0.026, *Tab. 06*) were significantly lower than those in non-periventricular lesions (0.338 ± 0.025, p<0.001), for both CIS and RRMS (*Fig. 11*).

	Overall (n=160) *mean±SD*	**CIS (n=87)** *mean±SD*	**CDMS (n=73)** *mean±SD*	**p-value** *CIS vs. CDMS*
PVL (MTR)	0.317 ± 0.026	0.311 ± 0.024	0.313 ± 0.027	0.621
nPVL (MTR)	0.338 ± 0.025	0.338 ± 0.021	0.336 ± 0.029	0.699
CMT (mm)	2.486 ± 0.138	2.502 ± 0.123	2.464 ± 0.152	0.233

Tab. 06 MTR results at baseline (disease phenotypes)

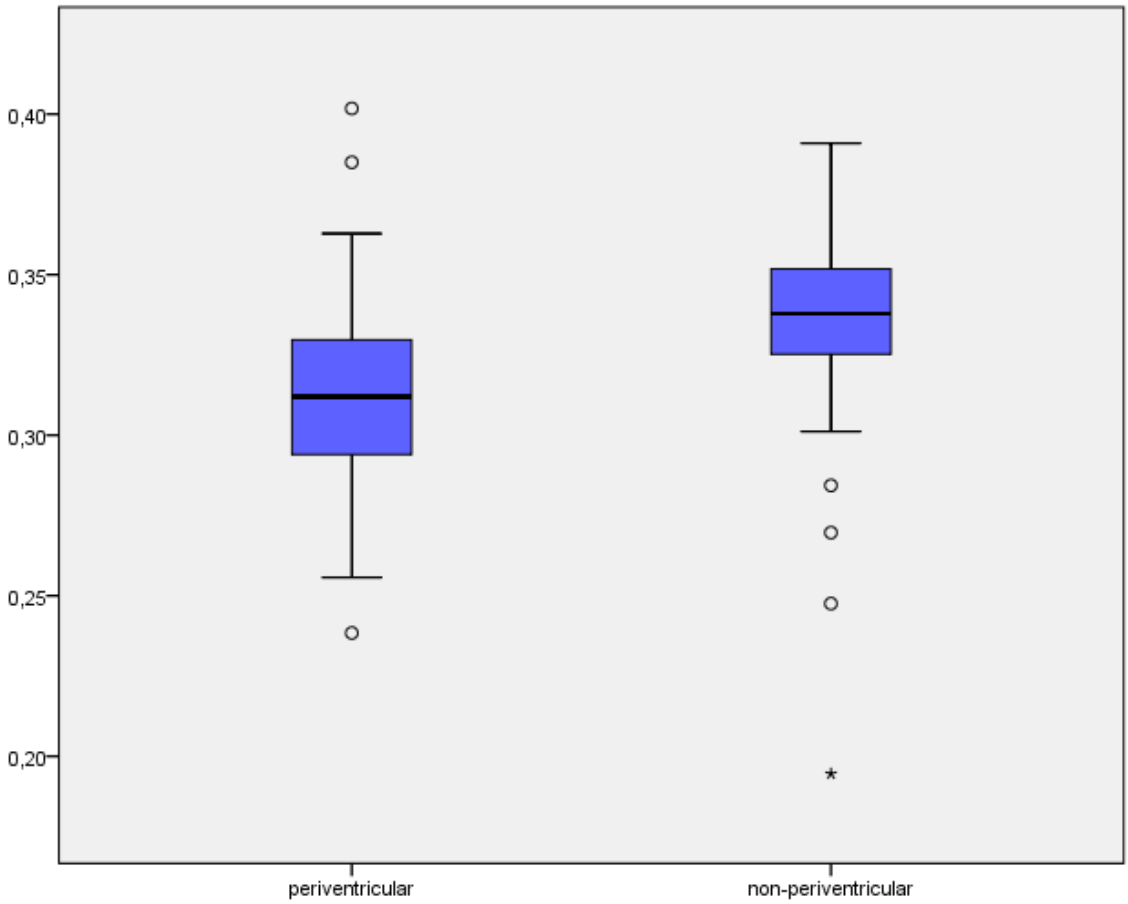

Fig. 11 Mean MTR in periventricular and non-periventricular lesions at baseline for both CDMS and CIS

The MTR in both PVL and non-periventricular lesions (nPVL) did not differ between the distinct MS subtypes as shown in *Figures 12* and *13* (p=0.621 in PVL and p=0.699 in nPVL), although both lesion groups showed a bigger variety in MTR in patients with MS

than those with CIS.

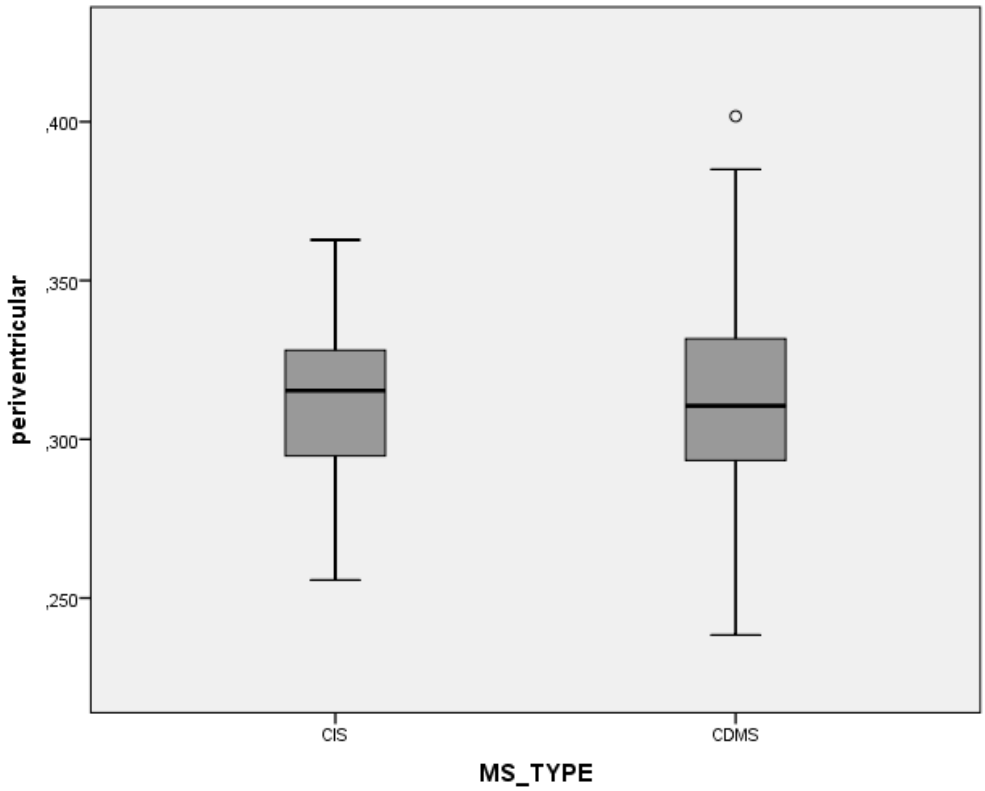

Fig. 12 Mean MTR in periventricular lesions- comparison of CIS and CDMS

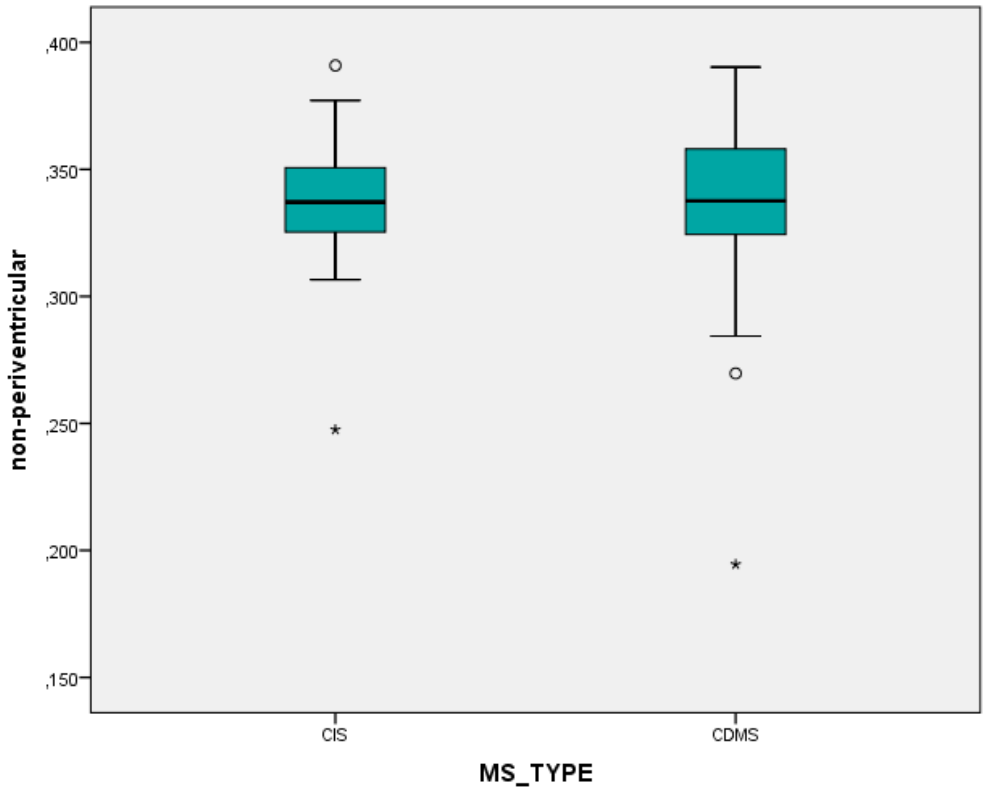

Fig. 13 Mean MTR in non-periventricular lesions - comparison of CDMS and CIS

3.2.2. Correlation of periventricular/non-periventricular lesions with cortical thickness

Neither within the overall cohort nor the two subgroups (MS and CIS, *Tab. 07*) there was a significant correlation between the mean MTR for PVL or nPVL with the mean cortical thickness (CMT, 0.043 ± 0.0028 cm) at baseline (p>0.05, *Fig. 14).*

Correlation	**Overall**	**CIS**	**CDMS**
MTR in PVL/CMT	r=0.028	r=-0.110	r=0.153
	p=0.738	p=0.339	p=0.215
MTR in nPVL/CMT	r=-0.082	r=-0.162	r=-0.027
	p=0.316	p=0.148	p=0.824

Tab. 07 Correlations between PVL and nPVL with cortical thickness at baseline

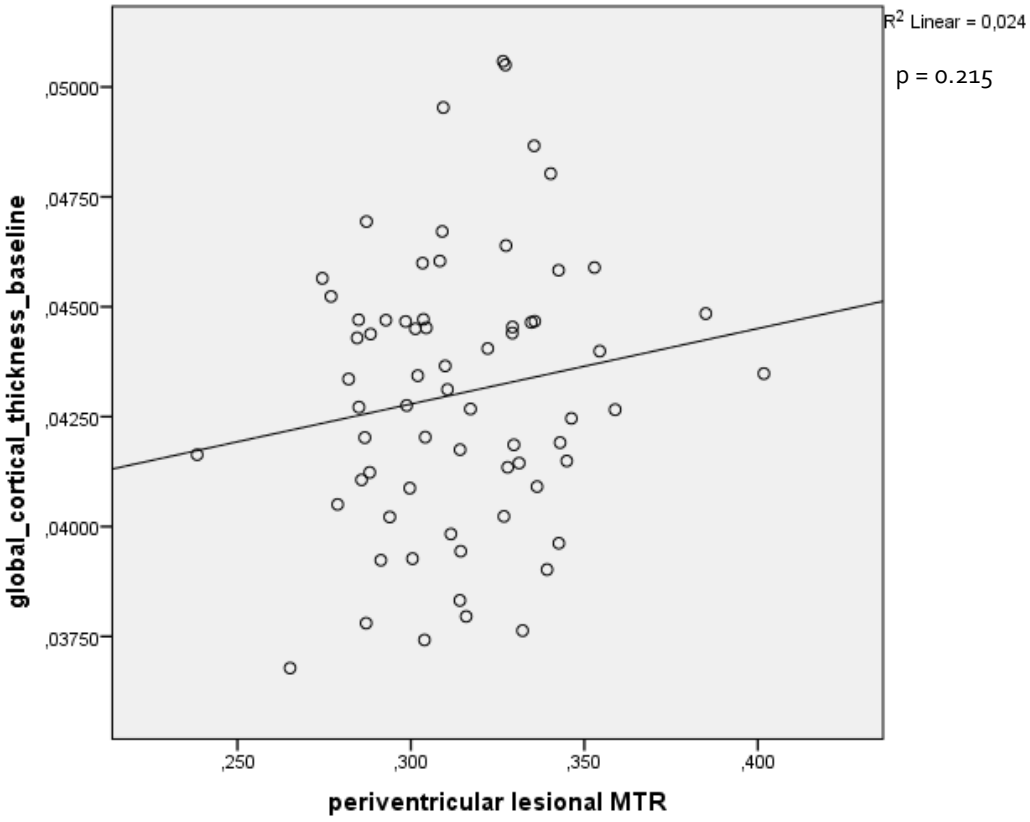

Fig. 14 A graph showing the lack of correlation of MTR in periventricular lesions with cortical thickness; results for patients diagnosed with MS

3.2.3. Difference in cortical thickness

The mean cortical thickness of patients with CIS (2.502 ± 0.123 mm) was not significantly different (p=0.233) than the cortical thickness of CDMS patients (2.464 ± 0.152 mm).

3.3. Results for Follow-up Cohort

Similar results were found in analysing the scans of the follow-up cohort (*Tab. 08*):

- The mean MTR in PVL (0.317 ± 0.026) were significantly lower than those of nPVL (0.338 ± 0.025) overall and for CIS and CDMS p<0.001.
- Again, there were no significant differences between CDMS and CIS using the lesional MTR (PVL p=0.469, nPVL p=0.31).
- The cortical thickness did not differ significantly between both phenotypes (2.493 ± 0.122 mm in CIS, 2.455 ± 0.150 mm in CDMS, p=0.266)

	Overall (n=132) *mean±SD*	**CIS (n=41)** *mean±SD*	**CDMS (n=91)** *mean±SD*	**p-value** *CIS vs. CDMS*
PVL (MTR)	0.317 ± 0.026	0.309 ± 0.024	0.313 ± 0.025	0.469
nPVL (MTR)	0.338 ± 0.025	0.345 ± 0.036	0.340 ± 0.021	0.310
CMT (mm)	2.467 ± 0.143	2.493 ± 0.122	2.455 ± 0.150	0.266

Tab. 08 MTR results at follow-up (disease phenotypes)

Patients with CIS at follow-up did not show a significant correlation of mean PVL or nPVL MTR with cortical thickness (*Tab. 09)*.

However, in patients with CDMS this correlation was significant (p=0.030) between PVL and cortical thickness (*Fig. 15*). Also, the correlation between PVL and cortical thickness for the whole cohort has turned significant at follow-up (r=0.216, p=0.017).

Correlation	**Overall**	**CIS**	**CDMS**
PVL/CMT	r=0.216	r=0.178	r=0.227
	p=0.017	p=0.268	p=0.030
nPVL/CMT	r=0.030	r=0.122	r=-0.045
	p=0.740	p=0.460	p=0.666

Tab. 09 Correlation between PVL and nPVL with cortical thickness at follow-up

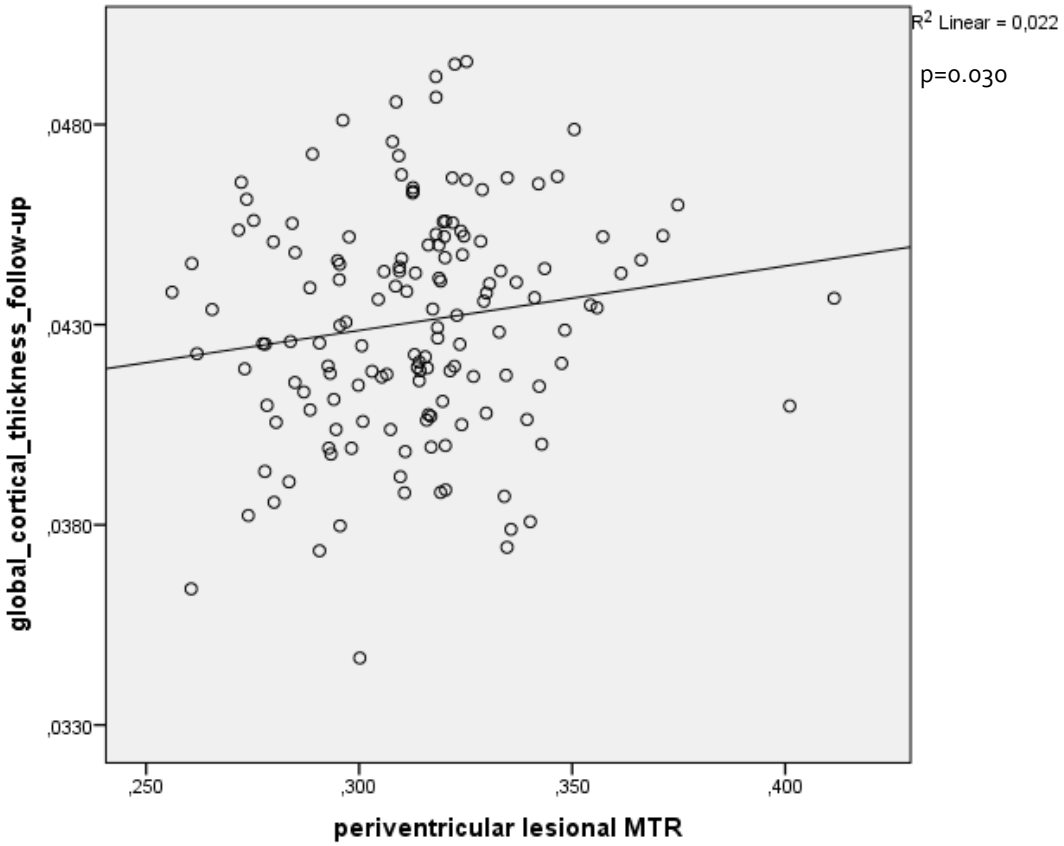

Fig. 15 Scatterplot demonstrating the relationship between the mean periventricular MTR and cortical thickness in patients with MS at follow-up

3.3.1. Changes in cortical thickness

The follow-up scan allowed to measure the changes in cortical thickness and its annual rate.

When comparing both phenotypes, neither the change in cortical thickness (p=0.115) nor its annual rate (p=0.071) showed a significant difference. The same result has been shown in comparing converters with non-converters (p=0.277 for the change in cortical thickness, p=0.346 for its annual rate).

A test for correlation revealed a significance of p=0.003 (r=0.257) of the mean MTR in nPVL at follow-up with the change of cortical thickness – the scatterplot shown in *Fig. 16* unveiled an outlier in patients with CIS who had a very high MTR as well as a very high rate of reduction of cortical thickness.

The MTR in PVL did not show a statistical significance with the reduction of cortical thickness in neither group, neither did the periventricular lesion load (*Tab. 10*).

Correlation	Overall	CIS	CDMS	Converter
PVL/Change of CMT	r=-0.036	r=-0.081	r=-0.016	r=0.004
	p=0.686	p=0.630	p=0.877	p=0.983
nPVL/Change of CMT	r=0.257	r=0.470	r=-0.064	r=0.117
	p=0.003	p=0.001	p=0.541	p=0.537
PVLL/Change of CMT	r=0.017	r=0.182	r=-0.163	r=0.117
	p=0.843	p=0.125	p=0.203	p=0.517

Tab. 10 Correlation of lesional MTR at follow-up and the change of cortical thickness

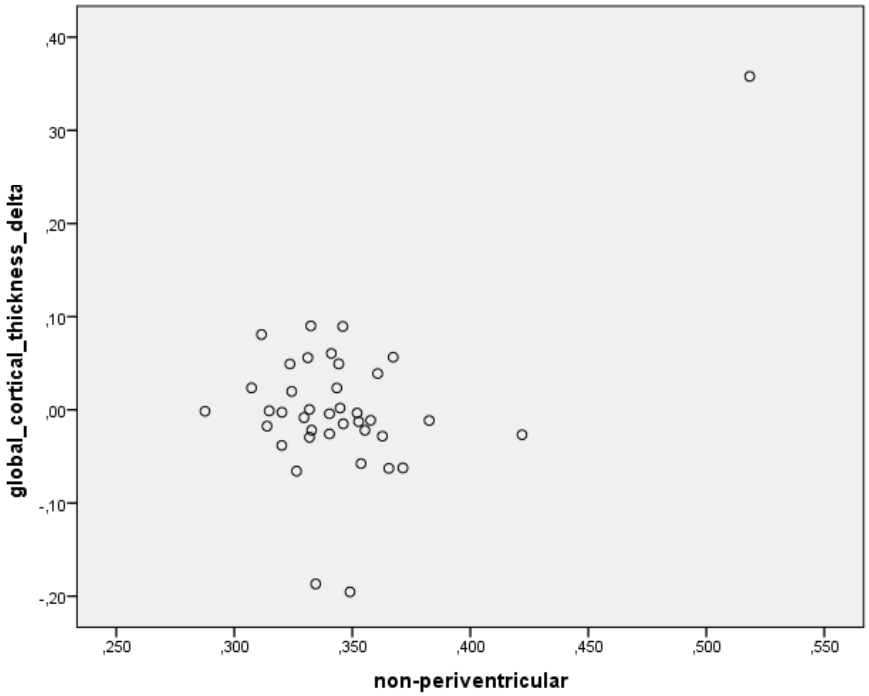

Fig. 16 Scatterplot of mean MTR in non-periventricular lesions to the reduction of global cortical thickness showing an outlier in the right upper corner

The change of MTR in both PVL and nPVL did not have an influence on the change of cortical thickness and its annual rate in neither the whole cohort nor the disease phenotypes ($p>0.05$). In converters, the correlation of the change of MTR in nPVL with the change of cortical thickness ($r=-0.429$, $p=0.026$) and its annual rate ($r=-0.392$, $p=0.043$) has shown to be significant.

3.3.2. Conversion

As described in chapter 2.1, 35 patients converted from CIS to CDMS between the two scans. We ran the same statistical analyses to distinguish converters from non-converters as we did for exploring differences between patients with CIS and CDMS.

	Converter (n=35) *mean±SD*	**Non-Converter (n=41)** *mean±SD*	**p-value** *Converter vs. Non-Converter*
PVL (MTR)	0.321 ± 0.020	0.306 ± 0.025	0.011
nPVL (MTR)	0.340 ± 0.016	0.335 ± 0.020	0.287
CMT (mm)	2.530 ± 0.137	2.494 ± 0.109	0.871

Tab. 11 MTR results at baseline (Conversion)

The mean MTR in PVL at baseline was significantly (p=0.011) higher in patients who converted to CDMS (0.315 ± 0.020, *Tab. 11*) than in non-converters (0.309 ± 0.024, *Fig. 17*). This result was not found at follow-up (p=0.343) although there was a trend towards lower MTR in non-converters (*Tab. 12*). In nPVL, the mean MTR did not show a significance in neither baseline nor follow-up.

	Converter (n=35) *mean±SD*	**Non-Converter (n=41)** *mean±SD*	**p-value** *Converter vs. Non-Converter*
PVL (MTR)	0.315 ± 0.020	0.309 ± 0.024	0.350
nPVL (MTR)	0.342 ± 0.021	0.345 ± 0.036	0.600
CMT (mm)	2.505 ± 0.137	2.493 ± 0.122	0.705

Tab. 12 MTR results at follow-up (Conversion)

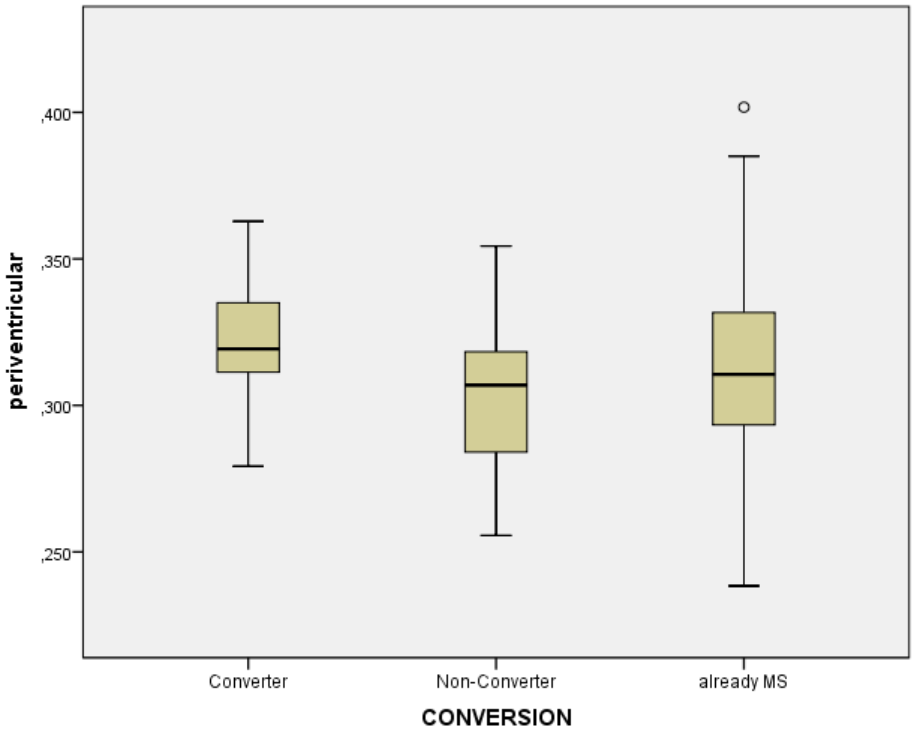

Fig. 17 Comparison of mean MTR in PVL in Converters and Non-Converters at baseline

Whether patients converted did not have an impact on the cortical thickness (p=0.871 at baseline, p=0.705 at follow-up, *Tables 11* and *12*) and its volumetric difference (p=0.277 for the overall change, p=0.346 for the annual rate), with the exception of described outlier in the CIS group, who necessarily are non-converters.

The periventricular lesion load was for both scans higher in non-converters (52.57% at baseline, 50.62% at follow-up) compared to converters (38.16% at baseline, 39.87% at follow-up), with a p-value of 0.021 for baseline and 0.071 for follow-up.

Patients, who have been diagnosed with CDMS during the course of this study, had a lower periventricular lesion load (39.87% at follow-up) than patients, who were initially diagnosed with CDMS (56.70%, p=0.003).

3.4. Correlations of MTR with Clinical Variables

Afterwards, we looked for associations between the imaging parameters and certain critical criteria (e.g. EDSS or age of onset).

3.4.1. EDSS

For the follow-up cohort of this study we particularly focused on the development of the EDSS over the years following the last scan. We investigated the impact of MTR in PVL, the PVLL and the cortical thickness on the EDSS and the EDSS progression.

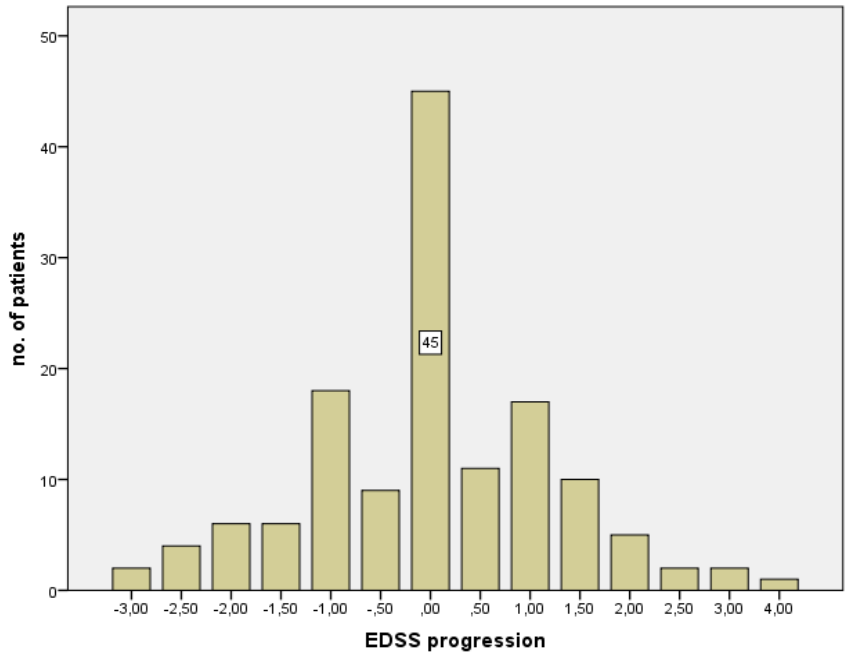

Fig. 18 EDSS progression of all patients throughout the study

14.5% of all patients (n=37) had an EDSS progression of at least 1.0 points, while 32.6% (n=45) remained at the same level throughout the study. Also, the EDSS of 45 patients (32.6%) decreased throughout the study (*Fig. 18*).

With the exception of the mean score in PVL with the EDSS at the second scan in patients with MS (p=0.025, r=0.291, *Fig. 19*), the MTR did not have an impact on the EDSS scores (p>0.05).

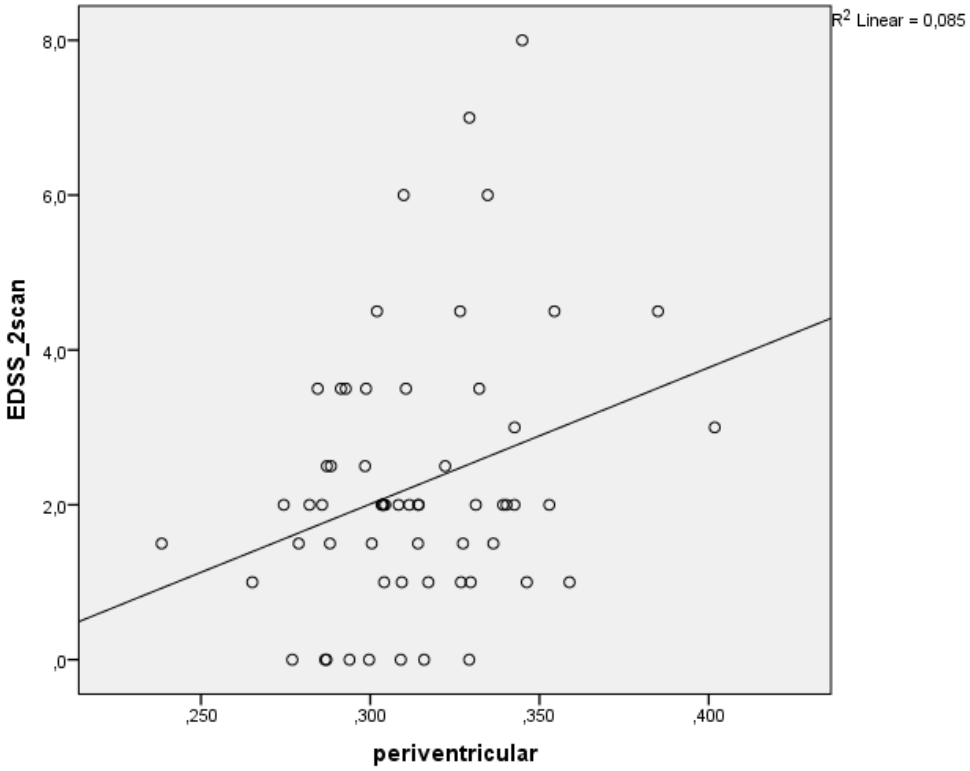

Fig. 19 Relation of mean MTR in periventricular lesions with the EDSS at the second scan in patients with CDMS

The periventricular lesion loads at both baseline and follow-up have not shown to have an influence (p>0.05) on the EDSS of the overall cohort or either subgroup (CIS – CDMS, converter – non-converter).

The progression of the EDSS between baseline and follow-up did not show any correlation with the cortical thickness (*Tab. 13*), while revealing a borderline significance of the EDSS progression with the cortical thickness at baseline in converters (p=0.066, r=-0.314, *Fig. 20*).

Correlation	Overall	CIS	CDMS	Converter
EDSS Progression/CMT1	r=-0.055	r=0.110	r=-0.092	r=-0.314
	p=0.532	p=0.498	p=0.390	p=0.066
EDSS Progression/CMT2	r=-0.100	r=0.062	r=-0.123	r=-0.280
	p=0.260	p=0.705	p=0.248	p=0.104
EDSS Progression/Change of CMT	r=-0.105	r=-0.061	r=-0.104	r=0.141
	p=0.235	p=0.709	p=0.327	p=0.420
EDSS Progression/Annual change of CMT	r=-0.133	r=-0.017	r=-0.154	r=0.140
	p=0.132	p=0.917	p=0.148	p=0.422

Tab. 13 Correlation of the EDSS progression with cortical thickness and its change

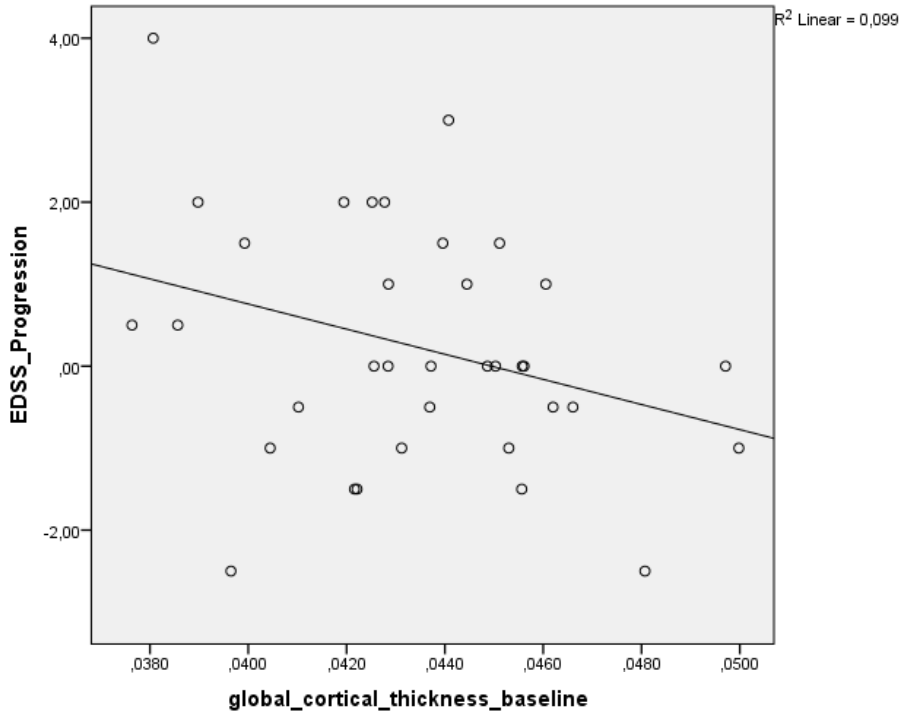

Fig. 20 Correlation of cortical thickness at baseline and EDSS progression in converters

3.4.2. Disease duration

There was a significant correlations of the disease duration with the mean MTR in PVL in patients with CDMS at follow up (p=0.041, r=-0.261), while such correlation could not be found for CIS at neither scan nor for CDMS at baseline.

The disease duration in patients with CDMS had an impact on the cortical thickness at both scans (p=0.001, r=-0.409 and p<0.001, r=-0.425, respectively), while the disease duration

in patients with CIS correlated significantly with the change of cortical thickness (p=0.009, r=0.299).

Correlation	Overall	CIS	CDMS	Converter
Disease duration/PVLL 1	r=0.296	r=0.012	r=0.427	r=-0.159
	p=0.001	p=0.942	p<0.001	p=0.376
Disease duration/PVLL 2	r=0.268	r=0.043	r=0.360	r=0.044
	p=0.002	p=0.797	p=0.001	p=0.809

Tab. 14 Correlations between the disease duration with the periventricular lesion load at baseline and follow-up

The disease duration of all patients at the beginning of this study had a statistical significant correlation with the lesion load (p=0.001, r=0.296 for baseline, p=0.002, r=0.268 for follow-up, *Tab 14*) – a result which was replicated solely in patients with CDMS (p<0.001, r=0.427 for baseline, p=0.001, r=0.360 for follow-up, *Fig. 21*), but not in patients with CIS (p>0.05).

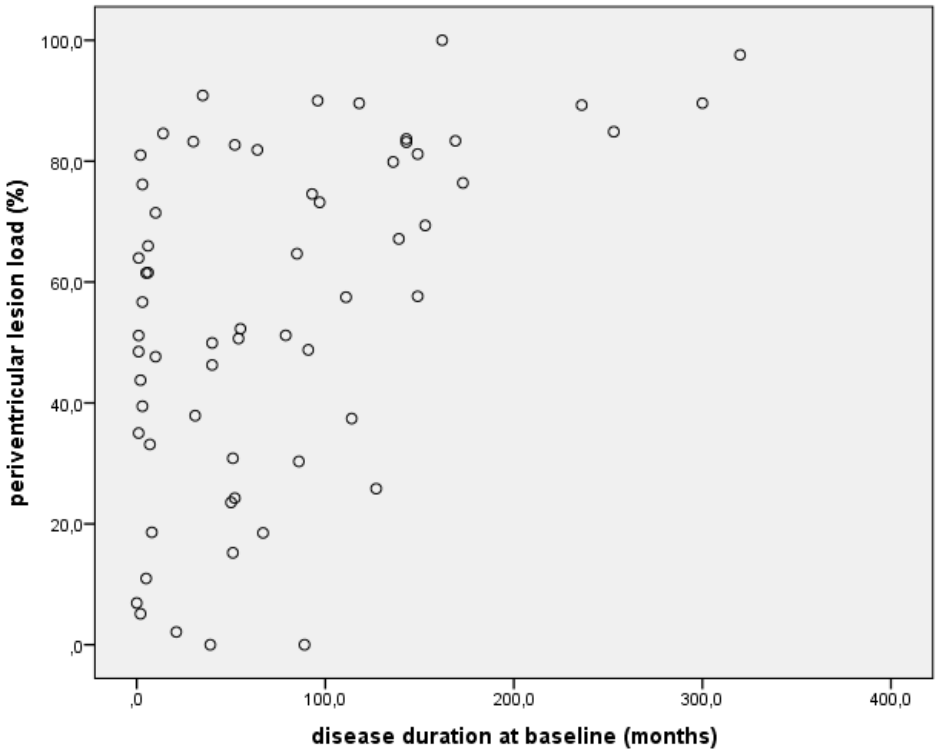

Fig. 21 Correlation of the disease duration in patients with CDMS with the periventricular lesion load

3.4.3. Disease modifying therapy

Whether patients received any disease modifying therapy (DMT) did not correlate with neither the mean MTR in PVL and nPVL, the periventricular lesion load, the cortical thickness or its change over time ($p>0.05$).

4 Discussion

The aim of this study was to, first, investigate if PVL demonstrate more severe microstructural changes as evidenced by MTR compared to nPVL; second, to assess how the lesional MTR correlates with cortical thickness as a marker for subpial grey matter pathology and how these changes evolve over the long-term and third, to test if correlations exist between these imaging parameters and clinical variables like disability or disease phenotype.

These hypotheses are based on the results of a previous study at our centre, which found that a higher lesion occupation of periventricular areas was associated with lower cortical thickness[10]. Also, we here investigated whether the MTR in PVL correlates with the rate of conversion in patients with CIS to CDMS over the course of this study.

Our results show that PVL have a significantly lower MTR compared to nPVL. Since a lower MTR is suggested as a marker of cellular damage[40], this result supports the hypothesis that brain lesions which are in direct contact with CSF show more pronounced microstructural changes[10]. Therefore, lesions in periventricular white matter appear to be affected more heavily by axonal loss and demyelination than non-periventricular lesions. The underlying hypothesis, that periventricular lesions show a larger degree of microstructural changes and axonal loss than non-periventricular lesions, represents an important extension to existing hypotheses that degeneration of the grey matter and also white matter changes might be mediated by CSF related factors[9,10]. Surprisingly, the subtypes CIS and CDMS did not show a significant difference in neither PVL or nPVL, suggesting that the damage in MS occurs early during the disease course and remains at a steady level. Fernando and colleagues have shown, that changes in NAWM are already detectable in patients with CIS[51], a result that was replicated in patients with optic neuritis[42].

We also tested whether the degree of microstructural changes within PVL correlates with the cortical thickness, which is a possible MRI marker for the cortical damage[52], in the whole cohort as well as the subgroups. However, we were not able to show such a correlation, neither in the whole cohort nor in the subgroups. A similar study by Tóth and colleagues, who focused on the relation of lesional demyelination and cortical pathology using diffusion-tensor imaging, found a correlation between periventricular lesions and

NAWM with the cortical thickness, which contrasts our findings[53]. They concluded that the underlying mechanism seems to be demyelination rather than axonal loss[53], a hypothesis supported by histopathological studies in mice indicating that demyelination is associated with axonal loss in perilesional white matter[54]. Brown and colleagues have also shown, using MTI, that NAWM of patients with CIS and CDMS is likewise more severely affected then in healthy controls[42]. Further, patients with optic neuritis already show lower MTR in NAWM than healthy controls[42]. The MTR of periventricular lesions increases from the inside out, a result, which was replicated in NAWM as well[41,42]. Liu and colleagues analysed the MTR of NAWM of both RRMS and SPMS in comparison to healthy controls and found them to be significantly lower in both subtypes[41]. When comparing both MS subtypes, the NAWM closest to the lateral ventricle was lower in SPMS, but not farther out into the white matter[41]. Also, the MTR of cortical lesions have been shown to be lower in SPMS than in CDMS[55].

Juxtacortical lesions also correlate with the cortical thickness, though not as strongly as periventricular lesions, as shown by Pareto and colleagues[52]. Why our results did not show a correlation between the MTR of PVL in contrast to nPVL with the cortical thickness, is not entirely clear. Rather than focusing on the local cortical thickness, we investigated the total volume of the cortex, which might have limited sensitivity. As histopathological studies have demonstrated, certain cortical areas (e.g. deep indentations of sulci and midline) to be more frequently affected than others[50]. An association between the MTR of PVL with the cortical thickness may be found on a local level. Also, further studies should assess whether MTR of PVL may be associated with the MTR of the cortex and cortical lesions.

It has not been shown that the cortex shows pathological changes in MS as well. It is also directly affected by possible pathogens in the CSF like cytokines[50]. Post-mortem studies have found B-cell follicle-like structures adjacent to grey matter pathology[50] in perivascular spaces and also infiltrating the meninges[56]. The presence of oligoclonal bands in the cerebrospinal fluid, which can be found in more than 85% of patients with MS[57], further supports the importance of B cells in MS pathogenesis[56]. Studies with patients suffering from progressive disease courses have found a possible link between cortical demyelination, which can extend to up to 25%[50], and leptomeningeal inflammation[58]. Histopathological studies have shown, that microstructural changes in the cortex occur

from the outside, which is in direct contact with CSF, to the inside[45]. This result has also been shown in MTR studies[47].

Furthermore, several biomarkers with a certain degree of correlation with MS have been discovered, like for example soluble CD163[59] or the myelin-specific antibodies[60], but a biomarker present in every MS patient has either yet to be found or does not exist, a possibility that cannot be excluded considering the heterogeneity and the complexity of the disease itself. Also, it remains unknown if the inflammation is a reaction to self-antigens[61] or if degenerative processes trigger an inflammatory response[62].

At follow-up, we indeed observed a correlation between cortical thickness and MTR values in PVL in patients with CDMS. In contrast, patients with CIS did once again not show such a correlation. This might indicate increasing microstructural damage with a longer disease duration. The median disease duration at the first scan was 10 months for both subgroups, 5 months for CIS and 50 months for CDMS. A cohort of patients with longer disease durations may show a stronger correlation. It also may be due to the steady decrease of overall brain volume in patients with CDMS[14].

The annualised change of cortical thickness during the observational period did not correlate with the MTR values in PVL and nPVL. This is not surprising, considering the lack of correlation between both lesional types with the cortical thickness in the first place. The correlations between the periventricular lesion load and the cortical thickness found by Jehna and colleagues[10] suggested a possible link of the PVLL% with the change of cortical thickness over time, but our results have not shown this association. Interestingly, there also was no difference in the annualised change of cortical thickness during the observation period between the disease subtypes. While this indicates that grey matter atrophy starts early in the disease course, a phenomenon described in several studies[7,14,39], it has to be noted that the mean time interval between the two scans was merely 38 months and the reduction of the cortical thickness does not occur over all brain regions equally, but first appears to be confined in few regions[63]. Also, the disease course of this cohort during the observation period and progression of disability was minute – the median EDSS of patients remained at a low level throughout the study (1.0 in CIS, 2.0 in CDMS, 1.5 overall).

Using the follow-up data, we also were able to assess the role of microstructural changes in periventricular and non-periventricular lesions in the conversion of patients from clinically isolated syndrome to clinical definite multiple sclerosis. We hypothesised MTR values for

PVL to be lower in converters than in non-converters, MTR in PVL to show a stronger correlation with the cortical thickness in converters and converter to have a higher grade of reduction of the cortical thickness. Not unexpectedly, the results did not allow us to predict which patient would eventually convert from CIS to CDMS. Other MTR studies also have shown a link between the conversion from CIS to CDMS over two years in NAWM, but not over five years[42]. Therefore, MTR assessed like in our study cannot serve as a viable predictor for the conversion of patients with CIS[42]. An established way for this prediction are the Barkhof/Tintoré-criteria[64,65] that consider focal tissue changes like T2- or contrast enhancing lesions:

a) presence of at least one Gadolinium-enhancing lesion or nine T2 hyperintense lesions
b) at least one juxtacortical lesion
c) at least one infratentorial lesion
d) at least three periventricular lesions[64].

Other findings regarding the reduction of brain volume and cortical thickness were as expected. The disease duration at baseline correlated with the cortical thickness in patients with CDMS, and age of onset as well as disease duration correlated with the reduction of cortical thickness over time in patients with CDMS. The correlation between disease duration and cortical thickness and its reduction in patients with CDMS was related to the reduction of the brain volume during the disease course[7,14], as described above.

The EDSS of patients remained at a low level throughout the study, and subsequently did not correlate with the PVLL% or the cortical thickness. A correlation between the EDSS at follow-up in patients with CDMS with the mean MTR in PVL has been observed, but the scatterplot shown in *Fig.20* suggests that a higher EDSS indicates a higher MTR, which is the opposite from one would expect. Other studies have shown correlations of white and grey matter brain lesions with the EDSS, but not with NAWM[66,67]. In both studies, the median EDSS of patients, 3.0[66] and 3.5[67] , was higher than the EDSS in this study, which for both subgroups combined was 1.5 and for CDMS 2.0. Also, the EDSS range in the study conducted by Lema and colleagues was 2.0 to 7.5[67], while the range of this study was 0 to 8.0. This suggests, that the MTR in PVL and nPVL possibly might have a correlation with the disability at a higher threshold than tested in our study, although PVL commonly do not harbour functional eloquence.

Furthermore, the patients' progression of disability, measured by the EDSS, showed an association with the reduction of cortical thickness for patients with CDMS, but not with CIS. This result is in line with other studies demonstrating that grey matter pathology correlates with future disability in patients with either disease type[48], suggesting volumetric changes of the cortex may even be a better predictor than cortical lesions[68], although these findings have to reproduced by subsequent investigations.

Whether the treatment with disease modifying therapeutics has an influence on the volumetric change of the cortical thickness, could not be tested in this study, since it was neither powered nor designed to detect therapeutic effects. A study by Kim and colleagues[69], that compared glatirameracetate treated to untreated patients concerning a possible difference in volumetric changes of the brain and cortical lesions, did not find such a difference[69].

Our study is not without limitations. We did compare two different phenotypes, CIS and CDMS, but did not include a healthy control group. This comparison could, however, have provided a better understanding of grey matter changes. Also, analyses for the so called normal appearing white matter are pending and could not be included in this study. There are also confounding factors like pseudoatrophy, which describes the swelling of the brain due to oedema around inflammatory lesions and the concurring reduction of the brain volume when the oedema disappears as a result of efficient therapy[70], and various degrees of tissue hydration[70] that cannot be accounted for. We also did not investigate differences of our cohort with patients with progressive disease courses. To understand the pathogenesis of progressive forms, further investigations are necessary.

Nevertheless, we were able to demonstrate that periventricular regions are more prone to microstructural pathological changes than non-periventricular regions in patients with MS, already starting at early stages of the disease, as these were seen in both CIS and CDMS.

5 Conclusion

We here demonstrated that periventricular lesions show a larger degree of microstructural changes compared to non-periventricular lesions, providing MRI evidence based on MTI that support the hypothesis that soluble CSF factors drive the pathogenesis in both white matter demyelination and grey matter pathologies. However, we did not find a correlation between the MTR values in periventricular lesion and the cortical thickness. Further, the MTR values within lesions did not offer a high predictive value for further disease progression or conversion from CIS to clinically definite MS, and the same held true for periventricular lesion load.

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