CLINICAL RELEVANCE OF BRAIN ATROPHY MEASURES IN MULTIPLE SCLEROSIS

Tomáš Uher

KAROLINUM

Clinical Relevance of Brain Atrophy Measures in Multiple Sclerosis

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Contents

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Abbreviations

95% CI	95% Confidence Interval
ASA	Avonex–Steroid–Azathioprine study
BICAMS	Brief International Cognitive Assessment for Multiple Sclerosis
BVMTR	Brief Visuospatial Memory Test Revised
CNS	Central Nervous System
CVLT2	California Verbal Learning Test, Second Edition
EDSS	Expanded Disability Status Scale
FA	Flip Angle
FIRST	FMRIB Integrated Registration and Segmentation Tool
FLAIR	Fluid-Attenuated Inversion Recovery
FOV	Field of View
GQ	Grant Quantitative study
HLA	Human Leucocyte Antigen
HR	Hazard Ratio
ICV	Intra-Cranial Volume
lin-R^2	coefficients of determination of individual linear model
MACFIMS	Minimal Assessment of Cognitive Function in Multiple Sclerosis
MHC	Major Histocompatibility Complex
MMSE	Mini Mental State Examination
MOG	Myelin Oligodendrocyte Glycoprotein
MRI	Magnetic Resonance Imaging
MS	Multiple Sclerosis
MSNQ	Multiple Sclerosis Neuropsychological Questionnaire
NEDA-4	No Evidence of Disease Activity-4
OCT	Optical Coherent Tomography
OR	Odds Ratio β

p (adjusted p)	p-value adjusted by Benjamini-Hochberg procedure
PBVC	Percent Brain Volume Change measured by SIENA method
QMRI	Quantitative Magnetic Resonance Imaging
quad-R^2	coefficients of determination of individual quadratic model
RNFL	Retinal Nerve Fiber Layer
PASAT	Paced Auditory Serial Addition Test
SET	Study of Early interferon beta-1a Teatment
SDMT	Symbol Digit Modalities Test
SDP	Sustained Disability Progression
SIENA	Structural Image Evaluation using Normalization of Atrophy
SIENAX	Structural Image Evaluation using Normalization of Atrophy cross-sectional
T1-WI/FFE 3D	T1-Weighted Images 3-Dimensional Fast Field Echo
TE	Time to Echo
THK	Slice Thickness
TI	Inversion Time
TR	Time to Repetition

Preface

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) presenting with a wide spectrum of clinical and radiological phenotypes.[1–4] Although MS was originally considered an inflammatory disease that predominantly affects the white matter,[5, 6] neurodegeneration resulting in accelerated brain and spinal cord atrophy is now recognized as an important determinant of disability.[1, 2, 4, 7–13] It is commonly understood that MS is a complex heterogeneous disease characterized by a broad spectrum of physical[14, 15] and cognitive[16, 17] symptoms, variable treatment response, radiological features, and neuropathology. This heterogeneous presentation of symptoms is likely attributable to complex interactions between external and hereditary factors,[18] resulting in limited predictability of the disease and its response to treatment. Therefore, there is an urgent need for personalized treatment.

Unfortunately, traditional clinical predictors are not sufficiently sensitive to reliably predict MS future and monitor ongoing disease activity. Contrastingly, abnormal magnetic resonance imaging (MRI) findings have been shown to be the most informative predictors and surrogate markers of disease activity.[19, 20] Not only the accumulation of the lesion burden but also the atrophy of the brain and spinal cord are important determinants of disease progression and associated with the development of physical[1, 4, 7–9, 21] and cognitive disabilities.[16, 17, 22, 23] Therefore, the assessment of the course of brain atrophy within individual patients could facilitate the identification of those with current disease activity and those at the highest risk of accumulating permanent disability.[14]

In this context, some efforts have been made to bring measurements of brain atrophy into clinical practice.[9, 24, 25] Unfortunately, the relatively high intra-individual variability of longitudinal brain atrophy measures renders the application of brain volumetric measures in individual patients with MS

challenging.[7–9, 25, 26] Therefore, brain atrophy measures are yet to be utilized routinely in clinical practice.

In this publication, we investigated how the high intra-individual variability of volumetric brain volume measures can be overcome and whether they have practical applications in clinical decision-making. We propose several approaches, including high-frequency MRI scanning, combined clinico-radiological composite scores, and the application of cross-sectional volumetric measures.

This publication is intended for neurologists, radiologists, and other specialists who treat patients with multiple sclerosis, as well as researchers in neuroimaging methods.

1. Introduction

1.1 Epidemiology

MS mainly affects young individuals and is a leading cause of disability in this age group,[27] with disease onset usually occurring between the ages of 20 and 40 years. Worldwide, there are approximately 2.8 million patients with MS.[28, 29] The prevalence of MS In the Czech Republic is approximately 170–200 per 100,000 (overall 20,000 patients) (ReMuS Registry data). Generally, the prevalence of MS increases with distance from the equator, that is, farther north in the Northern Hemisphere or farther south in the Southern Hemisphere. As such, MS is most prevalent in northern Europe, North America, Australia, and New Zealand.[30, 31] Women are diagnosed with MS at least two to three times as often as men.[32, 33] In the last decade, the incidence of MS has increased, especially in women; however, the reason for this remains unknown.[34]

1.2 Disease pathogenesis

MS is an immune-mediated disease of the central nervous system (CNS) caused by a neuroinflammatory autoimmune response to self-antigens in genetically susceptible individuals. MS is characterized by demyelination, inflammation, lesion formation, and neurodegeneration. Progressive neurodegeneration of the CNS is believed to be mostly a consequence of neuroinflammation rather than an independent process. Additionally, a peripheral immune response targeting the CNS is particularly important in the early phases of disease progression. Furthermore, the immune pathophysiological processes within the CNS are predominant during the late progressive phases of the disease.[35–38]

There is no specific antigen known to induce the oligoclonal expansion of B and T cells. Traditionally, MS is thought of as a T cell-driven disease in which inflammatory T cells cross a damaged blood-brain barrier, react with myelin, and induce inflammatory and neurodegenerative processes.[39, 40] However, an emerging body of evidence suggests B cells play an important role in the pathogenesis of MS.[41–43] Initially, a compromised blood-brain barrier allows for the invasion of monocytes and T cells into the brain or spinal cord parenchyma. Peripheral monocytes and activated microglia are primarily responsible for demyelination in MS lesions. Focal inflammatory demyelination occurs in both the white and gray matter. White matter lesions occur particularly in the peri- and periventricular regions. Contrastingly, lesions in the gray matter are often located along the subpial surface of the cortex and are topographically related to inflammation in the adjacent meninges. In this respect, it is likely that soluble factors from the cerebrospinal fluid trigger the development of white periventricular and subpial cortical lesions.[44, 45]

Active MS lesions contain broken down myelin, clonally expanded CD8+ T cells and, to a lesser degree, CD4+ T, gamma delta T, monocytes, rare B, plasma, and dendritic cells expressing major histocompatibility complex (MHC) class II molecules, as well as monocytes, large numbers of macrophages containing myelin debris, and immunoglobulin deposition. Additionally, there are pathological changes in oligodendrocytes (cytopathic changes, apoptosis, phagocytosis of apoptotic oligodendrocytes, swelling of cells with abnormal nuclei, complement deposition, and lysis) as well as eventual signs of astrocytic damage. Cortical lesions display fewer inflammatory infiltrates and microglial activation than white mater lesions.[38, 44, 46] Some axons are still preserved, even in cases of total myelin loss. Remyelination may occur in some lesions, but this is usually very limited.[47] In this context, a possible new subtype of MS, named myelocortical MS, is characterized by no brain white matter demyelination, but loss of myelin in the brain cortex and spinal cord.[48]

Several hypotheses of neurodegenerative processes have been proposed, including Wallerian degeneration secondary to demyelination, oligodendrocyte loss, axonal degeneration, damage from reactive oxygen species, nitric oxide, and energy failure from mitochondrial dysfunction.[35, 49, 50]

1.3 Risk factors

Genetic factors

MS is not the result of a single-gene defect. Several genes with incomplete penetrance are associated with an increased risk of MS. Currently, dozens of genetic variants (single-nucleotide polymorphisms – SNPs) have been identified, mostly related to adaptive immunity. Most of these susceptibility loci for MS are also shared with other autoimmune disorders. Additionally, these susceptibility genetic loci appear to have only a modest influence on the risk of developing MS.[51, 52] For example, human leukocyte antigen (HLA) class II alleles DRB1*1501, 0301, and 1303 (expressed on innate immune cells and important for antigen recognition by CD4+ and CD8+ T lymphocytes) are associated with a 3-fold increased risk of having MS.[53] Taken together, genetic predisposition accounts for only 15–25% of the lifetime risk of MS. In other words, twin studies have shown that a homozygous twin of an MS patient has, on average, a 15–25% risk of developing MS.[51, 54] Contrastingly, siblings of patients with MS have, on average, only a 3% risk of developing MS.[55] Families with a high number of patients with MS are rare. Even in these families, no single gene has been identified as being responsible for the development of MS.[56]

Environmental risk factors

Several environmental risk factors, especially those associated with the sustained activation of the immune system, have been proposed. It is hypothesized that environmental factors modulating peripheral adaptive immunity may play a key role in increasing the risk of developing MS.[36, 57] The most important risk factors include Epstein–Barr virus infection, decreased sunlight exposure, low vitamin D levels (since vitamin D modulates differentiation of T lymphocytes), and smoking.[32, 58–61] Other environmental risk factors such as obesity,[62, 63] psychological stress,[64–67] vaccinations,[68] unhealthy diet,[69–72] and gut microbiome abnormalities have also been suggested.[73–75]

1.4 Clinical presentation

Neurological symptoms

The clinical presentation of MS includes a wide range of neurological signs and symptoms originating from focal or diffuse brain or spinal cord

damage. With the exception of optic neuritis (not often observed in other diseases), the neurological presentation is not specific for MS. This makes the diagnosis of MS based solely on clinical criteria challenging to almost impossible. Neurological symptoms most often include:

- Muscle weakness (paresis) and spasticity, associated with lesions in the corticospinal or cortico-bulbar tracts of the brain or spinal cord.
- Neo- and paleocerebellar syndromes with ataxia, tremor, and abnormalities in stance and gait associated with the pathology of the cerebellum and its connections.
- Dysarthria, dysphagia, nystagmus, peripheral facial palsy, diplopia, internuclear ophthalmoplegia, and other cranial nerve involvements associated with lesions in the brainstem.
- Sensory symptoms such as paresthesia (numbness), hypoesthesia (loss of sensation), dysesthesia, and neuropathic pain, are associated with brain or spinal cord lesions in the spinothalamic or dorsal column pathways.
- Bowel and bladder dysfunctions, such as retention, incontinence, and urgency, usually associated with spinal cord lesions.
- Visual dysfunction, mostly caused by optic neuritis but can sometimes be associated with lesions in the temporal or occipital lobes.
- Walking difficulties, usually of multifactorial etiology.
- Fatigue and affective disorders.

The expanded disability status scale (EDSS) quantifies neurological signs and symptoms in MS and is used to monitor clinical disease activity.[76] EDSS is a widely accepted tool used in clinical practice and clinical trials. However, the EDSS has several limitations. First, there is a relatively high inter-rater variability in EDSS scores due to the detailed scoring system and the subjective nature of some parts of the neurological examination. Second, the relationship between the actual disability status and EDSS scores is not linear. For example, there is a smaller change in objective disability between EDSS 1.0 and 2.0 than between EDSS scores 5.0 and 6.0. The EDSS is mostly focused on walking ability, with scores above 4.5 especially depending solely on walking performance. However, the EDSS is not sufficiently sensitive to minor changes in walking performance and hand motor function. Finally, the cognitive and affective functions are not sufficiently assessed using this metric. For example, patients with severe cognitive dysfunction (dementia) and no walking problems would have the same EDSS score as a cognitively

preserved patients who are able to walk without assistance for at least 200 meters.[77–79]

Therefore, new measures have been developed and tested in both research and practice to improve the clinical monitoring of patients with MS. These include:

- The 9-hole peg test quantifies upper extremity function; the patient picks up nine pegs and puts them in nine holes as quickly as possible, the time to finish the task is recorded, and the total administration time is 10 minutes.[80]
- In the 25-foot walk test, the patient was instructed to walk 25 feet (8 m) as quickly as possible, the time to finish the task is recorded, and the total administration time is 1–5 min.[80]
- SLOAN visual acuity charts, evaluate visual impairment; the number of letters that are identified correctly is scored, and the administration time is 5 min.[81]
- Various cognitive tests (see below).

Although the use of extended monitoring with additional clinical measures has the potential to improve the sensitivity of clinical disease activity detection, it has some important drawbacks. First, additional clinical studies are required to define and quantify the value of additional testing. Second, extensive monitoring is time-consuming and associated with higher patient and financial burdens. Thus, further research is desperately required to address these questions.

The clinical course of MS has four basic disease patterns:[82]

- Radiologically isolated syndrome has findings on brain MRI typical of MS; however, there are no clinical signs or symptoms suggestive of MS. This stage is associated with a significantly increased risk of developing MS.[83]
- Clinically isolated syndrome is the first clinical episode suggestive of MS. According to the McDonald 2010[84] and 2017 criteria,[85] a proportion of patients with enhancing lesions on brain MRI or positive oligoclonal bands may already have been diagnosed with MS at this stage.
- Relapsing-remitting MS is the most common disease pattern (80%). It is characterized by clinical attacks (relapses) of new neurological symptoms. These attacks are followed by remissions associated with partial or full recovery.
- Secondary progressive MS follows relapsing-remitting MS. It occurs in the late disease stages and is characterized by severe neurological

disability, lack of relapses, disability progression unrelated to relapses, and poor response to immunomodulatory treatments.
- Primary progressive MS is characterized by disability progression from the onset of symptoms, but without clinical relapse or remission (except in relapsing-progressive MS). This type of MS also shows poor response to the majority of immunomodulatory treatments.

The MS classifications currently in use purely reflect the clinical course (disease activity and progression) and comprise only a limited number of clinical subtypes (radiologically isolated syndrome, clinically isolated syndrome, relapsing-remitting, primary, and secondary progressive). However, the narrow spectrum of clinical subtypes is not the only limitation. There is also great heterogeneity in the clinical course, treatment response, and objective biological markers within the established clinical subgroups. As a result, the translation of clinical classifications into clinical practice for treatment decision-making and prognosis estimation in individual patients is limited. In this context, reliable imaging and laboratory surrogate markers that can provide objective criteria for the identification of specific disease patterns are required.

Cognitive symptoms

Cognitive impairment is a common neuropsychiatric symptom in MS, with a prevalence rate between 10% and 70% depending on the disease duration. Cognitive dysfunction may already be detected in patients with clinically isolated syndrome in the early stages of MS.[86–88] Impairment of cognition is increasingly recognized as an important determinant of employment status and associated societal costs;[89, 90] adverse effects on social functioning, coping, quality of life; and treatment adherence among MS patients.[91, 92] The core domains of cognition – including verbal and visual memory, information processing speed, semantic fluency, sustained attention, and executive functions – are most often affected.[93–95] Neuropsychological deficits are related to brain structural MRI measures in patients with MS.[16] Numerous studies of early MS have shown an association between cognitive impairment and white matter lesions and[96] whole brain,[97] cortical,[98, 99] and subcortical deep gray matter atrophy including thalamic volume loss.[100–102]

Routine neurological examination does not detect cognitive impairment in the majority of patients with MS, resulting in cognitive impairment often being underdiagnosed, even though it could be an important symptom of MS progression.[23] The usefulness of brief cognitive screening batteries, such

as the Mini-Mental State Examination (MMSE)[103] or the Multiple Sclerosis Neuropsychological Questionnaire (MSNQ),[104] has been questioned because of their low sensitivity in the detection of MS-specific cognitive impairment. However, detailed psychometric assessment of cognitive impairment requires considerable time and resources. The implementation of screening batteries of intermediate length, such as the Minimal Assessment of Cognitive Function (MACFIMS),[93] may also be limited because of their time-consuming nature and the need for administration by experienced neuropsychologists. All of the above considerations emphasize the need for a short, validated, and accepted instrument that can capture cognitive impairment in patients with MS and can also be administered by staff without neuropsychological training. Hence, the Brief International Cognitive Assessment for MS (BICAMS)[105, 106] or Single Symbol Digit Modalities Test (SDMT)[107] has been suggested as suitable for use in routine clinical practice.

1.5 Paraclinical measures

Magnetic resonance imaging

Among the different paraclinical measures, brain magnetic resonance imaging (MRI) is one of the most accepted and sensitive tools used to monitor subclinical disease activity and diagnose MS.[19, 20] Moreover, MRI measures have become common radiological endpoints in clinical research.

Conventional MRI measures include the number and location of T1-hypointense, T2-hyperintense, and T1 contrast-enhancing lesions, whereas sophisticated software can also assess the T1 and T2 of contrast-enhancing lesion volumes. Lesions are typically distributed in the spinal, infratentorial, peri-ventricular, and juxta-cortical locations.[85] However, the majority of cortical lesions are not seen on standard MRI scanners.[108] Because of this, MS was originally considered to be a disease that predominantly affects the white matter.[5, 6] Currently, pathological changes in the gray matter are increasingly recognized as an early[10, 109–119] and an important determinant of disease activity in MS patients.[109, 120–122] Although brain lesions in MS represent a histo-pathologically heterogeneous and dynamic group of focal brain pathology, ranging from edema and inflammation to demyelination and axonal loss, their neuro-inflammatory origin is well accepted.[46]

Not only accumulation of lesions but also global and regional brain atrophy are important aspects of disease progression associated with

physical[1, 4, 7–9, 13, 21] and cognitive disability.[16, 17, 22, 23] In MS, loss of brain volume is driven by several mechanisms including tissue loss (i.e., loss of myelin, glial cells, neurons, and axons due to inflammatory demyelination and neurodegeneration), as well as changes in non-tissue components (i.e., fluid shift due to inflammation, hydration, endocrine influences, or environmental factors).[7–9, 24, 123, 124] Currently, there are a number of manual, semi-automated, and automated techniques[7–9] used for the assessment of global and regional brain volumes such as Structural Image Evaluation using Normalization of Atrophy Cross-sectional (SIENAX),[125] FreeSurfer,[126, 127] NeuroQuant, MS metrix,[128] or model-based segmentation/registration tool – FMRIB Integrated Registration and Segmentation Tool (FIRST).[128, 129] Longitudinal methods are also available, such as Structural Image Evaluation using Normalisation of Atrophy (SIENA), which are employed to directly measure relative volume changes over time.[125, 130, 131] Unfortunately, high intra-individual variability of longitudinal MRI measures due to a number of biological and technical biases does not allow for the confident evaluation of brain atrophy in clinical practice.

The major limitation of traditional lesion and volumetric MRI measures lies in the fact that focal MRI lesions and regional or global brain volume changes are only partially reflective of the disseminated pathology in MS.[17, 132, 133] For example, specific topography rather than lesion or brain volume may play a role in the pathogenesis of disability in MS. Hence, more advanced MRI techniques, such as magnetization transfer ratio, diffusion tensor imaging, proton MRI spectroscopy, and functional MRI measuring various aspects of MS pathology[17] are likely to further improve our understanding of the associations between MRI and disability progression at different MS stages. Unfortunately, there is also a remarkably high intra-individual variability associated with these advanced MRI methods, which is a major limitation for their application in clinical practice.

Finally, the spinal cord is heavily affected in patients with MS and contributes substantially to the disease progression. In MS, the spinal cord is usually characterized by focal and diffuse lesions as well as global atrophy.[12, 134] However, spinal cord MRI is performed in clinical practice and studies much less frequently than brain MRI. This is mostly due to technical challenges such as an inhomogeneous magnetic field in this region, the small physical dimensions of the spinal cord, and motion artifacts within the spinal canal, together with the flow of cerebrospinal fluid and periodic motion due to respiratory and cardiac cycles.[135] Moreover, spinal cord MRI

is usually not sensitive to changes in spinal cord pathology over short-term follow-ups, and there are no established cut-offs for spinal cord atrophy. Hence, quantitative assessment of the spinal cord is usually performed only for research purposes and is not monitored regularly in most patients with MS.

Biochemical

Cytological (plasmatic cells, lymphocytic pleocytosis) and biochemical (normal protein, normal albumin quotient, increased IgG index, and IgG quotient) studies of cerebrospinal fluid play an important role in the differential diagnosis of MS. Particularly, the identification of cerebrospinal fluid-restricted oligoclonal bands typical for MS and is widely used for diagnosis confirmation.[136, 137] Furthermore, anti-aquaporin-4[138] and anti-myelin oligodendrocyte glycoprotein (MOG)[139] antibodies are helpful in distinguishing between MS and neuromyelitis optica and MOG antibody disease. Serum neurofilament light chain level is an exceedingly promising predictor and marker of disease activity. Several studies are currently underway to investigate its potential use in clinical practice.[140–145]

Optical coherent tomography

Optical coherent tomography (OCT) measures the thickness of the retinal nerve fiber layer (RNFL), which contains only non-myelinated axons. The RNFL thickness is associated with disability, relapse, and brain atrophy. Importantly, OCT is helpful in distinguishing between MS and neuromyelitis optica.[146] However, further studies are needed to determine whether OCT is suitable for monitoring or predicting disease progression.[147]

1.6 Diagnosis

There is no single diagnostic test for MS. Diagnostic processes include medical history, neurological examination, and paraclinical tests, such as MRI, cerebrospinal fluid analysis, and eventual evoked potentials or OCT.

For diagnosis of MS, to the following criteria must be met:[85]

- Neurological symptoms arising from involvement of brain, optic nerve, or spinal cord.
- Confirmed dissemination of disease in space (new relapse implicating different CNS site, $\geq$ 1 lesions in $\geq$ 2 MS-typical regions of the CNS

including: spinal cord, infratentorial, periventricular, and juxtacortical/cortical region).
- Confirmed disease dissemination over time (new relapse, new lesion, simultaneously contrast-enhancing lesion together with non-enhancing lesion on brain MRI; oligoclonal bands in the cerebrospinal fluid can be used instead of dissemination in time).
- All different diagnoses have been ruled out.

1.7 Management

Currently, there is no causative cure for MS. Current therapies focus mainly on the prevention or reduction of neuroinflammation. A wide range of immune therapies with specific mechanisms of action and immune targets have been approved for MS. New immunomodulatory (disease-modifying) treatments are currently the most effective drugs for MS. A major issue is that most therapies are effective only during early disease stages, with minimal effects in late progressive phases.

Most therapies for MS significantly alter the survival and trafficking of immune cells. For example, the pharmacological effects of fingolimod result in sequestration of lymphocytes in lymph nodes. Natalizumab, a monoclonal antibody, which binds to the α4 integrin sub-unit present in antigen-4 on leukocytes, inhibits the adhesion interactions of leukocytes with the vascular cell adhesion molecule present on the activated vascular endothelium of the blood-brain barrier. Rituximab and ocrelizumab, both monoclonal antibodies that target the CD20 antigen (a membrane-embedded surface molecule) present in most B cells (except terminally differentiated plasma B cells), cause B cell death. Alemtuzumab targets the CD52 antigen and depletes the T, B, and NK cell populations. Monocytes, NK, and B cells repopulate the immune system more rapidly than T cells after this treatment.[148] Dimethyl fumarate treatment causes lymphopenia that reduces CD3 T cell counts with preferential depletion of CD8 cells.[149] Interferon beta, which has anti-proliferative activities, also causes short- and long-term changes in diverse cell populations, particularly activated NK cells.[150, 151] Glatiramer acetate has many immunological effects, including its ability to alter T cell differentiation, leading to a shift from a Th1 (pro-inflammatory) to a Th2 (anti-inflammatory) immune profile, which may dampen inflammation within CNS.[152] Teriflunomide selectively and reversibly inhibits

dihydro-orotate dehydrogenase, a key mitochondrial enzyme in the de novo pyrimidine synthesis pathway, leading to a reduction in proliferation of activated T and B lymphocytes without causing cell death.[153]

Additionally, cytostatic therapies – such as cyclophosphamide, mitoxantrone, or azathioprine – are rarely used as off-label immunosuppressive treatments, usually for patients not indicated for disease-modifying treatment. High-dose steroids and plasma exchange are typically used to manage relapses. Furthermore, a wide range of symptomatic drugs including analgetics, spasmolytics, anti-spastics, antidepressants, or anxiolytics are used for relief of various symptoms associated with CNS dysfunction. Finally, psychotherapy, aerobic and anaerobic exercises, rehabilitation, physiotherapy, and ergotherapy are important elements of the successful and comprehensive care of MS patients.[154, 155]

1.8 Prediction of disease activity

MS is characterized by a broad spectrum of phenotypes. Therefore, treatment efficacy may be improved by identifying MS subpopulations at a high risk of disability progression or lack of treatment response. Given that irreversible acute axonal damage is most extensive in the early disease stages,[156] it is extremely important to identify individuals who do not respond to treatment as early as possible, even if the mechanisms by which this occurs are not completely understood yet.[2] Therefore, an important, yet currently unmet, need for modern MS treatment is to determine applicable predictors of subsequent disease activity.

In clinical practice, traditional clinical markers such as early disability progression or high relapse activity are used to predict disease activity. However, clinical predictors are neither sensitive nor specific enough for use as reliable surrogate markers of disease activity over time. Contrastingly, recent studies have shown that the characteristics of MRI pathology are very informative for future disease activity in short-,[11, 157–159] mid-,[158, 160, 161] and long-term studies.[158, 162–165] Particularly, the most important predictors of disability progression in relapsing-remitting MS have been suggested to be the occurrence of new T2 lesions,[158–162, 166] accumulation of T2 lesion volume,[167, 168] T1 lesion volume,[169, 170] whole brain,[168, 171] and central atrophy[168, 169] as well as gray matter[172] and thalamic volume changes.[173, 174]

In addition to MRI, serum neurofilament light chain levels show relatively good predictive value for concurrent and future disease activity. Importantly, neurofilament levels are easy to measure in the serum and act as predictors in statistical models independent of MRI markers.[144] Therefore, serum neurofilament light chain levels are a promising new predictor of disease course. Interestingly, measures of blood-brain barrier function[175] before interferon treatment initiation and early serum lipid profile changes during interferon therapy,[176] seem to predict clinical and radiological disease activity over long-term follow-up.

However, further research is required to confirm any added value of these new laboratory markers in clinical practice.

1.9 Disease monitoring

It is well accepted that clinical monitoring (EDSS, relapses) of new disease activity is insufficient for reliable assessment of disease progression. However, more detailed clinical monitoring using quantitative assessments of vision, hand function, walking ability, or cognitive performance is not standardized in clinical practice and is time-consuming.

It is well known that most new active lesions on brain MRI are clinically asymptomatic but are clinically relevant from a long-term perspective. Therefore, among different paraclinical measures, brain MRI is one of the most accepted and sensitive tools suitable for monitoring MS progression.[19, 20] Specifically, the occurrence of new, enlarging, or contrast-enhancing lesions on MRI is a widely used surrogate marker of radiological disease activity.[20] Additionally, global and regional brain atrophy are also an important part of disease progression that is associated with the development of physical[1, 2, 4, 21] and cognitive[16, 17, 86, 177] disabilities. Recently, efforts have been made to incorporate brain atrophy measurements into clinical practice for decision-making in patients.[9, 24] Unfortunately, the high variability of longitudinal MRI measures in individual patients over time, resulting from a wide range of biological and technical biases, does not allow for a confident evaluation of brain atrophy in clinical practice.

2. Main aims of the current work

The high intra-individual variability of cerebral atrophy measures makes their applicability in individual patients with MS questionable.[7–9, 25] Therefore, current brain atrophy measures are not prepared for application in clinical practice. In this study, we investigated possible methods to overcome this high variability, thereby enabling atrophy measures to become important decision-making tools in clinical practice. The main aims of this study were:

1. To investigate the agreement between MRI volumetric measures obtained using various software techniques for the assessment of lesions and brain volumes, and their changes over time.
2. To describe the dynamics of brain volume loss at different stages of MS, the association between brain volume loss and clinical measures, and investigate the effects of treatment escalation on the rate of brain volume loss.
3. To establish cutoff values for global and regional brain volume loss that can discriminate between healthy people and patients with MS.
4. To quantify the prevalence and factors associated with brain volume increase.
5. To investigate the occurrence of linear and non-linear trajectories of brain volume loss in patients with MS during follow-up.
6. To describe the proportion of patients with dissociation between brain atrophy and lesion burden using a new definition.
7. To quantify the degree to which the precision of brain volume loss assessment can be improved with high-frequency brain MRI monitoring over a short-term follow-up.
8. To investigate the predictive role of early changes in MRI outcomes with respect to the relapse and progression of disability in patients with early MS.

9. To assess the accuracy of a wide range of early MRI markers in predicting disease activity in a homogeneous sample of relapsing-remitting MS patients using interferons. Furthermore, we investigated whether a combination of volumetric MRI markers with clinical predictors could facilitate the identification of patients with poor long-term disability outcomes.
10. To develop an MRI-based algorithm that allows the identification of patients in need of neuropsychological evaluation and those at the highest risk of cognitive decline.
11. To investigate whether the strength of the association between MRI metrics and cognitive outcomes differed among the various MS subpopulations.
12. To describe the effects of pregnancy on the lesion activity and brain volume in women with MS.

3. Methods

3.1 Brain MRI

MRI acquisition
All MRI scans were performed on the same scanner (1.5-Tesla Gyroscan; Philips Medical Systems, Best, Netherlands) using the same protocol. The MRI protocol included fluid-attenuated inversion recovery (FLAIR) and T1-weighted 3-dimensional fast field echo (T1-WI/FFE 3D) sequences (Figure 1). Volumetric assessments were performed at the Department of Radiodiagnostics, First Faculty of Medicine, and General University Hospital in Prague or at the Buffalo Neuroimaging Analysis Center, NY, USA.

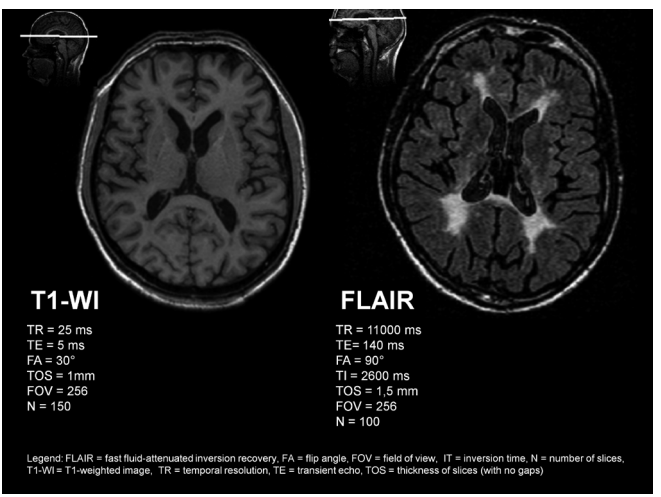

Figure 1: MRI protocol

MRI analysis in BNAC

Image analysis was performed at the Buffalo Neuroimaging Analysis Center. The volume of T2 lesions was measured using semi-automated edge-detection contouring-thresholding technique in Jim software (http://www.xinapse.com).[178, 179] T2 lesion analysis was performed using the aid of a "subtraction image." Whole brain, gray matter, white matter, and lateral ventricle volume were calculated using SIENAX, which normalizes measurements for head-size. Lesion filling was performed before segmentation using an in-house developed method. The SIENA technique was applied to assess longitudinal whole-brain volume changes.[125]

MRI analysis in Prague

Image analysis was performed at the General University Hospital in Prague with ScanView software. ScanView is a semi-automated software developed by Jan Krasensky.[180] This software was used for measurement of the volume of T1 and T2 lesions, the parenchymal fraction, the whole brain, and the corpus callosum volume using a segmentation-based approach.[1, 11, 22, 157, 175, 181] T2 lesion volume was measured from FLAIR and the volume of the whole brain from the T1-WI/FFE 3D. Normalized whole brain volumes needed to be normalized regarding the total intracranial volume (ICV). The normalized partial volume of the corpus callosum was measured using ScanView software and estimated in seven (4th slice being in the central position) sagittal reconstructions of T1-WI/3D/GE slices.[11, 157, 175, 181] More details of ScanView software were provided elsewhere.[12]

3.2 Clinical assessment

Neuropsychological assessment

The patients were evaluated with the Czech-validated version of the BICAMS.[105, 182] Cognitive processing speed was assessed with the Symbol Digit Modalities Test (SDMT)[107] and the oral response form was recorded using the Paced Auditory Serial Addition Test-3 seconds (PASAT-3). Memory was tested using the Brief Visuospatial Memory Test Revised (BVMTR)[183] in the visual modality and the California Verbal Learning Test Second Edition (CVLT2)[184] in the auditory sphere.

Impairment for a single test was defined at a level of 1.5 standard deviations (z-score < 1.5 compared to a healthy population), using the

regression-based norms of 134 healthy controls adjusted for age, sex, and education.[105] Patients with at least one pathological BICAMS subtest were considered cognitively impaired.[105, 106] Confirmed cognitive decline was defined as a new pathological outcome of BICAMS at 12 months and confirmed at 24 months. The Beck Depression Inventory was used to assess depressive symptoms.[185]

Neurological assessment

Disability status was assessed using EDSS.[76] Sustained disability progression 1 (SDP1) was defined as an increase in EDSS by 1.0 point (baseline EDSS > 0) or 1.5 point (baseline EDSS = 0), which was confirmed at 6- or 12-month follow-up. Sustained disability progression 2 (SDP2) was defined as an increase in EDSS of 2.0 points (baseline EDSS > 0) or 3.0 points (baseline EDSS = 0) confirmed after 12 months. A small proportion of patients was evaluated using the 9-hole peg test, 25-foot walk test, and visual acuity SLOAN chart.

4. Sample

4.1 SET study

The early interferon beta-1a treatment (SET) in high-risk patients after clinically isolated syndrome (CIS) study was an investigator-initiated, multicenter, prospective observational study (EudraCT identification number 2005-001281-13). The study included 220 patients who were consulted after their first demyelinating event suggestive of MS to any of eight participating centers within the Czech Republic (149 patients from General University Hospital, Prague; the rest of the patients from the KZ Hospital, Teplice; University Hospitals in Brno, Pilsen, and Olomouc; St. Anne's University Hospital, Brno; University Hospital in Motol, Prague, and Královské Vinohrady University Hospital, Prague) between 2005 and 2009, who were 18–55 years of age, enrolled within 4 months from the clinical event, had an EDSS score of 3.5, displayed the presence of two or more T2-hyperintense lesions on diagnostic MRI, and had the presence of two or more oligoclonal bands in cerebrospinal fluid obtained at the screening visit prior to steroid treatment.[2, 109, 157] The exclusion criteria for this study were lack of clinical and MRI follow-up data after baseline or pregnancy. The study included clinical visits every 3 months for a total of 48 months and subsequent long-term follow-ups in routine clinical practice. Disability was assessed at baseline and every 6 months thereafter, whereas sustained disability progression (SDP) was determined at 24 weeks after the 48 months examination. MRI was performed at baseline, 6 months, and annually thereafter (Figure 2). All patients started treatment at baseline with 30 mg of intramuscular interferon beta-1a once a week, which has been shown to delay the conversion to clinically definite MS.[18] All patients were treated with 3–5 g of methylprednisolone for the first symptom before study enrollment, and a baseline MR examination was performed at least 30 days

after steroid administration. Relapses were treated with 3–5 g methylprednisolone during the study. Treatment changes were made in accordance with the SET study protocol: patients showing inadequate treatment response (i.e., 2 moderate relapses or 6 months sustained progression of one EDSS step during 12 months of treatment) or lack of tolerance (unacceptable flu-like symptoms despite symptomatic treatment or a 3-fold increase in liver enzyme concentrations). The study protocol was approved by the local ethics committees of all participating centers and informed consent was obtained from all patients. This study was supported by the Czech Ministry of Education and Health [NT13237-4/2012, MSM 0021620849, PRVOUK-P26/LF1/4, RVO-VFN64165/2012] and Biogen Idec (http://www.biogenidec.com/). The funders had no role in the study design, data collection and analysis, decision to publish, or manuscript preparation.

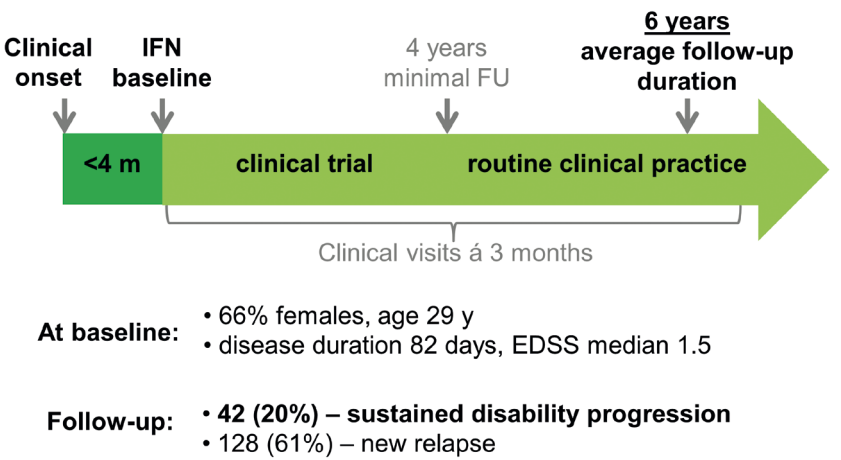

Figure 2: SET trial design

4.2 Avonex-Steroid-Azathioprine (ASA) study

The ASA study was an investigator-initiated, 2-year, double-blind, placebo-controlled trial investigating the clinical and imaging measures of relapsing-remitting MS patients treated with intramuscular interferon beta-1a treatment alone or in combination with low-dose azathioprine or

low-dose azathioprine and prednisone.[186, 187] The original study included 181 patients from two participating MS centers within the Czech Republic enrolled between 1999 and 2003.[10, 186, 188] The inclusion criteria also included two oligoclonal bands in the cerebrospinal fluid (CSF), ≤ EDSS 3.5, and two relapses in the last 12 months or 3 relapses in the last 24 months. The long-term extension of this study was an open-label study with routine follow-up. The extension involved clinical visits every 3 months and an MRI every 12 months. The baseline of the study was the time of intramuscular interferon beta-1a initiation (Figure 3). The study protocol was approved by the ethics committee and all patients provided written informed consent. Funding: This study was supported by the Czech Ministry of Education and Health (MSM 0021620849) and Biogen Idec.

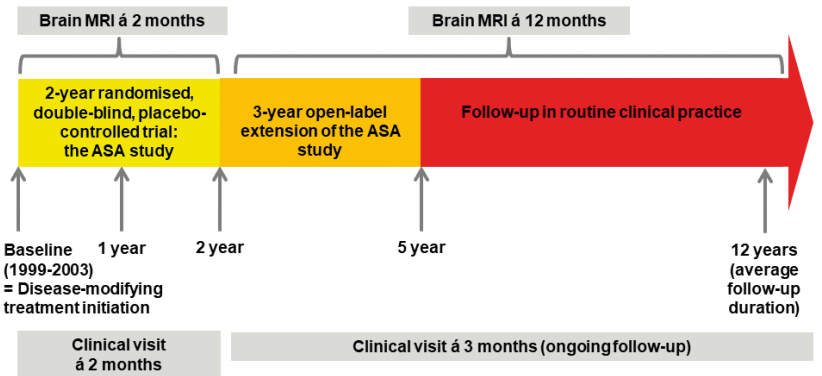

Figure 3: ASA study design

4.3 Grant Quantitative (GQ) study

The GQ study was an investigator-initiated, single-center, 3-year prospective observational study that included 1,226 patients with MS. This study investigated the clinical value of a series of clinical and paraclinical measures for evaluating MS progression in routine practice. The inclusion criteria were diagnosis of MS, native Czech speaker, and being an adult (>18 years of age). Patients with brain diseases other than MS or psychiatric disorders were excluded. Enrollment in the GQ study began in June 2012 and ended in October

2015. Clinical follow-up included visits every 3 or 6 months for patients with stable disease.[22, 189] The study protocol was approved by the medical ethics committee, and patients provided written informed consent. This study was supported by the Czech Ministry of Education and Health (grant numbers NT13237-4/2012, PRVOUK-P26/ LF1/4 and RVO-VFN64165).

4.4 The quantitative magnetic resonance imaging (QMRI) program

All patients with MS who underwent brain MRI performed at the Department of Radiodiagnostics of the General University Hospital in Prague starting in March 2000 were included in the QMRI program. This was

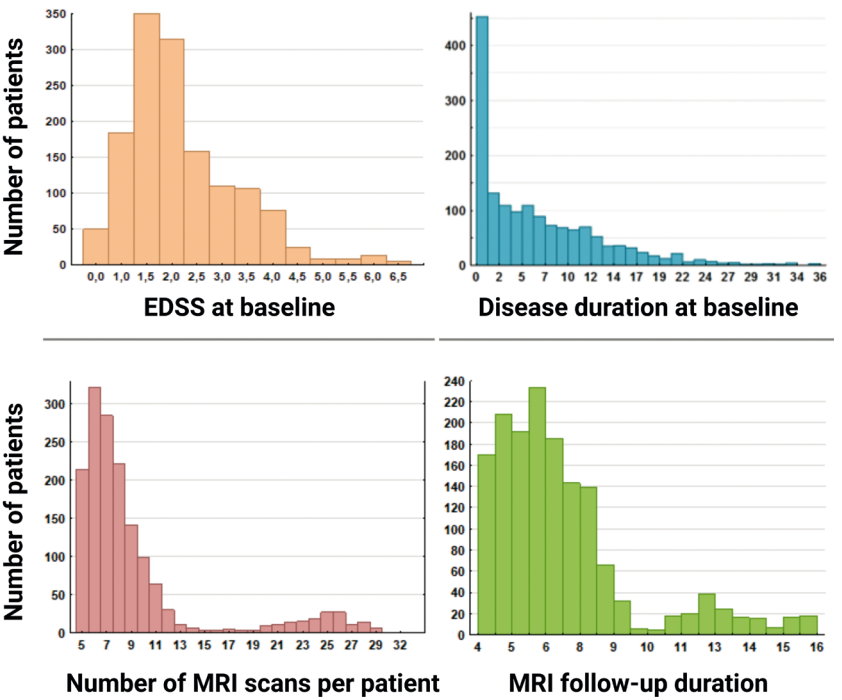

Figure 4: Sample characteristics

a real-world cohort (compared to clinical studies: no regular MRI time points, changes in medication, and different treatment strategies). Of the 3,430 MS patients (with 20,053 brain MRI scans) enrolled in the QMRI program; 1,757 MS patients had ≥ 3 MRI scans and ≥ 4 years of MRI follow-up duration (Table 1–3; Figure 4). MRI scans of all patients were performed on the same 1.5-Tesla scanner (Gyroscan, Phillips) using the same protocol (T1-WI, 1 mm; FLAIR, 1.5 mm).[190]

Table 1: Baseline and follow-up characteristics of patients

BASELINE CHARACTERISTICS	N=1,564 (≥4 years, ≥5 scans)
Number of females	1,122 (71.7%)
Age at first MRI scan	34.2±9.0
Disease duration (years) at first MRI scan	6.4 (median 4.6)
Natalizumab or fingolimod during follow-up	420 (26.9%)
EDSS at baseline	Median 2.0 (range 0-6.5)
T2 lesion volume at baseline (ml)	4.1±7.3 (median 1.3)
BPF at baseline (%)	85.7±2.2
FOLLOW-UP CHARACTERISTICS	
Annualized EDSS change	0.07±0.15
Annualized relapse rate	0.4±0.4
MRI follow-up duration	7.0 (median 6.3)
Number of MRI scans per patient	9.3 (median 7.0)
Annualized T2 volume absolute change (ml)	0.26±0.66
Annualized whole brain % volume change	–0.28±0.25
Annualized gray matter % volume change	–0.56±0.53
Annualized corpus callosum % volume change	–0.74±1.00

Table 2: Overview of missing MRI volumetric measures

MISSING VALUES	
Brain parenchymal fraction	0.9%
Brain volume	0.9%
T2 lesion volume	2.7%

MISSING VALUES	
T1 lesion volume	12.6%
Thalamic volume	24.7%
Corpus callosum volume	28.7%
Gray and white matter volume	29.9%
Lateral ventricle volume	35.8%
Brain parenchymal fraction	0.9%
Brain volume	0.9%
T2 lesion volume	2.7%
T1 lesion volume	12.6%

Table 3: Number of patients with longitudinal MRI scans

NUMBER OF PATIENTS					
Number of MRI scans per patient	**MRI follow-up duration (years)**				
	≥4	≥5	≥6	≥8	≥10
≥4	1,688	1,304	924	356	161
≥5	1,564	1,245	904	355	161
≥6	1,354	1,146	860	348	158
≥8	745	701	621	320	155
≥10	383	371	351	267	151

4.5 Healthy controls

The enrollment of healthy people began in 2001 and was completed in 2014. The exclusion criteria were as follows: a history of neurological disorders affecting brain atrophy, abnormal brain MRI findings, and use of chronic anti-inflammatory or immunomodulatory medication. The original healthy control database included 133 participants (410 MRI scans). Overall, 58 participants underwent ≥ 2 years of MRI follow-up and ≥ 3 MRI scans during the follow-up period.

5. Summary of selected studies

5.1 A novel semiautomated pipeline to measure brain atrophy

Background: Several techniques were used[7–9] for the assessment of brain volume. Direct comparison of different methods is limited by methodological issues, such as the lack of a gold standard for image acquisition, short follow-up, and small sample size.[191–194]

Objective: We investigated the agreement between volumetric MRI measurements from two software packages, including the in-house developed ScanView, and commonly used techniques for the evaluation of T2 lesions and whole brain volume and their changes. Furthermore, we evaluated the intra-individual variability in brain volume loss progression between the two methods.

Methods: The study included patients with MS from the SET[2, 11, 157, 175, 195] and ASA [1, 4, 10, 186, 195, 196] studies. Together, 3340 MRI scans were included from 209 patients after the first demyelinating event suggestive of MS, 181 patients with relapsing-remitting MS, and 43 controls. Although all MRI scans were performed using the same scanner and protocol, the volumetric evaluations were performed independently at two different neuroimaging centers using different software packages. Volumetric analysis using the ScanView software was performed in Prague. Commonly used techniques, such as SIENA, SIENAX, and Jim software, have been used in Buffalo (Figure 5). Individual variability in the longitudinal MRI data was estimated using the mean squared error.

Results and conclusions: The absolute volumes of the brain and lesions from both volumetric methods were significantly different but were strongly correlated. This observation underscores the fact that absolute and relative volumetric data obtained using different software cannot be adopted by other clinicians or researchers when different volumetric methods or MRI scanners are used.[197, 198]

The variability of the relative whole-brain volume loss as assessed using SIENA[125] was expectedly lower than that of ScanView.[22, 157] The higher variability of ScanView may be explained by the segmentation-based character of the method. More specifically, we found that the mean deviation of the volume change in the whole brain was approximately 0.30%. Considering that this residual error (± 0.30%) was similar to the cutoff value for pathological whole brain volume loss (± 0.40%), reliable identification of patients with accelerated brain volume loss in practice is very challenging.

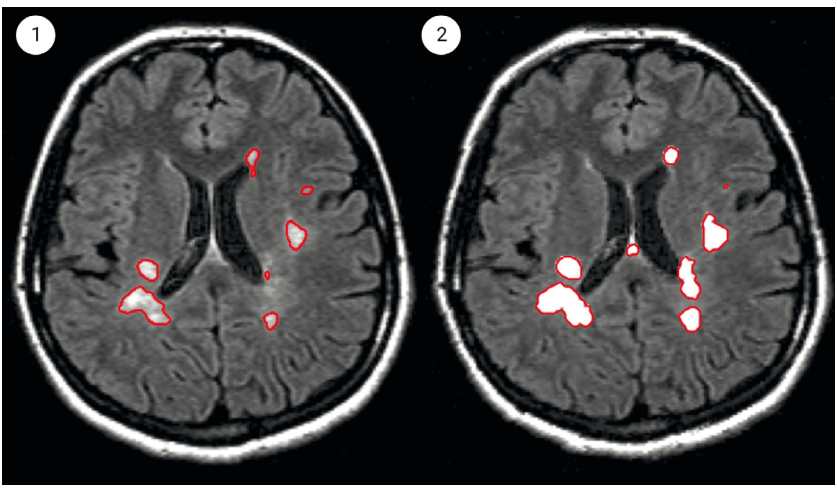

Figure 5: Example of lesion segmentation provided by Jim (1) and ScanView (2) software

Details are provided in the publication: Uher T, Krasensky J, Vaneckova M et al. A novel semiautomated pipeline to measure brain atrophy and lesion burden in multiple sclerosis: A long-term comparative study. *Journal of Neuroimaging* 2017; 27(6):620-629.[180]

5.2 Evolution of brain volume loss rates in early stages of multiple sclerosis

Background: A better understanding of brain volume loss trajectories throughout the course of MS is required to improve the assessment of brain atrophy in clinical practice and research.

Objective: To describe the dynamics of brain volume loss at different stages of relapsing-remitting MS, the association between brain volume loss and clinical measures, and investigate the effects of treatment escalation on the rate of brain volume loss.

Methods: We included 1903 patients predominantly with relapsing-remitting MS from the ASA (N = 166), SET (N = 180), and QMRI (N = 1557) cohorts with ≥ 2 MRI scans and ≥ 12 months of follow-up. Brain MRI scans (N = 7203) were performed using a single 1.5 Tesla scanner. The relationships between age or disease duration and global and tissue-specific brain volume loss rates were analyzed using mixed models.

Results and conclusions: Although the rate of brain volume loss declined with longer disease duration, the effect of disease duration on brain volume loss was small. Greater brain volume loss was observed in patients with recent relapses and greater disability, although the strength of these associations was small. We found a stronger association between the loss of brain volume and the accumulation of T2 lesions. Importantly, brain volume loss was decreased by the escalation of immunomodulatory therapy.

Establishing a threshold for pathological loss of brain volume in the context of normal aging is a key step toward the clinical interpretation of brain volume loss in MS. In this context, it is important to emphasize that the drivers of brain volume loss differ over time. Aging and comorbidities contribute more to overall brain atrophy in older people than in young patients with MS.[199, 200] Therefore, in young patients with MS, higher rates of brain atrophy are more likely to indicate high disease activity that requires therapeutic intervention. Together, there is no clinically relevant relationship between the rate of brain volume loss and age or disease duration (Figure 6). Accelerated loss of brain volume is weakly associated with a concurrent increase in the level of disease activity and eventually leads to more profound neurological disability. Evidence of a higher rate of brain volume loss should prompt the consideration of a change in disease-modifying treatment to prevent neurological disability.

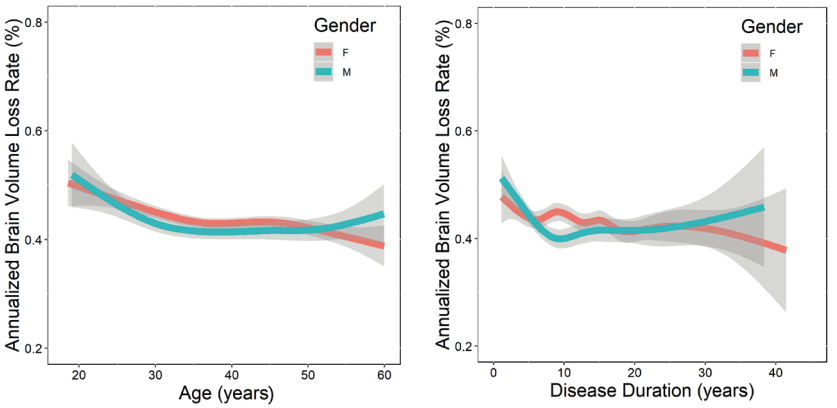

Figure 6: Relationship between whole-brain % volume loss and age (left) or disease duration (right)

Details are provided in the publication: Uher T, Krasensky J, Malpas C, et al. Evolution of brain volume loss rates in early stages of multiple sclerosis. *Neurology, Neuroimmunology, & Neuroinflammation* 2021; 8(3):e979.[13]

5.3 Pathological cut-offs of brain volume loss

Background: Thresholds for regional and global pathological loss of brain volume have not yet been defined. Monitoring brain atrophy rates can improve the identification of patients with progressive diseases.

Objective: We aimed to define cut-off values of brain volume loss capable of discriminating between controls and MS patients by establishing cut-off values for the whole brain[7–9, 124] gray matter, thalamus,[109, 201] and corpus callosum volume loss rates.[157, 180, 202–204]

Methods: This study included the following cohorts:386 patients after the first clinical event suggestive of MS from the SET study[2, 11, 157, 205] or the QMRI program; 964 relapsing-remitting MS patients from the ASA study,[1, 186, 195, 196, 205, 206] or the QMRI program; 63 secondary progressive MS patients from the QMRI program; and 58 age-matched controls. In total, 11,438 MRI scans were evaluated.

Results and conclusions: Cut-off values for brain volume loss rates were identified as possible discriminators between controls and patients with MS. We found similar brain, gray matter, thalamic, and corpus callosum volume loss pathological cut-offs. The corpus callosum volume loss cut-offs showed a slightly higher sensitivity to discriminate between controls and patients with MS, as compared with other regional or global brain structures. Owing to the relatively low accuracy of the identified cutoffs, any increase in their sensitivity led to a decrease in their specificity. Therefore, highly specific cutoffs may reliably identify pathological brain atrophy in only a proportion of individuals with the highest rates of brain volume loss (Figure 7). We hypothesize that the accuracy of the identified cutoff values might be even lower in clinical practice due to greater intra-individual fluctuations of brain volume measures

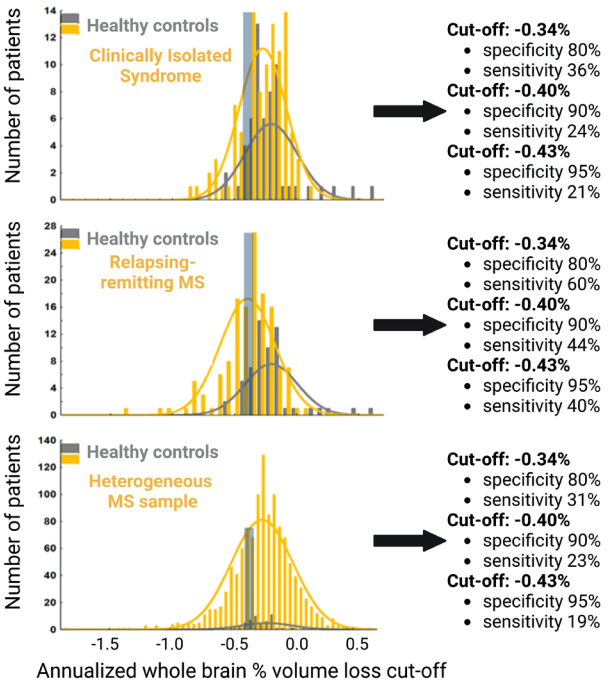

Figure 7: Distribution of annualized relative changes of whole brain volume loss with the cut-offs discriminating controls from MS patients

in real-world practice as a result of various biases.[7, 8, 124, 180] We suggest that defined cutoffs are not yet prepared for use in clinical practice.

Details are provided in the publication: Uher T, Vaneckova M, Krasensky J, et al. Pathological cut-offs of global and regional brain volume loss in multiple sclerosis. *Multiple Sclerosis Journal* 2019; 25(4):541-553.[180]

5.4 Interpretation of brain volume increase in multiple sclerosis

Background: MS is typically associated with accelerated brain atrophy,[8, 9, 25] but brain volume increase (BVI) can also occur,.[207] This can complicate the interpretation of changes in brain volume. However, the clinical relevance of the BVI in patients with MS has not been investigated.

Objective: To quantify the prevalence and factors associated with BVI in MS patients.

Methods: We examined 366 patients with MS (2,317 scans) and 44 controls (132 scans). MRI was performed on the same 1.5-Tesla scanner using an identical scanning protocol. Volumetric analysis of brain volume changes was performed using SIENA and ScanView software. BVI was defined as a percentage of brain volume change > 0%. We compared the clinical and MRI outcomes between patients with and without BVI.

Results and conclusions: We found a high prevalence (15.9%) of MRI scans with BVI (Figure 8). This is in contrast to the well-known accelerated brain volume loss in MS.[8, 9, 25] We do not have direct evidence, but considering the measurement error of volumetric analysis[180, 208] and average rate of brain volume loss in MS (from –0.4 to –1.0%),[8, 9, 24, 209] the majority of cases of BVI seemed to be a result of measurement errors. This assumption was also supported by the observation that consecutive BVI were not associated with disease stabilization. The frequency of BVI identified by ScanView and SIENA software was between 31.7% and 44.5%, indicating that even small differences in methods[180] have a relatively strong effect on the classification of MRI scans. Nevertheless, we hypothesize that in some cases, BVI is associated with a real increase in brain volume, especially when considering the effect of biological factors such as fluid shift due to inflammation,[210] hydration status,[124] daytime,[205] endocrine influences[211] or environmental and cardiovascular factors.[212, 213] The neuroprotective effects

of highly effective treatments leading to BVI might be another relevant option warranting further investigation.[207] Taken together, clinicians should be aware of the frequent occurrence of BVI, interpret it with great caution, and use precise and accurate MRI volumetric techniques.

Details are provided in the publication: Uher T, Bergsland N, Krasensky J, et al. Interpretation of brain volume increase in multiple sclerosis. *Journal of Neuroimaging* 2021; 31(2):401-407.[26]

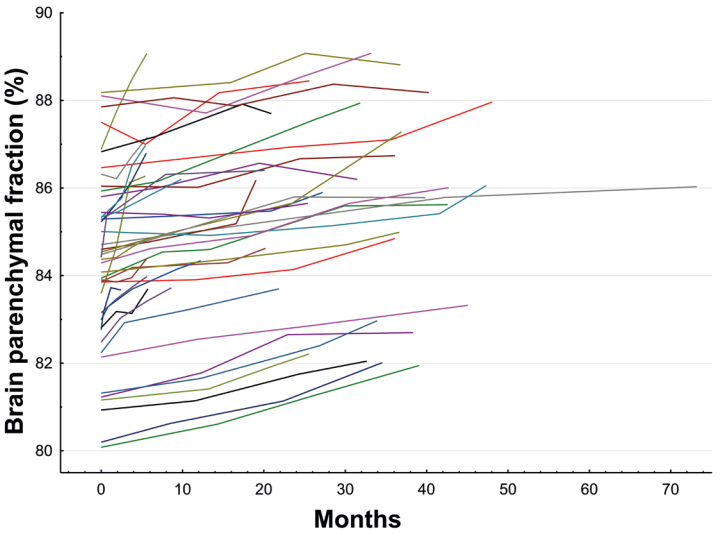

Figure 8: Examples of increasing normalized brain volume trajectories.

5.5 Occurrence of non-linear brain volume loss in patients with MS

Background: In recent MS studies with repeated MRI measurements, linear regression slopes have been used to estimate individual rates of brain volume loss. Although a linear course of brain volume loss has been assumed, the potential occurrence of non-linear brain volume loss trajectories has not been investigated.

Objective: To investigate the frequency of the non-linear course of brain volume loss in MS.

Methods: We included 1,546 MS patients from the QMRI program with ≥ 5 MRI scans (mean = 9.3, median 7.0 scans) and ≥ 4 years (mean = 7.0, median 6.3 years) follow-up. Most patients were treated with disease-modifying agents. Brain volume loss was measured using the SCANVIEW software. We calculated the coefficients of determination for the individual linear regression models (lin-R^2) and quadratic regression models. A Non-linear trajectory was assumed if the quadratic model fit the trajectory of brain volume loss better than the linear model (quad-R^2 > 5% or > 10% higher than lin-R^2; $P < 0.01$). The characteristics of patients with linear and non-linear brain volume loss were compared using the Mann-Whitney U test and adjusted logistic regression.

Results and conclusions: A total of 98 (6.3%) patients showed non-linear brain volume loss (quad-R^2 > 5% higher than lin-R^2) (Figure 9). The prevalence of non-linear brain volume loss decreased to 63 (4.0%) when a stricter definition (> 10% higher) was applied. Non-linear brain volume loss showed deceleration in 44 (2.8 %) (Figure 10) and acceleration in 19 (1.2 %) patients (Figure 11). Occurrence of non-linear brain volume loss was 27.3% (> 5% higher) or 11.3% (> 10% higher) in patients with ≥10 years follow-up. The incidence of non-linear brain volume loss was 29.3% (> 5% higher) and 12.6% (> 10% higher) in patients with ≥ 15 MRI scans, respectively (Figure 12). Patients with non-linear deceleration of brain volume loss (> 5% higher) had a higher brain parenchymal fraction at baseline ($p = 0.003$), a higher rate of brain volume loss ($p < 0.0001$), increased volume of T2 lesions ($p < 0.001$), greater progression of disability ($p = 0.001$), younger age ($p = 0.002$), and shorter disease duration ($p = 0.017$) than patients with linear brain volume loss. Patients with non-linear brain volume loss acceleration (> 5%) were similar to those with linear brain volume loss (Figure 13). In summary, most patients with MS had a linear trajectory of brain volume loss over a short-term follow-up period. However, a considerable proportion of non-linear brain volume loss trajectories was found in patients with a longer follow-up and a higher number of MRI scans. Therefore, the assumption of linearity in brain volume loss needs to be verified, particularly in long-term MRI studies. Factors associated with non-linear brain volume loss need to be investigated.

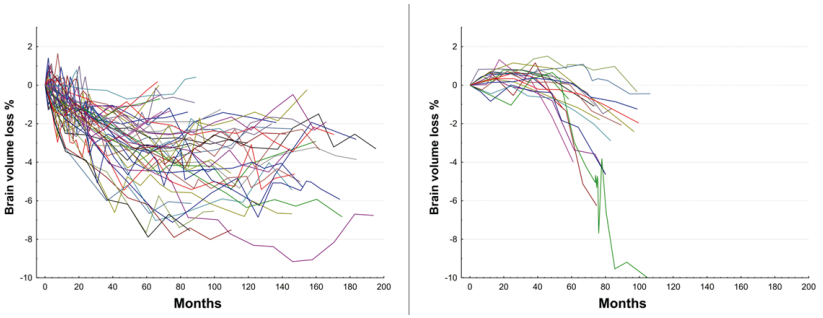

Figure 9: Brain volume loss trajectories with deceleration and acceleration over follow-up

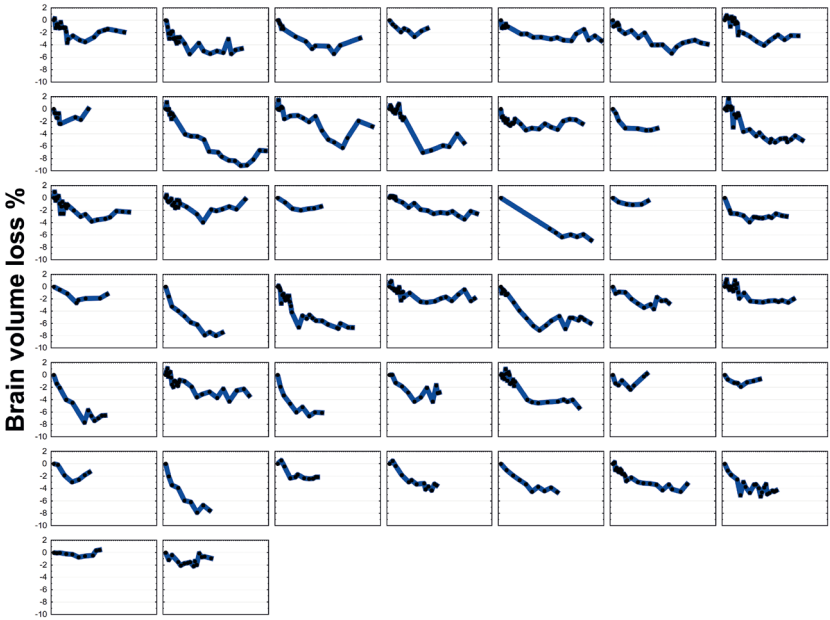

Figure 10: Individual brain volume loss trajectories with deceleration over follow-up

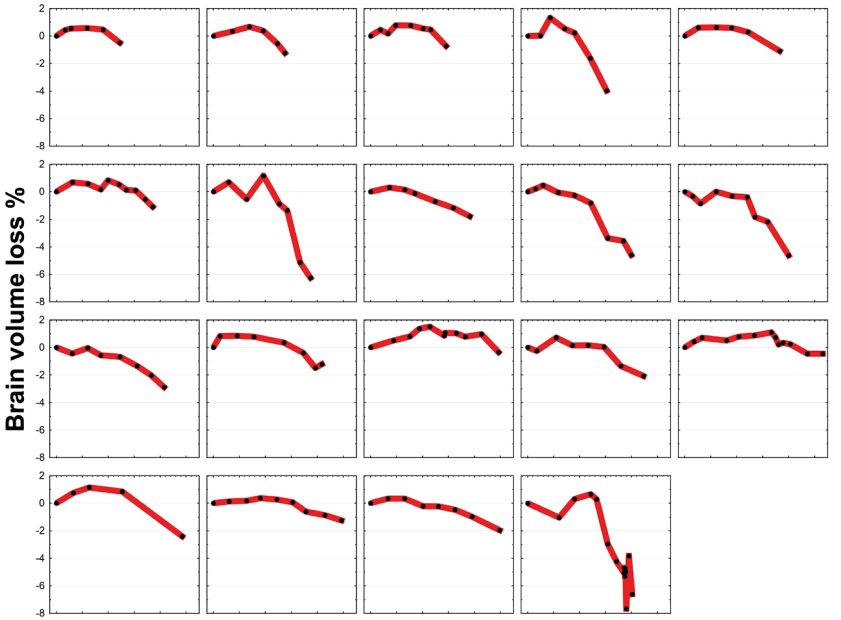

Figure 11: Individual brain volume loss trajectories with acceleration over follow-up

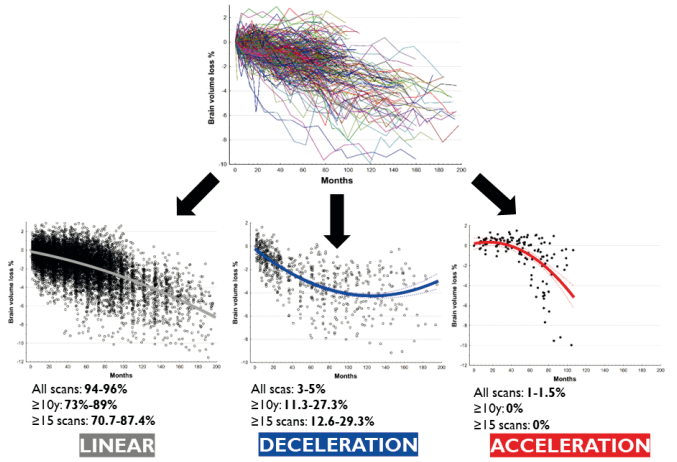

Figure 12: Linear and non-linear trajectories of brain volume loss

Characteristics	MRI trajectory subtype		
	Linear	Deceleration	Acceleration
Number of participants	1448	76	22
Females %	71.5%	77.6%	77.3%
Age at baseline (years)	34.3±9.0	31.0±8.0	36.8±10.4
Disease duration at baseline (days)	6.5±6.7	4.4±4.6	8.2±8.4
EDSS at baseline †	2.0 (1.5-2.8)	2.0 (1.5-2.5)	2.0 (1.5-3.9)
Annual. EDSS change †	0.05 (0-0.15)	0.10 (0.03-0.21)	0.10 (0-0.22)
Relapse rate	0.38±0.37	0.47±0.34	0.29±0.27
Treatment escalation %	26.3%	31.6%	31.8%
T2 lesion volume at baseline (cm^3) †	1.3 (0.4-4.3)	1.7 (0.5-4.4)	2.8 (0.7-10.8)
Annual. T2 lesion volume change (cm^3) †	0.06 (0-0.3)	0.19 (0.06-0.5)	0.13 (0.04-0.5)
Brain parenchymal fraction (%)	85.7±2.2	86.5±1.9	84.9±2.8
Annual. brain atrophy (%)	-0.27±0.25	-0.41±0.28	-0.42±0.35

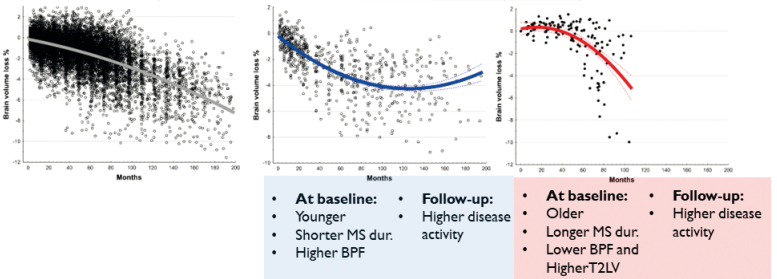

Figure 13: Characteristics of patients with deceleration and acceleration trajectories of brain volume loss

The details are provided in the abstract presented at the 34th Congress of the European Committee for Treatment and Research on Multiple Sclerosis (ECTRIMS) in Berlin, Germany: Uher T, Krasensky J, Sobisek L, et al. Occurrence of non-linear brain volume loss trajectories in multiple sclerosis patients. *Multiple Sclerosis Journal*; 24(2_suppl): ECTRIMS 2018; poster number 91.

5.6 MRI phenotypes according to dissociation between the brain atrophy and lesion burden using a new definition

Background: Few attempts have been made to systematically investigate specific MRI phenotypes in patients with MS.

Objective: To describe the proportion of patients with dissociation between brain atrophy and lesion burden using a new definition.

Methods: We included 3,083 patients with relapsing-remitting and secondary progressive MS. All brain MRI scans (N = 17,009) were performed using a single 1.5-Tesla machine. For each MRI scan, we calculated

the dissociation score (d) between the brain parenchymal fraction and the volume of the T2 lesion, which was defined as the distance (in percentiles) between the measurement and the regression line (between the brain parenchymal fraction and T2 lesion volume) estimated from all brain MRI scans and adjusted for its variability. Dissociation was defined as $d > 90^{th}$ or $d < 10^{th}$ percentile, identifying 20% of the MRI scans with the lowest association between the brain parenchymal fraction and T2 lesion volume.

Results and conclusions: At baseline, 20.1% (620 of 3,082) of patients had a dissociation between the brain parenchymal fraction and the volume of the T2 lesion. This proportion decreased from 5 to 6 years to 16.2% (176 of 1,089) and increased again after 15 years (21.6%; 135 of 625). Although the proportion of dissociated scans at the time of disease onset and after 15 years of follow-up was similar, there was a difference in the prevalence of the different types of dissociation. At the onset of the disease, the highest proportion of patients had a proportionally higher brain parenchymal fraction than the volume of concurrent T2 lesions (178 vs. 44 of 1,020). Conversely, at advanced disease stages, the dissociation phenotype was more prevalent, with a disproportionally low brain parenchymal fraction in relation to concurrent T2 lesion volume (105 vs. 30 of 625). Dissociation status was not stable over time (5–10 years) in a large proportion of patients (13.2–16%). In addition to the expected brain and lesion volumes, only disease duration was associated with a change in the dissociation score. Three dissociation score trajectories were identified. The "high brain volume dissociation" type (n = 1,305; 42.3%) had a high score at baseline and major decrease of the score over time, the "no dissociation" (n = 890; 28.9%) type had a moderately high score at baseline and mild decrease of dissociation score over time, and the "low brain volume dissociation" type (n = 888; 28.8%) had a low dissociation score at baseline and no decrease of the score over follow-up. Patients with a "high brain volume dissociation" trajectory had younger age, shorter disease duration, lower disability, lower T2 lesion volume, and higher normalized brain volume at baseline (all $p < 0.001$). The dissociation score at baseline was not associated with the probability of 12-months confirmed EDSS worsening over the long-term follow-up (HR = 0.95; 95% confidence interval [CI]:0.04; –1.26; p = 0.207). In summary, most patients had a moderately strong association between brain atrophy and lesion burden. Dissociation between brain atrophy and lesion burden occurs in 15–22% of patients and is not specific only for different patients but also for different stages of the disease.

The details are provided in the abstract presented at the 37th (virtual) Congress of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS) in Vienna, Austria: Uher T, Krasensky J, Capek V, et al. Dissociation between brain atrophy and lesion burden in patients with multiple sclerosis: A new definition. *Multiple Sclerosis Journal*; 27(2_suppl): ECTRIMS 2021; poster number 510.

5.7 The role of high-frequency MRI monitoring in the detection of brain atrophy

Background: Measuring brain volume loss in practice for decision-making in MS is challenging because of the high variability in brain volume loss measures.[7–9, 25]

Objective: We investigated whether high-frequency brain MRI monitoring over a short-term follow-up period can increase the precision of brain volume loss measures.

Methods: We included 157 patients with relapsing-remitting MS with 1,585 MRI scans performed every 2 months using a single 1.5-Tesla scanner. Each patient underwent 7 consecutive MRI scans over 12 months. Linear regression analysis was applied to estimate the rate of annualized brain volume loss. Brain volume loss greater than 0.4% was considered pathological. We then compared the number of patients with pathological brain volume loss obtained by the inclusion of different numbers of MRI scans during follow-up.

Results and conclusions: MRI monitoring every 6 months led to only a minimal improvement (≤ 2.6% accuracy change) in estimating brain volume loss compared to MRI monitoring every 12 months. Hence, 6-monthly MRI monitoring is probably not an add-on value in clinical practice. Although bi-monthly MRI monitoring was associated with 10.5–22.2% accuracy change in the detection of abnormal brain atrophy rates, high-frequency monitoring would be associated with high patient and financial burdens. The assessment of a higher number of annual MR scans over a longer period is currently an option to improve the precision of brain volume loss estimates.

Details are provided in the publication: Uher T, Krasensky J, Sobisek L, et al. The role of high-frequency MRI monitoring in the detection

of brain atrophy in multiple sclerosis. *Journal of Neuroimaging* 2018; 28(3):328-337.[214]

5.8 Early MRI and clinical predictors of disability progression over 6 years in patients after first clinical event suggestive of multiple sclerosis

Background: Active MRI lesions and clinical disease activity are surrogate markers of disease progression in MS. It is not yet clear whether assessment of the rate of brain volume loss could help further improve the prediction of future disease activity.

Objective: We investigated the predictive role of baseline, 6-month, and 12-month clinical and imaging measures with respect to SDP in patients with MS.

Methods: This prospective study included 210 patients with CIS who received weekly intramuscular interferon beta-1a. Adjusted Cox proportional hazard models were used to identify predictors of SDP (Figure 14).

Results and conclusions: After 6.0 years (range from 4.0 to 8.1), 42 (20%) patients had disability progression and 128 (61%) patients had a new relapsing activity. The best predictors of future disability progression were WB and corpus callosum volume loss during the first six months after treatment initiation, greater T1 lesion volume at 12 months, and worsening of EDSS during the first 12 months of treatment (Figure 15). The combination of single predictors into a composite prediction score further improved our ability to predict the disease course in individual patients (Figure 16). Therefore, brain volume measurements may help improve clinical decision-making.

The details are provided in the abstract presented at the 31th (virtual) Congress of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS) in Barcelona, Spain: Uher T, Vaneckova M, Sobisek L, et al. Early MRI and clinical predictors of disability progression over 6 years in patients after first clinical event suggestive of multiple sclerosis. *Multiple Sclerosis Journal*; 21(11_suppl): ECTRIMS 2015, poster number 32.

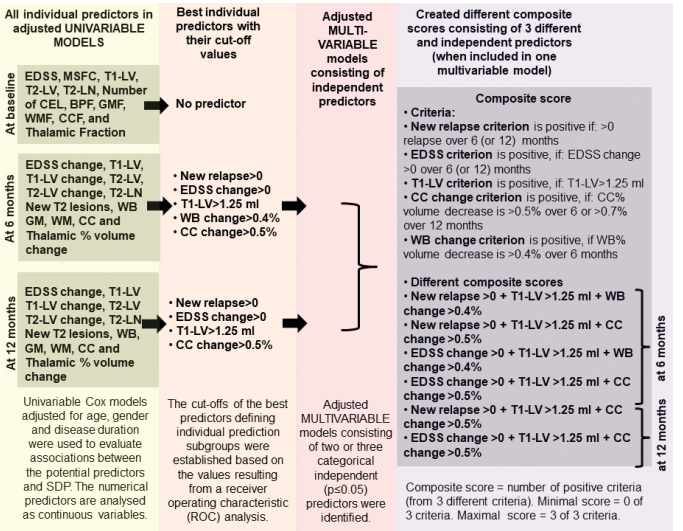

Figure 14: Statistical design of the study

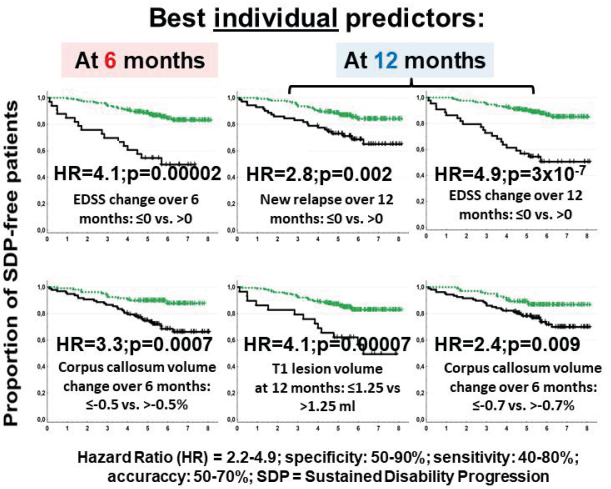

Figure 15: Kaplan–Meier curves of SDP by the best dichotomized clinical and MRI predictors

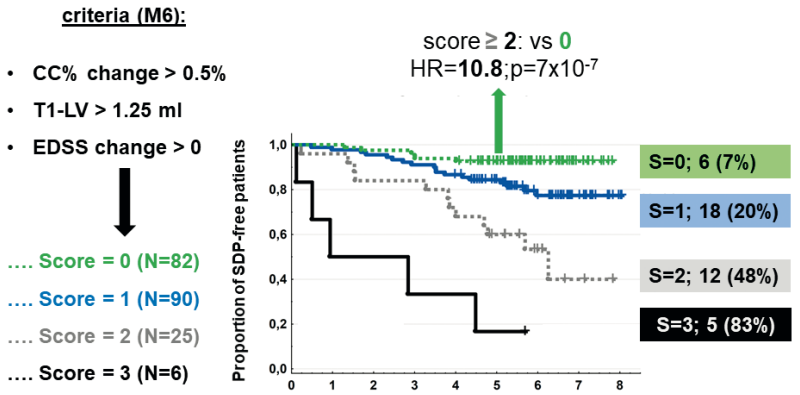

Figure 16: Kaplan–Meier curves of SDP based on individual composite prediction scores

5.9 Early MRI predictors of clinical progression after 48 months in CIS patients treated with intramuscular interferon beta-1a

Background: An important yet unmet need for modern MS treatment is to determine MRI predictors of subsequent activity in the early stages of MS. MRI pathology predicts new relapsing activity.[2, 109, 157, 165, 215–217] Considering the occurrence of irreversible CNS damage in the early phases of the disease,[156] it is important to identify patients with the highest risk of disease progression and lack of treatment response.[2]

Objective: We investigated the ability of baseline and 6-month MRI outcomes to predict relapse activity and development of confirmed disability progression in patients treated with weekly intramuscular interferon beta-1a after MS onset over 48 months.

Methods: A prospective observational SET study was conducted in 210 patients after their first clinical attack. Adjusted Cox proportional hazards models were used for statistical analyses. The investigated MRI predictors included the number of active T2 lesions, number and volume of gadolinium-enhancing lesions, volume changes of the cortical and deep gray matter, thalamus, hippocampus, and lateral ventricle volume.

Results and conclusions: Several MRI predictors of disease activity were identified. Approximately half of the patients who had relapsing

activity during follow-up had already experienced a new relapse activity during the first 6 months of the study. This observation supports previous findings of the early occurrence of new relapse activity with a subsequent decline in its incidence.[18, 218–221] Presence of gadolinium-enhancing lesions, high T2 lesion burden, accelerated corpus callosum volume loss, and accelerated lateral ventricle volume enlargement over the 6 months after treatment initiation with interferons helped to identify patients with the highest risk for disease activity. In summary, our results support the findings of previous studies.[2, 217, 218, 221] Hence, further controlled or comparative studies are needed to confirm the clinical relevance and reliability of MRI surrogate markers of treatment failure, and to investigate the effectiveness of new immunomodulatory drugs in high-risk patients in the early stages of MS.

Details are provided in publication: Uher T, Horakova D, Kalincik T, et al. Early magnetic resonance imaging predictors of clinical progression after 48 months in clinically isolated syndrome patients treated with intramuscular interferon β-1a. *European Journal of Neurology* 2015; 22(7):1113-23.[11]

5.10 Combining clinical and MRI markers enhances prediction of 12-year disability

Background: Abnormal MRI measures are the best predictors of disease activity in short-,[11, 157–159] mid-,[158, 160, 161] and long-term follow-up studies.[158, 162–165] Based on previous research, the most discussed predictors of future disease activity in relapsing-remitting MS were the following imaging markers: active T2 lesions,[158–162, 166] increase in the volume of the T2 lesion volume,[167, 168] and the volume of the T1 lesion volume,[169, 170] whole brain[168, 171] and central atrophy,[168, 169] gray matter,[172] and changes in thalamic volume changes.[173, 174] Importantly, many of the previous studies investigated only a limited number of MRI measures,[166, 168, 169, 173] investigated patient in heterogeneous anti-inflammatory treatments or in different disease stages.[168, 169, 173]

Objective: We evaluated the predictive accuracy of a wide range of MRI markers in patients with relapsing-remitting MS treated with intramuscular interferon beta-1a over long-term follow-up. We hypothesized that the combination of different MRI and clinical markers,[162, 222] may improve the long-term identification of patients with SDP.

Methods: We included 177 patients from the observational ASA study who were treated with intramuscular interferon beta-1a alone or in combination with oral steroids or azatioprine.[186] Adjusted Cox proportional hazards models were used for statistical analyses.

Results and conclusions: The best predictors of SDP included: T2 lesion number and volume; T1 lesion volume; corpus callosum and thalamus volumes at 12 months; EDSS score and EDSS change; number of new or newly enlarging T2 lesions; and relative change in corpus callosum volume (Figure 17 and 18). Importantly, the accuracy of single MRI predictors is relatively low, ranging from 52% to 68%. However, the combination of single MRI findings and clinical predictors in the composite score increased the predictive accuracy at the individual patient level. For example, the

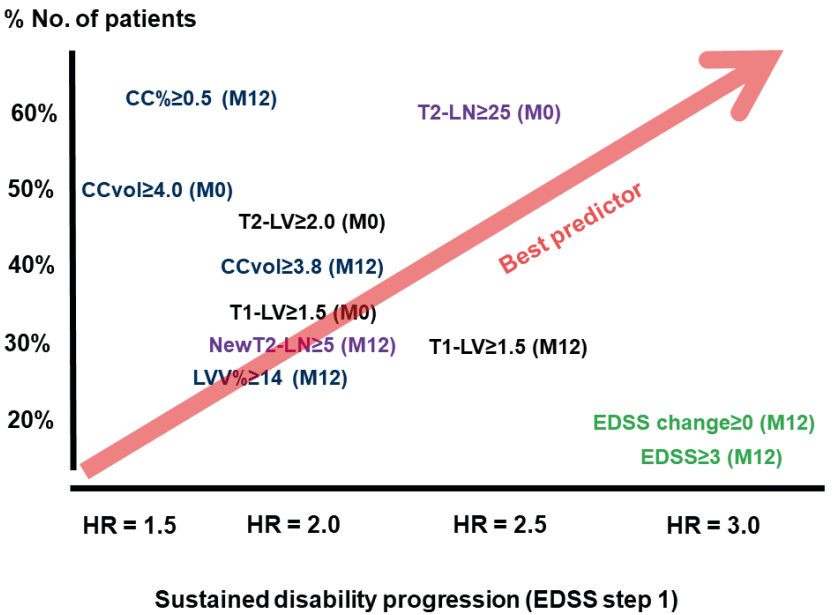

Figure 17: Proportions of patients fulfilling individual risk criteria for prediction of sustained expanded disability status scale (EDSS) progression by 1 step (CC = corpus callosum; HR = hazard ratio; LVV = lateral ventricle volume; T1 or T2-LN = T1 or T2 lesion number; T1 or T2-LV = T1 or T2 lesion volume)

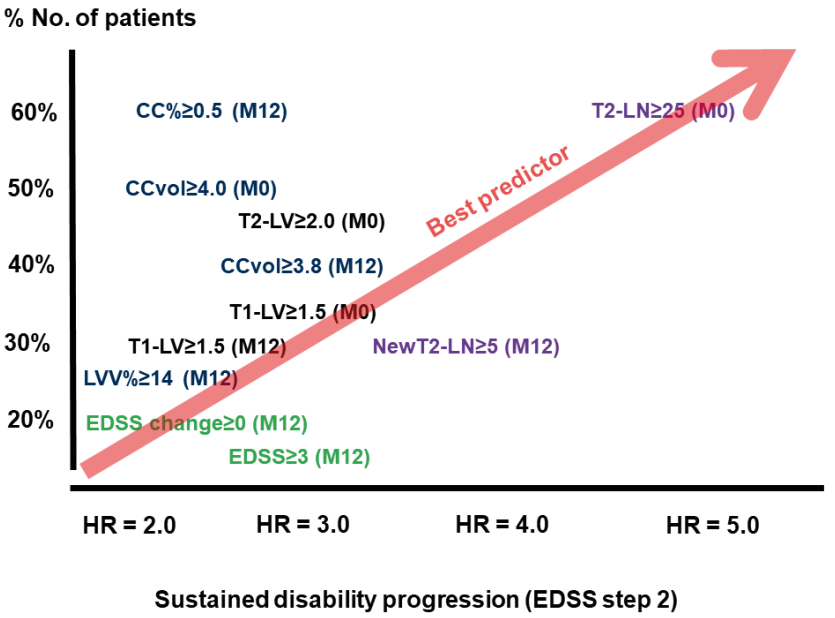

Figure 18: Proportions of patients fulfilling individual risk criteria for prediction of sustained expanded disability status scale (EDSS) progression by 2 steps (CC = corpus callosum; HR = hazard ratio; LVV = lateral ventricle volume; T1 or T2-LN = T1 or T2 lesion number; T1 or T2-LV = T1 or T2 lesion volume)

progression of the risk of disability in 12 years was five-fold higher in patients with three positive predictors than in patients without any positive predictors. Therefore, a combination of clinical and conventional imaging predictors with regional volumetric markers (such as corpus callosum and thalamic volumes) may improve the identification of patients at the highest risk of disability progression who may benefit from the early initiation of effective immunomodulatory treatment.

Details are provided in the publication: Uher T, Vaneckova M, Sobisek L, et al. Combining clinical and magnetic resonance imaging markers enhances prediction of 12-year disability in multiple sclerosis. *Multiple Sclerosis Journal* 2017; 23(1):51-61.[1]

5.11 Identification of MS patients at highest risk of cognitive impairment using integrated brain MRI assessment approach

Background: Cognitive impairment is an important determinant of employment status and associated societal costs,[89, 90] and adversely affects social functioning, coping, quality of life, and treatment adherence among MS patients.[91, 92] Although abbreviated neuropsychological batteries such as BICAMS,[106] have been suggested for use in routine practice, they are still not accessible to most patients with MS. Although there is a correlation between brain MRI measures and worse cognitive functioning,[16, 17, 223] single MRI markers reflect only a small part of the neuropathology, resulting in cognitive impairment in MS patients with.[17] Hence, it remains to be investigated whether the integration of MRI measures reflecting inflammatory and neurodegenerative processes may improve our identification of patients with either cognitive dysfunction or at the highest risk of cognitive decline in the future.

Objective: We examined whether assessing the burden of lesions together with atrophy of the whole brain on MRI improves our ability to identify MS patients with cognitively impairment.

Methods: Of 1,253 patients enrolled in the study, 1,052 patients with all cognitive and volumetric MRI and clinical data available were included in the analysis. Brain MRI and neuropsychological assessments were performed using BICAMS. MRI volumetric measures, such as T1 and T2 lesion volumes and normalized whole brain volume measured by brain parenchymal fraction, were examined in this study because of their relatively good availability in practice.[3, 224–227] Multivariable logistic regression and individual prediction analysis were used to investigate the associations between MRI markers and cognitive impairment. The results of the primary analysis were validated at two subsequent time points (12 and 24 months).

Results and conclusions: Lesion and normalized brain volumes were independently correlated with cognitive function in patients with MS. High T2 lesion volume (> 3.5 ml) resulted in a three-fold greater prevalence of cognitive impairment in patients with high brain volume but a six-fold greater prevalence of cognitive impairment in patients with low normalized brain volume (brain parenchymal fraction < 0.85) (Figure 19). Considering the negative and positive predictive values of the combined MRI markers, we

suggest that the MRI algorithm may improve identification, particularly in patients with a low probability of cognitive impairment. MRI markers were also associated with a higher risk of cognitive decline in the following year. The risk of confirmed cognitive decline at follow-up was greater in patients with a high volume of T2 lesions (odds ratio [OR] = 2.1; 95% CI 1.1–3.8) and a low brain parenchymal fraction (OR = 2.6; 95% CI 1.4–4.7). In summary, a combination of the assessment of lesion burden and normalized brain volume improves the identification of patients with MS and cognitive impairment and can help clinicians select patients suitable for the assessment of cognitive functions.

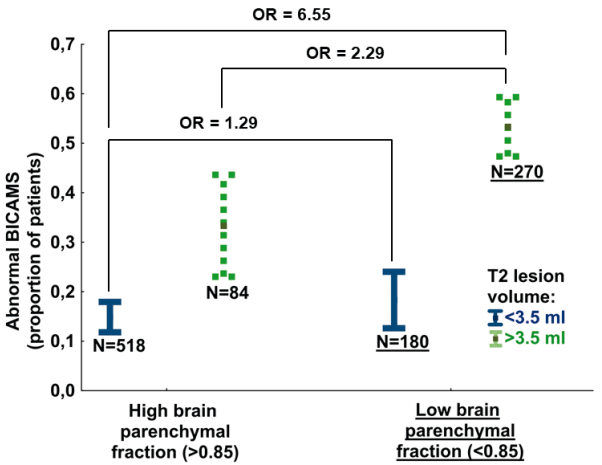

Figure 19: Proportions of cognitively impaired patients in subgroups of patients defined by dichotomized T2 lesion volume and brain parenchymal fraction at baseline of the study

Details are provided in the publication: Uher T, Vaneckova M, Sormani MP, et al. Identification of multiple sclerosis patients at highest risk of cognitive impairment using an integrated brain magnetic resonance imaging assessment approach. *European Journal of Neurology* 2017; 24(2):292-301.[22]

5.12 Cognitive clinico-radiological paradox in early stages of multiple sclerosis

Background: Brain MRI measures, such as the volume of T1 and T2 lesions,[96, 228] pathologies of normal-appearing white matter,[132, 133] cortical lesions,[229, 230] gray matter,[201, 231] or thalamic atrophy[100, 133] correlate with cognitive functioning in patients with MS. However, several previous studies have not found an association between MRI pathology and cognitive performance.[86, 232–241] Furthermore, the magnitude of previously published correlations varies considerably between studies.[16, 17, 242] We hypothesized that the strength of the correlation between imaging and cognitive measures is influenced by disease stage, disease burden, and patient characteristics.

Objective: We investigated whether the strength of the association between MRI metrics and cognitive outcomes differed among various subpopulations of patients with MS.

Methods: This study included a large sample of 1,052 patients with predominantly relapsing-remitting MS. All patients underwent brain MRI using a single scanner with volumetric assessment of T1 and T2 lesion volumes and brain parenchymal fractions. All patients were evaluated using the BICAMS battery and PASAT.

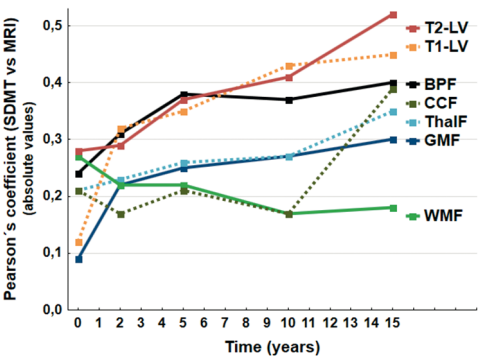

Figure 20: The strength of associations between brain MRI (brain normalized volumes and T1 and T2 lesion volume) and cognitive measures (Symbol Digit Modalities Test) in MS subpopulations. Subgroups of patients stratified by disease duration (CCF = corpus callosum fraction, GMF = gray matter fraction; ThalF = thalamic fraction; WMF = white matter fraction)

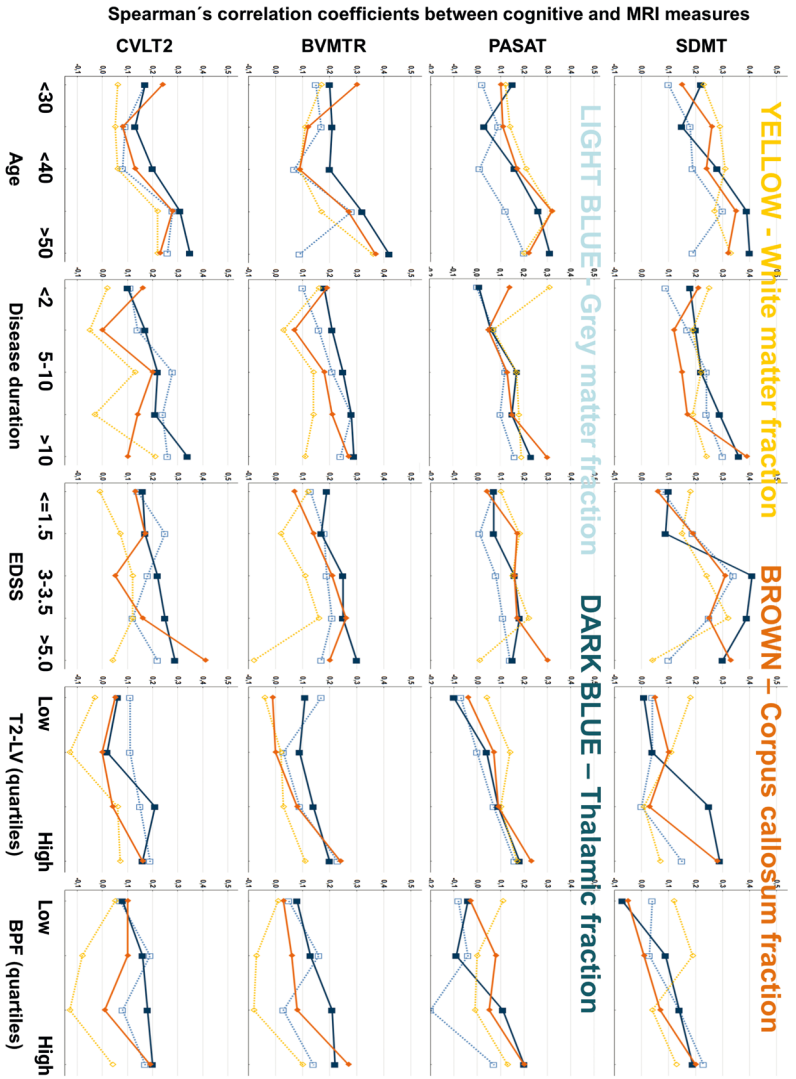

Figure 21: The strength of associations between normalized regional brain volumes and cognitive measures in MS subpopulations. Subgroups of patients stratified by age, disease duration, EDSS, T2 lesion volume, or brain parenchymal fraction were used for graphical purposes

Results and conclusions: We found that the strength of the correlation between brain MRI and cognitive measures increased with advanced disease. The correlations between imaging and cognitive measures were low in patients with a low disease burden but significantly stronger in patients with a high disease burden with a long duration of the disease, older age, greater disability, greater lesion, and lower brain volume (Figure 20 and 21). Taken together, our results suggest that greater brain damage is associated with greater cognitive dysfunction, especially in patients with a greater cumulative burden of preexisting diseases. The results of this study have several implications. First, the characteristics of a patient's disease should be considered when interpreting the results of cognitive research. In this context, there is also a need for balanced recruitment of patients into clinical trials. Finally, asymptomatic radiological disease progression cannot be considered benign, because the clinical consequences of subclinical brain damage may be delayed. We believe that the results of this study could help explain the reasons for the lack of associations found in the literature between imaging and cognitive measures in several cross-sectional[233, 238–241] and longitudinal studies,[86, 97] performed in patients with a low disease burden.

Details are provided in the publication: Uher T, Krasensky J, Sobisek L, et al. Cognitive clinico-radiological paradox in early stages of multiple sclerosis. *Annals of Clinical & Translational Neurology* 2017; 5(1):81-91.[189]

5.13 Pregnancy-induced brain MRI changes in women with multiple sclerosis

Background: The effects of pregnancy on the CNS in women with MS are not well understood.

Objective: To describe the effects of pregnancy on the lesion activity and brain volume in women with MS.

Methods: We included 62 women with relapsing-remitting MS and 221 women with available MRI time points. All women underwent clinical visits at baseline (< 24 and > 6 months before pregnancy), pre-pregnancy (< 6 months before pregnancy), postpartum (< 3 months after delivery), and during the follow-up period (> 12 and < 24 months after delivery) (Figure 22).

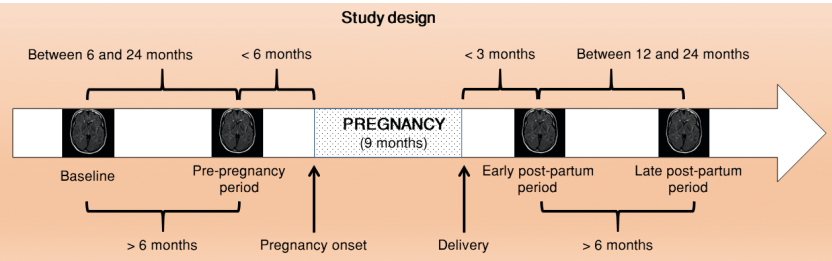

Figure 22: Study design

Results and conclusions: Women in the postpartum period showed a higher volume of T2 lesions, lower normalized brain volume, and greater acceleration of brain volume loss than those in the pre-pregnancy period. Furthermore, at 12-24 months after delivery, 41 women had a higher volume of T2 lesions and lower normalized brain volume than before pregnancy (Figure 23 and 24). Taken together, pregnancy considerably affects

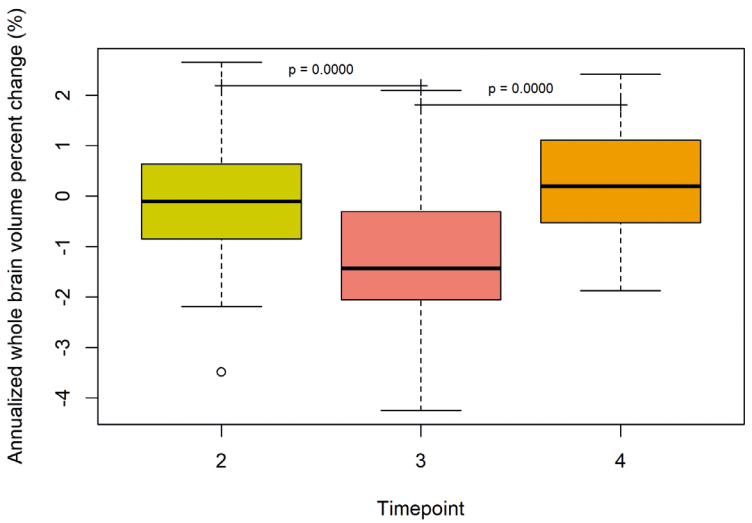

Figure 23: The rate of annualized brain volume at prepregnancy period (2), at early postpartum period (3), and at follow-up (e.g., late postpartum period) (4)

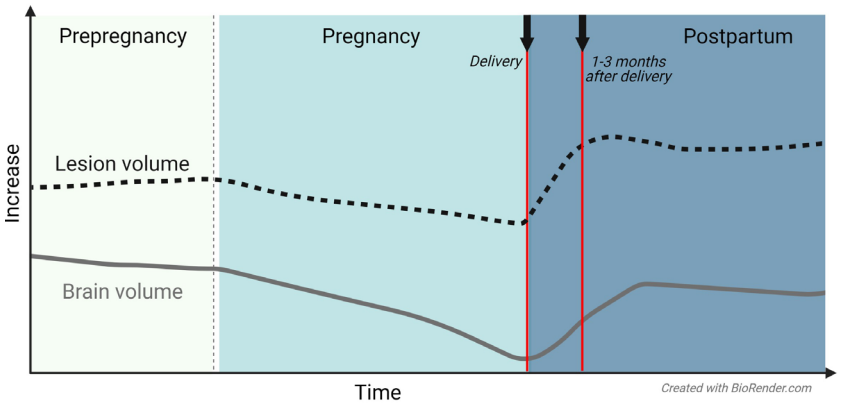

Figure 24: Proposed model of brain and lesion volume evolution through pregnancy

lesion activity and brain volume. Pregnancy-induced changes were also observed at 12–24 months after delivery. The results of this study have several practical implications. First, the late postpartum period should be considered an exclusion criterion in clinical trials with brain volumetry as a study outcome measure. The study results are also important for routine practice in which the assessment of brain volume loss is sometimes used to monitor disease activity.

Details are provided in the publication: Uher T, Kubala Havrdova E, Vodehnalova K, et al. Pregnancy-induced brain magnetic resonance imaging changes in women with multiple sclerosis. *European Journal of Neurology* 2022; 29(5):1446-1456.[243]

6. Summary and future directions

6.1 Time course and stability of brain atrophy

There is a relatively high intra-subject variability in global and regional MRI volumetric change measures. However, the variability in patients with MS differed substantially among MRI markers. For example, compared to the whole-brain atrophy rate, the percent volume loss of the gray matter and thalamus was approximately two-fold. The variability in the corpus callosum volume loss was up to three-fold higher. Considering the high measurement error of MRI volumetric measures, we hypothesized that MRI markers with high intersubject variability (such as the corpus callosum, thalamus, or lateral ventricle) could represent the most sensitive markers for monitoring disease activity.[180, 190]

Patients with MS have a relatively stable rate of brain volume loss throughout the disease course; however, in elderly patients, the aging process is likely to contribute more to the overall brain volume loss than in younger patients. Accelerated brain volume loss is weakly associated with a concurrent increase in disease activity, and escalation of disease-modifying treatments can help normalize the increased rate of brain volume loss.

Most patients exhibit a linear time course of global and regional brain volume loss and lesion burden accumulation. However, a non-linear time-course of brain volume loss occurred in a proportion of patients with a high rate of brain volume loss, long follow-up duration, and high number of MRI scans over time. Additional factors associated with non-linear brain volume loss should be addressed in future studies. It is possible that, in datasets with longer follow-up periods and a higher number of MRI scans, the proportion of individuals with non-linear MRI trajectories was higher. Therefore, the assumption of linearity in brain volume loss needs to be verified, particularly in long-term MRI studies.

Surprisingly, a considerable proportion of brain MRI scans performed during the short-term follow-up period showed an increase in brain volume. Although an increase in brain volume can be caused by several factors, the results indicate that measurement errors may contribute to an increase in brain volume in the majority of cases. Therefore, clinicians should be aware of the frequent occurrence of brain volume increases during short-term follow-ups and interpret brain volume changes in individual patients with great caution.

Although some subgroups of patients did not differ in the rate of brain atrophy, there were significant differences in the intra-individual variability of the longitudinal MRI outcomes. In this context, the identification of preventable factors (time of day when a patient is scanned, hydration status, medication history, and recent steroid administration) responsible for the increased intra-individual variability of MRI outcomes in some patients might help improve the accuracy of MRI volumetric measures in clinical research and routine practice.

We identified cut-offs for annualized global and regional brain volume loss rates that could discriminate between physiological and pathological brain atrophy. Our findings suggest that the predictive accuracy of the proposed volumetric cut-offs for discriminating pathological brain volume loss may differ among various MS populations. Generally, the precision of the cutoffs was greater in MS subgroups with higher rates of brain atrophy, such as in highly active relapsing-remitting MS, or in patients receiving disease-modifying treatment with lower efficacy, such as interferons (80% specificity and 60% sensitivity). Contrastingly, the general precision of the established cutoffs was relatively low in clinically stable patients after the first demyelinating event suggestive of MS and in patients receiving natalizumab treatment, with a mild rate of loss of brain volume (80% specificity and 30% sensitivity).[190]

6.2 Relationship between brain atrophy and lesion burden accumulation

We found a strong association between lesion burden accumulation and brain atrophy progression. Only a small proportion (15–22%, depending on the definition) of patients with MS showed a weak association between lesion volume accumulation and brain atrophy over long-term follow-up.

Although the proportion of patients with MRI dissociation at the time of disease onset and after 15 years of follow-up was similar, there was a difference in the prevalence of the different types of dissociation. At the beginning of the disease, the highest proportion of patients had disproportionally higher brain volume than those with concurrent T2 lesions. Conversely, at advanced disease stages, the dissociation phenotype was more prevalent, with a disproportionally low brain volume in relation to the concurrent T2 lesion volume. The dissociation status was unstable over time in a large proportion of patients (approximately 15%). Three dissociation score trajectories were identified. Taken together, the dissociation between brain atrophy and lesion burden was not specific only for different patients but also for different stages of the disease.

6.3 Prognostic role of MRI atrophy measures

Patients with MS with a high lesion burden, as assessed by T1 or T2 lesion volume or T2 lesion number, high brain corpus callosum or thalamic atrophy, early accumulation of new T2 lesions, and high early whole brain or corpus callosum volume loss after disease-modifying treatment initiation (interferon beta-1a), were at the highest risk of disability accumulation over long-term follow-up. The statistical predictive capacity of these early MRI phenotypes was improved by combining them with early clinical outcomes. A composite score was generated from a subset of the best MRI findings and clinical predictors of disability progression, which may provide valuable information for therapeutic decisions, even in individual patients.[1]

Specific cross-sectional MRI phenotypes (high lesion load and high brain atrophy evaluated by the brain parenchymal fraction and their combinations) were also able to identify patient subgroups at the highest risk of cognitive impairment and deterioration during short-term follow-up. Importantly, the statistical approach was based on the evaluation of cross-sectional MRI measures with higher variability and lower measurement errors than longitudinal MRI markers. Therefore, we suggest that this integrated brain MRI-using assessment approach has the potential to be used for risk estimation of cognitive impairment in individual patients.[22]

Finally, we hypothesize that a high brain reserve, estimated as a high brain parenchymal fraction and low lesion volume, may explain why patients lacking advanced brain atrophy or with a small lesion burden are

relatively resistant to the development of measurable cognitive deterioration after an increase in brain injury. Contrastingly, patients with high preexisting brain damage (a high degree of brain atrophy and lesion load) are relatively sensitive to any additional structural brain damage, a sign indicative of exhausted brain reserves.[189]

6.4 Recommendations for use of brain atrophy measures in clinical practice

Currently, it is widely accepted that the high intra-subject variability of longitudinal MRI measures resulting from biological and technical biases does not allow for a confident estimation of brain atrophy rates in individual patients.[180] Furthermore, we observed an unsatisfactory accuracy of established brain atrophy cutoffs that attempted to discriminate between physiological and pathological brain atrophy, even in models where linear regression slopes were fitted to all available measurements of the percentage volume change within the individual subject. Hence, the overall predictive accuracy of the suggested cut-offs may be even lower in clinical practice because of the considerable intra-individual fluctuations in brain atrophy outcomes occurring in patients due to various biases. Despite this, we suggest that complex brain MRI phenotype monitoring could still be feasible in individual patients with MS if the following points are considered.

1. Application of a single longitudinal volumetric measure of global or regional brain atrophy for individual predictions or longitudinal monitoring is not feasible. Therefore, a combination of different types of traditional and volumetric MRI and clinical outcomes, as shown in the predictive composite score,[190] may have the potential to outweigh the high intra-subject variability of single imaging markers and provide high measurement precision, enabling its application in individual patients.
2. The accuracy of brain volume loss measures may be improved in individual patients when a series of consecutive MRI scans is conducted over a short-term follow-up period. This approach represents another option to minimize the high intra-subject variability of single imaging and clinical markers and provide measurement accuracy, enabling its applicability in individual patients. The minimal number of MRI scans needed and the shortest follow-up duration required to obtain a reasonable measurement error substantially lower than the suggested pathological

cutoffs for brain atrophy remain to be confirmed by further research. Preliminary findings have shown that longer follow-up (≥ 2 years) rather than a higher number of MRI scans may provide better precision in measuring brain volume loss in individual patients.

3. Cross-sectional measures, such as absolute T2 lesion volume or brain parenchymal fraction, have substantially lower relative measurement errors and higher inter-subject variability than longitudinal outcomes. Therefore, these cross-sectional MRI markers can be used to predict or estimate the risk of disability progression in individual patients. However, because of the cross-sectional nature of the brain parenchymal fraction measure, it cannot be used for the longitudinal monitoring of brain atrophy.[1, 20]

6.5 MRI phenotypes

The identification of specific MS phenotypes may be of great importance in estimating the risk of disability progression and individually tailored treatment. Determining specific disease patterns may also improve our understanding of the disease mechanisms of MS and would contribute to the current research on genetic associations with disease progression.[244]

Finally, the identification of specific MS subgroups could also influence future research by testing new relevant hypotheses, re-evaluating the findings of previous research, and improving patient recruitment into clinical trials.

However, the currently used MS classification purely reflects the clinical course and comprises only a limited number of clinical subtypes.[82] However, this narrowed spectrum of clinical subtypes is not the only limitation. There is also significant heterogeneity in the clinical course, response to treatment, and objective biological markers within established clinical subgroups. Subsequently, the translation of clinical classifications into clinical practice for treatment decision-making and prognosis estimation in individual patients is limited. In this context, reliable imaging and laboratory surrogate markers that can provide objective criteria for identifying specific disease patterns are lacking.

Several previous studies have suggested that brain imaging markers may potentially modify or complement the current clinical MS classification.[82] Especially MRI which clinical importance is growing rapidly, is considered

a more accurate, pathologically representative, and objective tool for the description of disease patterns compared to clinical assessment confused by several biases. MRI was hypothesized to identify MS phenotypes that are directly influenced by pathophysiological mechanisms.[244] Furthermore, a combination of MRI and clinical results was suggested to improve the identification of MS phenotypes.

To the best of our knowledge, few attempts have been made to systematically investigate specific MRI phenotypes in MS. Moreover, previous studies were cross-sectional, employed small sample sizes, and investigated only conventional or global MRI volumetric measures.[226, 245, 246] For example, recent cross-sectional studies described four MRI phenotypes based on brain imaging measures of inflammation (assessed by T2 lesion volume or the occurrence of contrast-enhancing lesions) and brain imaging measures of axonal/tissue loss (assessed by brain parenchymal fraction). Although a higher T2 lesion burden was associated with greater brain atrophy in most patients, a considerable proportion of patients had either a high T2 lesion load or high brain atrophy. Different pathophysiological mechanisms, individual regeneration and repair capacity, neuronal and axonal integrity, or dominant gray matter pathology may explain the appearance of different MRI patterns. Particularly, the correlation between disability and MRI outcomes was different in the MRI subgroups, indicating the potential prognostic role of MRI phenotypes.[226] It is also worth noting that MRI phenotypes were not related to the current clinical classification of MS. This agrees with other studies showing MRI variability between individuals with established clinical MS subtypes.

In summary, the findings of previous research emphasize that the current clinical classification does not closely overlap with MRI phenotypes and that further research is needed to identify specific disease patterns in more detail.

6.6 Summary of the main findings

- In most patients, there is high intra-individual variability in longitudinal brain MRI volumetric measures.
- Established cut-offs for pathological brain atrophy have a relatively low accuracy.
- The measurement error of brain atrophy estimates is comparable to the suggested cut-offs of the pathological brain atrophy rate.

- A considerable proportion of MRI scans present with brain volume increase.
- Assessment of MRI trajectories (based on multiple MRI scans over long-term follow-up) and complex evaluation of a spectrum of global and regional brain atrophy and lesional volumetric markers may allow for the use of volumetric measures in individual patients in the future.
- Some regional brain atrophy measures (corpus callosum and lateral ventricle) may be more suitable for disease monitoring because of their greater disease specificity and higher inter-individual variability but similar intra-individual variability compared to whole-brain atrophy measures.
- Cross-sectional volumetric measures (e.g., lesion volume or brain parenchymal fraction) have higher interindividual variability and lower intra-individual variability than longitudinal volumetric measures. Therefore, it can be reliably used to predict or estimate the risk of disability progression in individual patients.

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