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HABILITATION

Clinical relevance of brain atrophy measures in multiple sclerosis

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Acknowledgements

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Abbreviations

ASA = Avonex–Steroid–Azathioprine study
BICAMS = Brief International Cognitive Assessment for MS
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
IFNß-1a = interferon beta-1a
lin-R^2 = coefficients of determination of individual linear model
MACFIMS = Minimal Assessment of Cognitive Function
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
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 fibre layer
PASAT = Paced Auditory Serial Addition Test
SET = Study of Early interferon b1-a Treatment
SDMT = Symbol Digiti Modalities Test
SDP = sustained disability progression
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 autoimmune disease of the central nervous system (CNS) associated with a broad spectrum of clinical and radiological phenotypes.[1-4] Even though MS was originally considered to be an inflammatory disease affecting predominantly the white matter,[5, 6] neurodegenerative processes resulting in accelerated brain and spine volume loss are increasingly recognized as an important determinant of neurological disability.[1, 2, 4, 7-11] It is commonly understood that MS is a complex heterogeneous disease characterized by a broad spectrum of physical[12, 13] and cognitive[14, 15] symptoms, variable treatment response, radiological features and neuropathology. This heterogeneous presentation of symptoms is most likely attributed to complex interactions between external and hereditary factors,[16] resulting limited predictability of disease course and treatment response. Therefore, there is an urgent need for individually tailored treatment.

Unfortuntaely, traditional clinical predictors are not sensitive enough to reliably predict future and monitor ongoing disease activity. In contrast, abnormal MRI findings have been shown as the most informative predictors and surrogate markers of disease activity.[17, 18] Not only accumulation of lesion burden, but also global and regional brain atrophy are important parts of disease progression and are associated with development of physical[1, 4, 7-9, 19] and cognitive disability.[14, 15, 20] As such, assessment of the course of brain atrophy within individual patients could facilitate the identification of those with current disease activity and those at highest risk of accumulating permanent disability in future.[12]

In this context, some effort has been made to bring measurements of brain atrophy into clinical practice.[9, 21, 22] Unfortunately, relatively high intra-individual variability of longitudinal brain atrophy measures renders its application in individual MS patients challenging.[7-9, 22] As such, brain atrophy measures are as of yet not utilized routinely in clinical practice.

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

1. Introduction

1.1 Epidemiology

MS affects mainly young individuals and is a leading cause of disability in this age group.[23] Disease onset usually occurs between 20 and 40 years of age. Worldwide, there are approximately 2,5 milion patients with MS.[24, 25] In the Czech Republic, the prevalence of MS is approximately 170-200 per 100,000 (overall 17-20,000 patients) (ReMuS Registry data). In general, the prevalence of MS rises as the distance from the equator increases; 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.[26, 27] Women are diagnosed with MS at least 2-3x as often as men.[28, 29] In the last decades, incidence of MS increases especially in woman, the reason is however unknown.[30]

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 a genetically susceptible individual. MS is characterized by demyelination, inflammation, lesion formation and neurodegeneration. It is believed that progressive neurodegeneration of the CNS is mostly a consequence of neuroinflammation, rather than an independent procsess. In addition, it is accepted that a peripheral immune response targeting the CNS is especially important in early phases of disease progression. Apart from that, immune pathophysiological proccess within the CNS are predominant in the late progressive phases of disease.[31-34]

There is no specific antigen known leading to 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.[35, 36] However, an emerging body of evidence suggests there is an important role for B-cells in the pathogenesis of MS.[37-39] At the beginning, a compromised blood-brain barrier allows for the invasion of monocytes and T cells to the brain or spine parenchyma. Peripheral monocytes and activated microglia are primarily responsible for the

demyelination in MS lesions. The formation of focal inflammatory demyelination occurs in both white and grey matter. Lesions in white matter occur particularly in perivascular and periventricul regions. On the other hand, lesions in gray matter are often located along the subpial surface of the cortex and are topographically related to inflammation in adjacent meninges. In this respect, it is likely that soluble factors from cerebrospinal fluid trigger development of white periventricular and subpial cortical lesions.[40, 41]

Active MS lesions contain myelin breakdown together with clonally expanded CD8+ T cells, and to a lesser degree CD4+ T cells, gamma delta T cells, monocytes, and rare B cells and plasma cells, dendritic cells expressing major histocompatibility complex (MHC) class II moleculles, large numbers of macrophages containing myelin debris, and immunoglobulin deposition. In additiona, there are pathological changes of oligodendrocytes (cytopathic changes, apoptosis, phagocytosis of apoptotic oligodendrocytes, swelling of cells with abnormal nuclei, complement deposition, and lysis). Furthermore, there can be also signs of astrocytic damage. Compared with white matter lesions, cortical lesions of display less inflammatory infilrates and microglial activation than white mater lesions.[34, 40, 42] Some axons are still preserved, even in cases of total myelin loss. Remyelination may occur in some lesion, but this usually is very limited.[43]

Several hypotheses of neurodegenerative proccesses have been put forward: Wallerian degeneration secondary to demyelination, oligodendrocyte loss and axonal degeneration, damage from reactive oxygen species, nitric oxide, or energy failure from mitochondrial dysfunction.[31, 44, 45]

1.3 Risk factors

Genetic factors

MS is not a result of any single-gene defect. Many different genes with incomplete penetrance are associated with an increased risk of MS. So far, dozens of genetic variants (single nucleotide polymorphisms, SNPs) have been identified, mostly related to adaptive immunity. Most of these susceptibility loci for MS are shared with other autoimmune disorders. In addition, these susceptibility genetic loci appear to have only a modest influence on the risk of developing MS.[46, 47] For example, human leucocyte antigen (HLA) class II alleles DRB1*1501, 0301 and 1303 (expressed on the innate immune cells and important for antigen recognition by CD4+ and CD8+ T lymphocytes) are asociated with a 3 fold increased risk of having MS.[48] Taken together, genetic predisposition accounts for only 15-25% of the life-time risk of MS. In other

words, twins studies have shown that a homozygous twin of an MS patient has, on average, a 15-25% risk of developing MS.[46, 49] In contrast, siblings of MS patient have, on average, only a 3% risk of developing MS.[50] Families with a high number of MS patients are rare. Even in these families, no single gene has been identified to bte responsible for he development of MS.[51]

Enviromental risk factors

Several environmental risk factors, especially those associated with the sustained activation of the immune system have been proposed. It is hypothesized that enviromental factors modulating the peripheral adaptive immunity may have a key role in increasing the risk of developing MS.[32, 52] The most important risk factors include Epstein-Barr virus infection at a young age, decreased sunlight exposure, low vitamin D levels (modulates differentiation of T-lymphocytes), and smoking[28, 53-56]. Other environmental risk factors such as obesity,[57, 58] psychological stress,[59-62] vaccinations,[63] unhealthy diet[64-67], and gut microbiome abnormalities were also suggested.[68-70]

1.4 Clinical presentation

Neurological symptoms

The clinical presentation of MS includes a wide range neurological signs and symptoms originating from focal or diffuse brain and spinal cord damage. With the exception of optic neuritis, the neurological presentation is not very specific for MS. This makes diagnosis of MS based solely on clinical criteria very challenging or almost impossible. Most often neurological symptoms include:

- muscle weakness (paresis) and spasticity, which is associated with lesions in pyramidal (cortico-spinal) tracts or partial transverse myelitis.
- neo and paleo-cerebellar syndrome with ataxia, tremor and abnormalities in stance and gait, which are associated with cerebellar or brainstem lesions.
- dysartria, dysphagia, nystagmus, peripheral facial palsy, diplopia, internuclear opthalmoplegia or other cranial nerve involvements associated with lesions in the brainstem.
- impaired sensory symptoms such as paresthesias (numbness), hypesthesia (loss of sensation), dysesthesia or neuropathic pain associated with brain or spinal lesions in spino-thalamic or dorsal column pathways.

- bowel and bladder dysfunction such as retention, incontinence and urgency, usually associated with spinal lesions.

- visual dysfunction, mostly due to unilateral optic neuritis or more rarely associated with lesions in the occipital lobe.

- 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 for monitoring of clinical disease activity.[71] EDSS is a widely accepted clinical tool used in clinical practice as well as in clinical trials. However, there are a number of limitations to EDSS. Firstly, there is a relatively high inter-rater variability of EDSS scores due to the detailed scoring system and the subjective nature of some parts of neurological examination. Secondly, the relationship between actual disability status and EDSS scores is not linear. For example, there is smaller change in objective disability between EDSS 1.0 and 2.0 than between EDSS 5.0 and 6.0. EDSS is mostly focused on walking ability, especially at scores above 4.5 which depend solely on walking performance. On the other hand, EDSS is not sensitive enough to minor changes of walking performance and hand motor functions. Finally, cognitive and affective functions are not sufficiently assessed using this metric. For example, patient with severe cognitive dysfunction (dementia) and no walking problems would have the same EDSS score as cognitively preserved patient able to walk at least 200 metres.[72-74]

In this respect, new clinical measures have been developed and tested in both research and clinical practice. These include:
- 9 hole peg test: quantifies upper extremity function, patient picks up 9 pegs and puts them in 9 holes, as quickly as possible, time is calculated, administration time is 10 minutes.[75]

- 25 foot walk test: quantifies mobility and leg function, patient is instructed to walk 25 feet (8 m) as quickly as possible, time is calculated, administration time is 1-5 minutes.[75]

- SLOAN visual acuity charts: evaluates visual impairment, the number of letters that are identified correctly are scored, administration time is 5 minutes.[76]

- Cognitive tests (see bellow).

While the use of extended clinical follow-up with additional clinical measures has the potential to improve monitoring of clinical disease activity, there are some important drawbacks. First, more clinical studies are needed to define and quantify the added value of additional testing. Secondly, extensive monitoring is time-consuming and is associated with higher patient and financial burden. Because of these factors, further research is needed to address these questions.

The clinical course of MS has four basic disease patterns:[77]

- Radiologically isolated syndrome has MS typical findings on brain MRI, however, there are no clinical signs or symptoms suggestive of MS. This stage is associated with significantly increased risk of developing MS.[78]

- Clinically isolated syndrome describes a first clinical episode suggestive of MS. According to McDonald 2010[79] and 2017 criteria,[80] a proportion of patients with enhancing lesions on brain MRI or positive oligoclonal bands may be diagnosed with MS already at this stage.

- Relapsing-remitting MS is the most common disease pattern (80%). It is characterized by clinical attacks (relapses) of new neurologic symptoms. The attacks are followed by remissions associated with partial or fully recovery.

- Secondary progressive MS follows relapsing-remitting MS. This occurs in late disease stages and is characterized by severe neurological disability, a lack of relapses, disability progression, and poor response to immunomodulatory treatments.

- Primary progressive MS is characterized by disability progression from the onset of symptoms but without early relapses or remissions. This MS type also has a poor response to the majority of immunomodulatory treatments.

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

Cognitive symptoms

Cognitive impairment is a common neuropsychiatric symptom in MS with a prevalence rate between 43% and 70%. Cognitive dysfunction is also detected in clinically isolated syndrome patients in early stages of MS.[81-83] Impairment of cognition is increasingly recognized as an important determinant of employment status and associated societal costs,[84, 85] adverse effects on social functioning, coping, quality of life and treatment adherence among MS patients.[86, 87] The core domains of cognition, including verbal and visual memory, information processing speed, semantic fluency, sustained attention and executive functions are most often affected.[88-90] Neuropsychological deficits are related to brain structural MRI measures in MS patients.[14] Numerous studies in early MS have shown an association between cognitive impairment and white matter lesions,[91] whole brain[92], cortical [93, 94], and subcortical deep gray matter atrophy, including thalamus volume loss.[95-97]

Routine neurological examination does not detect cognitive impairment in the majority of MS patients resulting in cognitive impairment often being underdiagnosed, even though it could be an important symptom of MS progression. The usefulness of brief cognitive screening batteries as a Mini-Mental State Examination (MMSE)[98] or a Multiple Sclerosis Neuropsychological Questionnaire (MSNQ)[99] has been questioned because of its low sensitivity in the detection of MS specific cognitive impairment. On the other hand, 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)[88] may also be limited due to its time consuming nature and the need for being administered by experienced neuropsychologists. All of the above considerations emphasize the need for a short, validated and accepted instrument, able to capture cognitive impairment of MS patients and which can also be administered by staff without neuropsychological training. Hence, Brief International Cognitive Assessment for MS (BICAMS),[100, 101] or single Symbol Digit Modalities Test (SDMT)[102] have been suggested as suitable for use in routine clinical practice.

1.5 Paraclinical measures

MRI

Among different paraclinical measures, brain MRI is one of the most accepted and sensitive tools used in monitoring of subclinical disease activity and diagnosis of MS and.[17, 18] 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, while sophisticated software is also able to assess T1, T2 of contrast-enhancing lesion volumes. Lesions are typically distributed in the spinal, intratentorial, peri-ventricular and juxta-cortical locations.[80] However, the majority of cortical lesions are not seen on standard MRI scanners.[103] Because of this, MS was originally considered to be a disease affecting predominantly the white matter.[5, 6] Nowadays, pathological changes of gray matter are increasingly recognized as an early[10, 104-114] and important determinant of disease activity in MS patients.[104, 115-117] Although brain lesions in MS represent a histo-pathologically heterogeneous and dynamic group of focal brain pathology, ranging from oedema and inflammation to demyelinitation and axonal loss, their neuro-inflammatory origin is well accepted.[42]

Not only an accumulation of lesions, but also global and regional brain atrophy are important parts of disease progression associated with development of physical[1, 4, 7-9, 19] and cognitive disability.[14, 15, 20] In MS, brain volume loss is driven by several mechanisms including tissue loss (i.e., loss of myelin, glial cells, neurons and axons due to the inflammatory demyelination and neurodegeneration), as well as changes in non-tissue components (i.e., fluids shift due to inflammation, hydration, endocrine influences or environmental factors).[7-9, 21, 118, 119] To date, there are a number of manual, semi-automated and automated techniques[7-9] used for the assessment of global and regional brain volume measures, including Structural Image Evaluation using Normalization of Atrophy Cross-sectional (SIENAX),[120] FreeSurfer,[121, 122] NeuroQuant, MS metrix,[123] and model-based segmentation/ registration tool - FMRIB Integrated Registration and Segmentation Tool (FIRST).[123, 124] Longitudinal methods are also available, such as SIENA, which are employed to directly measure relative volume changes over time.[120, 125, 126] 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 lesional 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.[15, 127, 128] For example, specific topography rather than lesional or brain volume may play a role in the pathogenesis of disability in MS. Hence, more advanced MRI techniques, such as magnetisation transfer ratio, diffusion tensor imaging, proton MRI spectroscopy, and functional MRI measuring various aspects of MS pathology[15] are likely to further improve our understanding of the associations between MRI and disability progression at different stages of MS. Unfortunately, a remarkably high intra-individual variability is also present in these advanced MRI methods, and 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 disease progression. In MS, the spinal cord is usually characterised by focal and diffuse lesions as well as global atrophy.[129] However, spinal cord MRI is measured in clinical practice and clinical studies much less frequently than brain MRI. This is mostly due to technical challenges, such as inhomogeneous magnetic field in this region, the small physical dimensions of spinal cord, and motion artefacts within the spinal canal together with the flow of cerebrospinal fluid and periodic motion due to respiratory and cardiac cycles.[130] Moreover, spinal cord MRI is ussually 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 performed only for research purposes and is therefore not monitored regularly in most MS patients.

Biochemical

Cytological (plasmatic cells, lymphocytic pleocytosis) and biochemical (normal protein, normal albumin quotient, increased IgG index and IgG quotient) studies of cerebro-spinal fluid have an importan role in the differential diagnosis of MS. Especially, identification of cerebro-spinal fluid restricted oligo-clonal bands is typical for MS and is widely used for diagnosis confirmation.[131, 132] Furthermore, anti-aquaporin-4[133] and anti-MOG (myelin oligodendrocyte glycoprotein)[134] antibodies are helpful to distinguish between MS and neuromyelitis optica. Serum neurofilamet light chain level is an exceedingly promising predictor and marker of disease activity. Currently, a number of studies are ongoing, investigating its potential use in clinical practice. [135-137]

Optical coherent tomography

Optical coherent tomography (OCT) measures the thickness of the retinal nerve fibre layer (RNFL), which contains only non-myelinated axons. RNFL thickness is associated with disability, relapse activity and brain atrophy. Importantly, OCT is helpful to distinguish between MS and neuromyelitis optica.[138] However, further studies are needed to show whether OCT is suitable for disease monitoring or prediction of disease progression.[139]

1.6 Diagnosis

There is no single diagnostic test of MS. Diagnostic proccesses include a medical history, neurologic exam, and para-clinical tests including MRI, cerebro-spinal fluid analysis, and eventually evoked potentials or OCT.

For diagnosis of MS, it is needed to:[80]

- Identify neurological symptoms arising from involvement of brain, optic nerve or spinal cord.
- Confirm dissemination of disease in space (new relapse implicating different CNS site, ≥ 1 symptomatic or asymptomatic CNS lesions in ≥ 2 MS-typical regions of the CNS including: spinal cord, infratentorial, periventricular and juxtacortical/cortical).
- Confirm dissemination of disease in time (new relapse, new lesion, simultaneously contrast-enhancing lesion together with non-enhancing lesion on brain MRI; oligoclonal bands in cerebrospinal fluid can be used instead of dissemination in time).
- Rule out a different diagnosis.

1.7 Management

As of yet, there is no cure for MS. Current therapies focus mainly on the prevention or decrease of neuroinflammation. A wide range of immune therapies with specificic mechanisms of actions and immune targets have been approved for MS. New immunomodulatory (disease-modifying treatments) are at this time the most effective drugs for MS. A major issue is the fact that most therapies are effective only during early disease stages, while they have minimal effect in late progressive phases.

Most MS therapies significantly alter the survival and trafficking of immune cells. For example, the pharmacological effects of fingolimod result in sequestration of lymphocytes in the lymph nodes. Natalizumab, a monoclonal antibody, which binds to the α4 integrin sub-unit present in very late 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 of which are monoclonal antibodies that target the CD20 present on most B cells (except terminally differentiated plasma B cells), causes B cell death. Alemtuzumab targets CD52 and depletes T, B and NK cell populations. Monocytes, NK and B cell repopulate the immune system more rapidly than T cells after treatment.[140] Dimethyl fumarate treatment causes lymphopenia that reduces CD3 T cell counts with preferential depletion of CD8 cells.[141] Interferon-beta, which has anti-proliferative activities, also causes short and long-term changes to diverse cell populations, particularly activated NK cells.[142, 143] Glatiramer acetate has many immunological effects including its capability to alter T-cell differentiation leading to a shift from Th1 (pro-inflammatory) to Th2 (anti-inflammatory) immune profile, which may dampen inflammation within CNS.[144] 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.[145]

In addition, cytostatic therapies such as cyclofosfamide, mitoxantrone or azathioprine are rarely used as off-label immunosupresive treatment, usually for patients not indicated for treatment with disease-modyfying treatment. High-dose steroids and plasma exchange are used for management of relapses. In addition, 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 exercise, rehabilitation, physiotherapy and ergotherapy are important elements of successful and comprehensive care for MS patients.[146, 147]

1.8 Prediction of disease activity

MS is a heterogeneous disorder with a broad spectrum of phenotypes. Because of this, treatment efficacy may be significantly improved by identifying specific MS subpopulations at high risk of disability progression in spite of using disease-modifying treatments. Given that irreversible acute axonal damage is most extensive in early disease stages,[148] it is exceedingly important to

identify those individuals who fail to respond to treatment as early as possible, even if the mechanisms by which this occurs remain poorly understood at this time.[2] Hence, an important, yet currently unmet, need of modern MS treatment is to determine reliable and in routine clinical practice applicable predictors of subsequent disease activity.

Nowadays, in clinical practice traditional clinical markers, such as early disability progression or high relapsing activity, are used for the prediction of disease activity. However, clinical predictors are not particularly sensitive nor specific enough to be used as reliable surrogate markers of disease activity over time. In contrast, recent studies have shown that abnormal MRI findings are the most informative predictors of future disease activity in short-term,[11, 149-151] mid-term,[150, 152, 153] and long-term studies.[150, 154-157] Particularly, the most important predictors of disability progression in relapsing-remitting MS have been suggested to be: the occurrence of new T2 lesions,[150-154, 158] accumulation of T2 lesion volume[159, 160] and T1 lesion volume,[161, 162] whole brain[160, 163] and central atrophy[160, 161] as well as gray matter[164] and thalamic volume changes.[165, 166]

In addition to MRI, serum neurofilaments light chain level shows a relatively good predictive value for disease activity. Importantly, neurofilaments levels are easy to measure in serum and acted as predictors in statistical models independent from MRI markers.[137] Therefore, serum neurofilaments light chain level is a promising new predictor of disease course. Interestingly, measures of blood-brain barrier function[167] before interferon treatment initiation, and early serum lipid profile changes during interferon therapy[168], predict clinical and radiological disease activity over long-term follow-up.

More research is however needed 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 not sufficient for reliable assessment of disease progression. However, more detailed clinical monitoring using quantitative assessment of vision, hand functions, walking abilities, or cognitive performance is not standardized in clinical practice and is time-consuming.

It is well known that majority of new active lesions on brain MRI are clinically asymptomatic but are clinically relevant from a long-term perspective. Therefore, among

different para-clinical measures, brain MRI is one of the most accepted and sensitive tools suitable for monitoring of MS progression.[17, 18] Specifically, the occurrence of new, enlarging or contrast-enhancing lesions on MRI are a widely used surrogate marker of a radiological disease activity.[18] In addition, global and regional brain atrophy are also an important part of disease progression that is strongly associated with development of physical[1, 2, 4, 19] and cognitive[14, 15, 81, 169] disability. In this context, in recent years efforts have been made to bring measurements of brain atrophy into clinical practice for decision-making in individual patients.[9, 21] Unfortunately, high intra-individual variability of longitudinal MRI measures resulting from a number of biological and technical biases does not allow for confident evaluation of the brain atrophy within individual patients.

2. Main aims of the current work

The high intra-individual variability of brain atrophy measures make its applicaticability in individual MS patients questionable.[7-9, 22] Therefore, as it stands, brain atrophy measures are not prepared for application in clinical practice. In this work, we investigated possible ways on how to overcome this high variability in order for atrophy measures to become important decision-making tools in clinical practice. The main aims of the work are the following:

1. To investigate the agreement between MRI measures obtained by various volumetric techniques for the assessment of T2 lesion and whole brain volumes, and their changes in MS patients. In addition, we assessed quantitative intra-individual variability of whole brain volume loss measures.

2. To establish cut-off values of global and regional brain volume loss able to discriminate between healthy controls and MS patients.

3. To investigate the occurence of linear and non-linear trajectories of brain volume loss in MS patients over follow-up.

4. To quantify the degree to which the precision of brain volume loss assessment could be improved with high-frequency brain MRI monitoring over short-term follow-up.

5. To investigate the predictive role of early changes in MRI outcomes with respect to relapse activity and the development of disability progression in patients after first demyelinating event suggestive of MS.

6. To evaluate the predictive accuracy of a broad spectrum of early MRI markers in a homogenous sample of relapsing-remitting MS patients on interferon treatment. Furthermore, to investigate whether the combination of volumetric MRI markers with established clinical predictors may facilitate timely identification of patients with poor long-term disability outcomes.

7. To generate an MRI-based algorithm that allows clinicians to identify MS patients in need of neuropsychological assessment and those at the highest risk of cognitive decline over short-term follow-up periods.

8. To investigate whether the strength of the association between MRI metrics and cognitive outcomes differs between various MS subpopulations.

3. Methods

3.1 Brain MRI

MRI acquisition

All MRI scans analyzed in this project were performed on the same scanner (1.5-Tesla Gyroscan; Philips Medical Systems, Best, the Netherlands) in the Department of Radiodiagnostics at General University Hospital in Prague with the same protocol. The standardized MRI protocol consisted of two sequences: fluid-attenuated inversion recovery (FLAIR) and T1-weighted 3-dimensional fast field echo (T1-WI/FFE 3D). Contiguous slices covering the whole brain were acquired with the following parameters: FLAIR sequence: time to echo (TE)=140 ms, time to repetition (TR) 11000 ms, inversion time (TI) 2600 ms, matrix size 256x181, flip angle (FA) 90°, slice thickness (THK) 1.5/0 mm (with no gaps), field of view (FOV)=256 mm) and T1-WI/FEE 3D (TE/TR: 5/25 ms, FA=30°, matrix size 256x256, THK1.0/0 mm, FOV=256 mm). Volumetric assessment was performed independently in the Department of Radiodiagnostic, First Faculty of Medicine and General University Hospital in Prague, Charles University, Czech Republic and in the Buffalo Neuroimaging Analysis Center, NY, USA.

FLAIR

TR = 11000 ms
TE= 140 ms
FA = 90°
TI = 2600 ms
THK/Gap = 1,5/0 mm
FOV = 256x256
Slice count = 100

T1W/3D

TR = 25 ms
TE = 5 ms
FA = 30°
THK/Gap = 1/0 mm
FOV = 204x256
Slice count = 150

Legend: FA = flip angle, FLAIR = fluid-attenuated inversion recovery, FOV = field of view, T1W/3D = T1-weighted 3-dimensional, TE = time to echo, THK/Gap = thickness of slices and gaps, TI = inversion time, TR = time to repetition
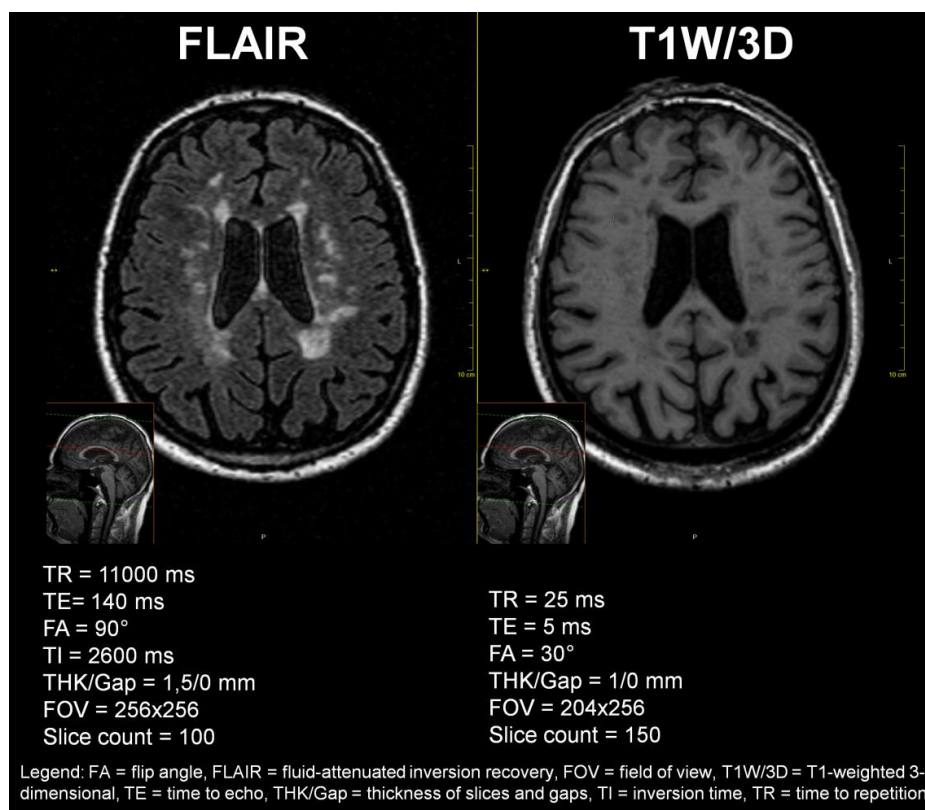
MRI analysis in BNAC

 T2 lesion volume was calculated by experienced operators applying a reliable semi-automated edge-detection contouring-thresholding technique in Jim software (http://www.xinapse.com) as previously described.[170, 171] Using FLIRT (FMRIB; http://www.fmrib.ox. ac.uk/),[172] all follow-up FLAIR images for a given subject were coregistered to his or her baseline FLAIR image with a 6 degree of freedom, rigid-body model. All subsequent lesion analyses were performed on coregistered images with trilinear interpolation. For each follow-up time point, T2 lesion analysis was performed via the aid of a "subtraction image." Images were bias corrected, but not normalized. Normalization was not deemed necessary as overall image intensity was consistent between time points. Briefly, the FLAIR image from the previous time point was subtracted from the corresponding current FLAIR image. The result was then smoothed with a Gaussian kernel of σ=0.5 mm.

Whole brain, gray matter, white matter and lateral ventricle volume was calculated using SIENAX, which normalizes measurements for head-size. Lesion filling was performed before segmentation using an in-house developed method. For longitudinal changes of the whole-brain volume, we applied the SIENA method[120] to calculate the percentage whole brain volume change.[126]

MRI analysis in Prague

Semi-automated image analysis was performed in the Department of Radiodiagnostics, General University Hospital in Prague with the ScanView software. ScanView is a semi-automated software tool for measurement of lesion volume, brain parenchymal fraction, whole brain and corpus callosum volumes via segmentation-based techniques.[1, 11, 20, 149, 167, 173] The software was developed in the Department of Radiodiagnostics General University Hospital in Prague (by Jan Krasensky).[174]

T2 lesion volume was measured from the FLAIR sequence. In the first step, we performed 3D space coregistration control to ensure the same positioning compared to the baseline allowing for follow-up monitoring. In the fully automated second step, skull stripping was performed to isolate the brain parenchyma. Here, we used automated algorithm with a visual quality control step which is a part of ScanView software. This step was applied on FLAIR sequences without fat suppression. Skull striping was based on a combination of enhancement techniques such as 3D Gaussian filter (kernel 3x3x3 mm), median filter (kernel 3x3x3 mm), edge-enhancing filter (kernel 7x7x7 mm) and morphological image operators (erode/dilate filter). Coregistered FLAIR sequences were then transferred to the T1-WI. In the

third step, step-wise homogenization procedures were applied to correct the field inhomogeneity and to reduce noise. Following standard image processing, the signal intensity of brain parenchyma was normalized (peak = 10000, white matter = 5000 arbitrary units). For every individual examination signal intensity of brain parenchyma was multiplied by the reciprocal of the estimated 3D bias field that was determined specifically for our MRI scanner (1.5-Tesla, Gyroscan, Phillips). Then the resulting brain parenchyma images were smoothed with a series of homogenization filters. We applied a 3D Gaussian filter (kernel 3x3x3 mm), then 3D median filter (kernel 3x3x3 mm) and combined filter consisting of 3D Gaussian filter (kernel 5x5x5 mm) and edge-enhancing filter (kernel 7x7x7 mm). Finally, T2 lesions were defined to consist of voxels with intensity >140% of the mean of white matter and having a minimum size of 11 voxels (corresponding to a sphere with a 3-mm diameter). Additional analysis showed that T2 lesions volume defined at the level of voxels intensity >130% corresponds best with T2 lesion volume estimates provided by the Jim software applied in the Buffalo Neuroimaging Analysis Center.

WB volume was measured from the T1-WI/FFE 3D sequence. Non-normalized, absolute whole brain volume was measured on T1-WI thresholded at above 4000 arbitrary units. The first three steps of the volumetric analysis were the same compared with measurement of T2 lesion volume. Coregistration of FLAIR sequence with T1-WI was performed by FLIRT.[172] Voxel intensities were homogenized to obtain whole brain volume (irrespective of gray and white matter) by applying a 3D Gaussian filter (kernel 3x3x3 mm) and 3D median filter (kernel 3x3x3 mm). ScanView provides only non-normalized, absolute values of whole brain volumes. Therefore, whole brain volumes were normalized with respect to the total intracranial volume (ICV). ICV was calculated as the sum of the total brain parenchymal volume and the total intra-ventricular and subarachnoidal cerebrospinal fluid volume. Normalized compartment volumes were calculated, as follows: brain parenchymal fraction = whole brain volume/ICV. ScanView does not provide a direct measure of brain change, but longitudinal measures are based on two independent, cross-sectional measurements.

The partial volume of the corpus callosum was measured by ScanView software and was estimated in seven (4th slice being in the central position) sagittal reconstructions of T1-WI/3D/GE slices per patient using an automated procedure. More specifically, the corpus callosum areas for each of the 7 slices were added and multiplied by the 1.0 mm slice thickness yielding an estimate of corpus callosum volume.[11, 149, 167, 173]

Absolute gray matter and white matter volumes were measured in T1-WI segmented with SIENAX automated image segmentation tool, version 2.60 (http://www.fmrib.ox.ac.

). T1 hypointense lesion volume was subtracted from the area outlined by ScanView.CZ as the gray matter tissue. Gray matter volume was assessed without lesion in-painting. To confirm that lesion in-painting had negligible effect on gray matter volume, we performed lesion in-painting on 90% of the scans acquired and found that in-painting resulted in change in gray matter volume of less than 0.1%. Thalamic volumes were measured on 3D T1-WI and segmented with Freesurfer (http://surfer.nmr.mgh.harvard.edu/). In addition to the absolute volumes, percentage changes relative to the baseline measurement in each patient were used for brain, gray matter, white matter and corpus callosum volume. Regional brain volumes were normalized with respect to the total ICV (calculated as the sum of the total brain volume and the total intra-ventricular cerebrospinal fluid volume). Normalized compartment volumes were calculated, as follows: brain parenchymal fraction = white matter volume + gray matter volume/ICV; white matter fraction = whit matter volume/ICV; gray matter fraction = gray matter volume/ICV; corpus callosum fraction = corspus calloum volume/ICV and thalamus fraction = thalamus/ICV. In all instances, re-test and inter-rater errors were below 0.5% and 0.3%, respectively.

3.2 Clinical assessment

Neuropsychological assessment

Participants were tested with the Czech-validated version of BICAMS.[100, 175] Cognitive processing speed was assessed with the Symbol Digit Modalities Test (SDMT)[102] with stimuli presented visually and only the oral response form recorded and by Paced Auditory Serial Addition Test-3 seconds (PASAT-3). Memory was tested with the Brief Visuospatial Memory Test Revised (BVMTR)[176] in the visual modality, and the California Verbal Learning Test Second Edition (CVLT2)[177] in the auditory sphere. For both BVMTR and CVLT2, only the initial learning trials of each test were administered.

Impairment for a single test was defined at the level of 1.5 standard deviation (z-score <1.5 compared with a healthy population), using the regression-based norms of 134 healthy controls adjusted for age, sex, and education.[100] Patients were evaluated as cognitively impaired when scoring outside the normal range in one or more of the BICAMS tests.[100, 175] Confirmed cognitive decline was defined as a newly abnormal BICAMS outcome at 12 months, confirmed at 24 months of the follow-up. Unconfirmed cognitive decline was defined as newly abnormal

BICAMS outcome at 12 or at 24 months. For the assessment of depressive symptoms, the Beck Depression Inventory was used.[178]

A proportion of patients was tested Minimal Assessment of Cognitive Function in Multiple Sclerosis (MACFIMS) battery.[88]

Neurological assessment

Disability was assessed using EDSS.[71] Sustained disability progression 1 (SDP1) was defined as an increase in EDSS by 1.0 point (if baseline EDSS>0) or 1.5 point (if baseline EDSS=0), confirmed after 6 or 12 months. Sustained disability progression 2 (SDP2) was defined as an increase in EDSS by 2.0 points (if baseline EDSS>0) or 3.0 points (if baseline EDSS=0) confirmed after 12 months.

A proportion of patients was tested with: 9 hole peg test, 25 foot walk test and Visual acuity-SLOAN chart.

4. Sample

4.1 SET study

The study of early interferon b1-a treatment in high risk subjects after CIS (SET) is an investigator-initiated, multi-centre, prospective observational study (EudraCT identification number 2005-001281-13). The study included 220 patients after first demyelinating event suggestive of MS in 8 centers within the Czech Republic (149 patients from General University Hospital, Prague; the rest of the patients from: KZ Hospital, Teplice; University Hospitals in Brno, Pilsen and Olomouc; St. Anne's University Hospital, Brno; Motol University Hospital, Prague and Kralovske Vinohrady University Hospital, Prague) between years of 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 or less, displayed the presence of two or more T2-hyperintense lesions on diagnostic MRI, and had the presence of two or more oligoclonal bands in cerebro-spinal fluid obtained at the screening visit prior to steroid treatment.[2, 104, 149] 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 48 months and subsequent long-term follow-up in routine clinical practice. Disability was assessed at baseline and every 6 months thereafter, while SDP was determined after 24 weeks from the 48 months examination. MRI examination was obtained at baseline, 6 months, and yearly thereafter. All patients started the treatment at baseline with 30 mg of intramuscular interferon beta-1a (IFNß-1a) once a week, which has been shown to delay conversion to clinically definite MS.[16] All patients were treated with 3–5 g of methylprednisolone for the first symptom before study entry, and a baseline MR examination was performed at least 30 days after steroid administration. Relapses were treated with 3–5 g of methylprednisolone during the study. The 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 on 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 in all participating centers, and all patients gave their informed consent.

The study was supported by Czech Ministries of Education and Health [NT13237-4/2012, MSM 0021620849, PRVOUK-P26/LF1/4, RVO-VFN64165/2012] and Biogen Idec

Figure 1: SET trial design.
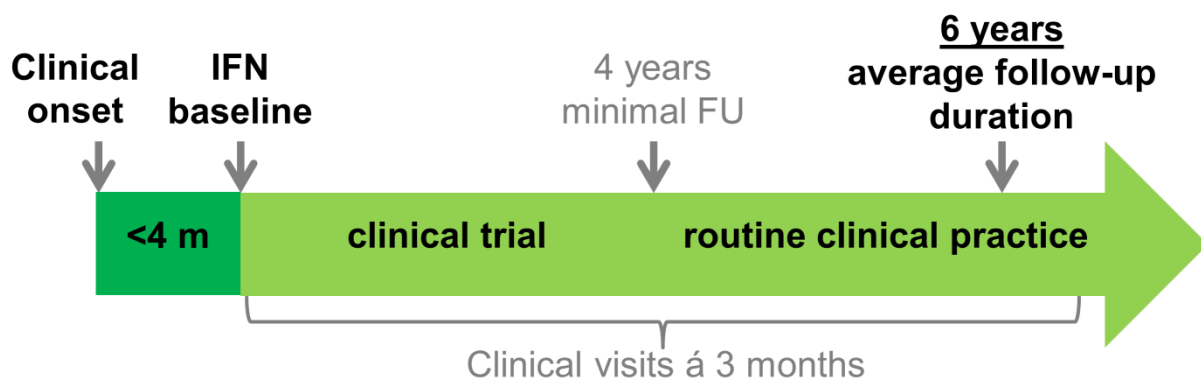
4.2 ASA study

The original Avonex–Steroid–Azathioprine (ASA) was an investigator-initiated, 2-year randomised, double-blind, placebo-controlled study investigating clinical and MRI outcomes of intramuscular IFNß-1a treatment either alone, or combined with low-dose azathioprine or low-dose azathioprine and prednisone in relapsing-remitting MS.[179, 180] The study included 181 patients with relapsing-remitting MS in 2 centers within the Czech Republic (163 patients from General University Hospital, Prague; 18 patiets from Motol University Hospital, Prague) between years of 1999 and 2003. Detailed description of the experimental protocol is provided elsewhere.[10, 179, 181] Inclusion criteria were as follows: clinically definite MS confirmed by MRI and at least 2 oligoclonal bands in the cerebrospinal fluid, age 18–55 years, RR course of MS, EDSS ≤3.5, and active disease defined by ≥2 relapses over the past 12 months or ≥3 relapses over the past 24 months.

The long-term extension of the study was an open-label study performed as part of the standardized clinical follow-up. The extension encompassed clinical visits every 3 months and quantitative MRI annually. Baseline of the study was defined as the time of IFNß-1a initiation.

The study protocol was approved by the Medical Ethics Committees of the General University in Prague and 1st Faculty of Medicine, Charles University in Prague, by the University of Buffalo and by the ethics committees in the participating centres. All patients provided their written informed consent.

Figure 1. ASA study design.

Design of the ASA study

Disease-modifying treatment initiation

MRI scan every 2 months | MRI scan every 12 months

2-year randomised, double-blind, placebo-controlled trial: the ASA study | 3-year open-label extension of the ASA study | Follow-up in routine clinical practice

Baseline (1999-2003) | 1 year | 2 year | 5 year | 12 years (average follow-up duration)

Baseline for the confirmatory analysis

Clinical visit every 2 months | Clinical visit every 3 months (ongoing follow-up)

4.3 GQ study

Grant Quantitative (GQ) study was an <u>investigator-initiated, single-centre, 3-year prospective</u> <u>observational study</u> including 1226 MS patients. The study investigated application of comprehensive battery of clinical and para-clinical measures to evaluate MS progression in routine clinical practice. Inclusion criteria were as follows: clinically isolated syndrome or clinically definite MS confirmed by MRI and cerebrospinal fluid examination, Czech native speaker, participation in brain MRI volumetric assessment programme and age 18 or more. The exclusion criteria were signs and symptoms suggestive of a disease other than MS and serious psychiatric disorder. Enrolment into the GQ study started in June 2012. For the present analysis, the database was locked in October 2015. The clinical follow-up included visits every 3 months for entire follow-up duration in routine clinical practice. Small proportion of patients with clinically and MRI stable disease had visits every 6 months.[20, 182]

The study protocol was approved by the Medical Ethics Committee of the General University in Prague and 1st Faculty of Medicine, Charles University in Prague. All patients provided their written informed consent.

The GQ study was supported by the Czech Ministries of Education and Health (NT13237-4/2012, PRVOUK-P26/ LF1/4, RVO-VFN64165).

4.4 QMRI programme

All MS patients with brain MRI performed at Department of Radiodiagnostics of General University Hospital in Prague starting in March 2000 were included in the quantitative magnetic resonance imaging (QMRI) programme.

This is a real-world clinical cohort (compared to clinical studies: no regular MRI time points, changes of medication, different treatment strategies). The inclusion criteria were: age >18 years, CSF assessment and diagnosis of clinically isolated syndrome, relapsing-remitting MS, secondary or primary progressive MS.

From the 3,430 patients (with 20,053 brain MRI scans) enrolled in the QMRI programme, 1,171 patients had confirmed diagnosis of MS, ≥4-years MRI follow-up and ≥5 MRI scans over follow-up. Together 1,757 MS patients had ≥3 MRI scans and ≥4 years of MRI follow-up duration.

All MRI scans in all patients performed on the same 1.5-Tesla scanner (Gyroscan, Phillips) with the same MRI protocol (T1-WI, 1 mm; FLAIR, 1.5 mm).[183]

Table 1 and Figure 1. Sample characteristics.

Baseline characteristics	N=1564 (≥4 years, ≥5 scans)
Number of females	1122 (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 GM % volume change	-0.56±0.53
Annualized CC % volume change	-0.74±1.00

NUMBER OF PATIENTS					
Number of MRI scans per patient	MRI follow-up duration (years)				
	≥4	≥5	≥6	≥8	≥10
≥4	1688	1304	924	356	161
≥5	1564	1245	904	355	161
≥6	1354	1146	860	348	158
≥8	745	701	621	320	155
≥10	383	371	351	267	151

MISSING VALUES	
Brain parenchymal fraction	0.9%
Brain volume	0.9%
T2 lesion volume	2.7%
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%
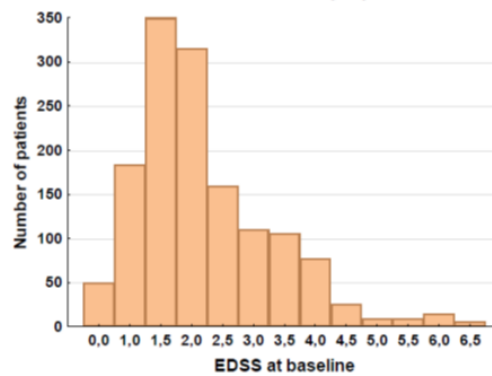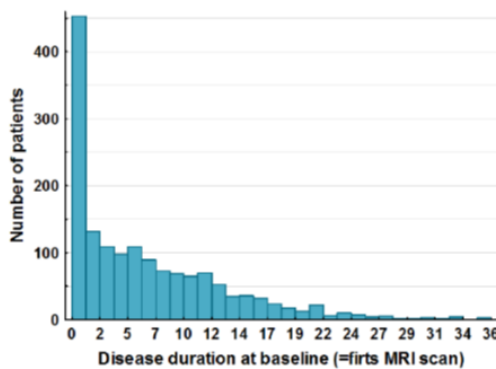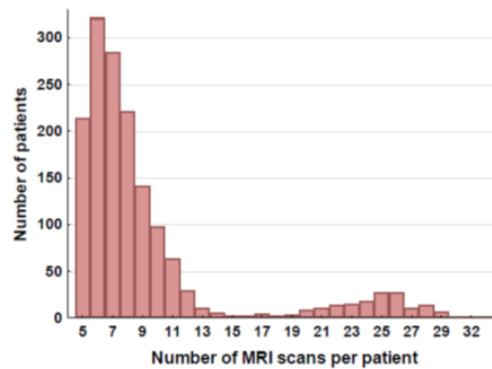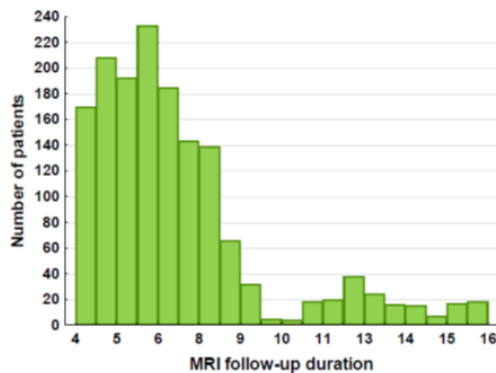
4.5 Healthy controls

Enrolment of healthy controls started in 2001 and was completed in 2014. The exclusion criteria were: history of neurological disorder affecting brain atrophy, abnormal brain MRI findings, and chronic medication with chronic anti-inflammatory or immune-modulatory properties. The original healthy controls database included 133 subjects (with 410 MRI scans). Together, 58 subjects had $\geq$2 years MRI follow-up and $\geq$3 MRI scans over follow-up.

5. Summary of selected studies

5.1 A novel semiautomated pipeline to measure brain atrophy

Background: To date, there are a number of manual, semi-automated and automated techniques[7-9] for the assessment of global and regional brain volume measures. Although the SIENA method is widely used in clinical trials and research studies in assessing brain atrophy in MS patients, there are number of studies using different volumetric techniques. However, comparative studies of different volumetric methods are limited and complicated by a number of methodological issues including the lack of a gold standard for image acquisition, short follow-up durations, and small sample sizes.[184-187]

Objective: The aim of this study was to investigate the agreement between MRI measures obtained by in-house developed ScanView software and commonly used volumetric techniques for the assessment of T2 lesion and whole brain volumes and their changes in a large, longitudinal sample of MS patients. In addition, we assessed intra-individual variability of whole brain volume loss changes provided by different software methods.

Methods: This study was conducted in two large observational cohorts: of patients after the first demyelinating event suggestive of MS, who participated in the original SET study[2, 11, 149, 167, 188] and of patients with relapsing-remitting MS who participated in the original ASA trial.[1, 4, 10, 179, 188, 189] Given that SIENA was the most widely used software for brain atrophy assessment when the ASA and SET studies were initiated, we analyzed volumetric data obtained by ScanView and SIENA in this study.

The study was performed on a large cohort of actively treated MS patients with long-term follow-up. Together 3340 MRI scans from 209 patients after first demyelinating event suggestive of MS, 181 relapsing-remitting MS patients and 43 controls were analyzed. The average number of MRI scans and follow-up duration was 8.2 and 6.5 years, respectively. All MRI scans were performed on the same scanner with the same protocol in the Department of Radiodiagnostics in Prague, however volumetric assessments were performed independently in two different neuroimaging centers using different software packages. Volumetric analysis by ScanView software was applied in Prague. Commonly used techniques, such as SIENA, SIENAX and Jim software were applied in Buffalo. Correlations between MRI measures were

evaluated using correlation coefficients. Intra-individual variability of longitudinal MRI data was estimated by mean squared error.

Results and discussion: Although the absolute values of MRI outcomes provided by both volumetric techniques were significantly different, they were strongly correlated. In this context, it is important to emphasize that absolute and relative volumetric data and its cut-off values obtained by different software cannot be simply adopted by other MS centers, where different volumetric packages or even different MRI scanners are applied.

The differences between absolute values of whole brain volumes are likely due to inherent differences in the processing methods used between the two centers. In Buffalo, whole brain volume was calculated using the SIENAX method, which first uses the Brain Extraction Tool to isolate the brain. All brain extractions were manually quality controled and corrections were made as necessary. The somewhat larger brain volumes seen in the Prague analysis may stem from differences in how the brain was isolated and that it was a fully-automated procedure without manual corrections. Regardless, the two measures show strong correlations and it is well known that different packages do not yield perfectly identical results.[190, 191] In addition, assessment of T2 lesion volume using the ScanView was performed using a fully automated procedure compared with the semi-automated Jim software. The ScanView method is essentially based on intensity thresholds selectively applied, where T2 lesions are defined to consist of voxels with intensity >140% of the mean of white matter. Additional analysis showed that T2 lesions volume defined at the level of voxels intensity >130% corresponds best with T2 lesion volume estimates provided by the Jim software applied in Buffalo Neuroimaging Analysis Center. This may explain the difference between absolute values of T2 lesion volumes obtained by these two techniques.

In general, substantially stronger correlations were found among cross-sectional measures with correlation coefficients being between 0.90 and 0.95. The correlations among longitudinal measures were approximately 0.83. In case of longitudinal measures, it is important to note that the correlation coefficients were slightly lower when restricting the statistical analysis to specific timepoints or using only a single MRI scan per patient. We suggest that stronger correlations among cross-sectional measures can be explained by lower inter-subject biological variability of longitudinal data. However, lower correlations for longitudinal measures could be also attributed to the fact that they are derived measures with higher statistical variance. This is especially true for segmentation-based techniques based on cross-sectional segmentations at two different time points with subsequent calculation of

percent change resulting in two sources of measurement error. While a strong correlation between cross-sectional data does not automatically implicate a strong correlation between longitudinal measures, we suggest that further validation studies of MRI volumetric techniques should also investigate correlations of longitudinal volumetric data.

Intra-individual variability of percent whole brain volume loss as assessed by the SIENA method[120] was lower compared with ScanView,[20, 149] which supports the findings of previous research. These results represent indirect evidence that suggests a higher individual accuracy of the SIENA method for the assessment of brain atrophy over both short-term and long-term follow-up. The higher variability of the ScanView technique, which relies predominantly on a segmentation-based approach, may be explained by the fact that segmentation at two different time points leads to different spatial and intensity distributions of the tissue classes involved. This is associated with a misclassification of brain voxels which have relatively ambiguous intensity. This may have a technical impact on the overall quality of the analysis. It should also be noted that confounding biological factors may also yield results which are not as accurate compared to inherently longitudinal analyses.[7-9]

In this context, the accuracy of longitudinal MRI volumetric measures in individual patients and its applicability in clinical practice is discussed. For example, a cut-off value of -0.40% in terms of annualized percent whole brain volume loss has been proposed as a threshold value for abnormal brain atrophy in several recent studies.[21, 192] However, it remains to be elucidated whether or not utilizing such a cut-off value is feasible in clinical practice. Although, the measurement error of whole brain % volume loss using longitudinal techniques, like SIENA is less than 0.2%,[120] this does not take into account an important biological variability. In this study, we found that a mean deviation of whole brain % volume change from estimated linear regression slope (average unstandardized non-squared residual error) in each timepoint is approximately ±0.30% over a short-term follow-up duration of 2 years in both techniques. Average mean deviations of the percent whole brain volume change observed in MS patients were slightly higher, but comparable with healthy controls (0.28% in ScanView and 0.20 in SIENA; p=0.003). We hypothesize that the slightly higher variability obtained on MS patients may be attributed to the higher biological variability associated with MS related pathophysiological brain tissue changes and immunomodulatory treatment.

Interestingly, significantly higher mean deviations (±0.61% for ScanView and ±0.72% for SIENA) were found in the analysis performed over a long-term follow-up duration. We hypothesize that this observation could be an effect of an imperfect estimation of residual errors over long-term follow-up due to non-linear trajectories of brain volume loss in some MS

patients. Given that the presented residual errors over short-term and long-term follow-up were similar compared to the suggested cut-off values for pathological percent whole brain volume change, application of these thresholds in clinical practice may be challenging. However, we propose that the accuracy of brain atrophy assessment may be improved substantially if an estimation of brain atrophy would be based on an analysis of multiple MRI scans. In this context, it remains to be elucidated what number of MRI scans and how long of a follow-up period is needed to significantly reduce percent whole brain volume change measurement errors. Such information would allow for increased confidence in the assessment of brain atrophy in individual patients.

Conclusion: Our study suggests that the ScanView technique presented in this study demonstrates comparable accuracy to already established methods for the assessment of T2 lesion burden and whole brain volume changes in MS. Although the correlation of longitudinal MRI measures between ScanView and SIENA was strong, the latter showed lower intra-individual variability over follow-up. With the growing recognition of the clinical relevance of brain volume loss in MS, standardization and validation of MRI volumetric techniques may be of great importance for both patients and physicians. Therefore, we suggest that further studies investigating the precision, reproducibility and intra-individual variability of MRI volumetric techniques over long-term follow-up are needed for use of MRI volumetric measures in clinical trials and daily practice.

Limitations: It needs to be acknowledged that ScanView is essentially based on intensity thresholds and was originally customized to be applied on our specific MRI scanner (1.5-Tesla Gyroscan; Phillips with quadrature coil) and specific MRI sequences (FLAIR, THK 1.5 mm and T1-W/FFE 3D, THK 1.0 mm). The applicability of the ScanView technique has already been tested in different MRI centers and different MRI scanners, such as: 1.5-Tesla Intera; Philips and 1.5T MAGNETOM Avanto; Siemens. However, the data has not yet been reported. Currently, we are testing applicability of this technique on data from a 3-Tesla MAGNETOM Skyra; Siemens with promising results. In spite of this, it is important to acknowledge that there are potentially technical difficulties in the adaptation of this technique on other MRI scanners and other MRI protocols. This represents one of the drawbacks of the ScanView approach, which was designed solely for academic purposes. Also, it must be emphasized that the estimation of mean square error in healthy controls was based on small number of subjects and small number of longitudinal MRI scans. Therefore, our results need to be confirmed in future

studies. Finnaly, linear regression slopes assumed a linear course of brain volume loss.[21] Although, the vast majority of patients have a linear trajectory of brain atrophy over short-term, considerable proportion of non-linear trajectories are found in patients over long-term follow-up. Hence, assumption of linearity of brain atrophy should be verified in future studies. Here, a nonlinear course of brain atrophy in some patients might potentially influence the estimation of intra-individual variability of MRI longitudinal measures using MSE, especially over long-term follow-up, as shown in our study.

Reference:

Uher T, Krasensky J, Vaneckova M, Sobisek L, Seidl Z, Havrdova E, Bergsland N, Dwyer MG, Horakova D, Zivadinov R. A Novel Semiautomated Pipeline to Measure Brain Atrophy and Lesion Burden in Multiple Sclerosis: A Long-Term Comparative Study. J Neuroimaging. 2017 Nov;27(6):620-629. doi: 10.1111/jon.12445.[174]

Figure 1. Schematic representation of the skull stripping by ScanView software.
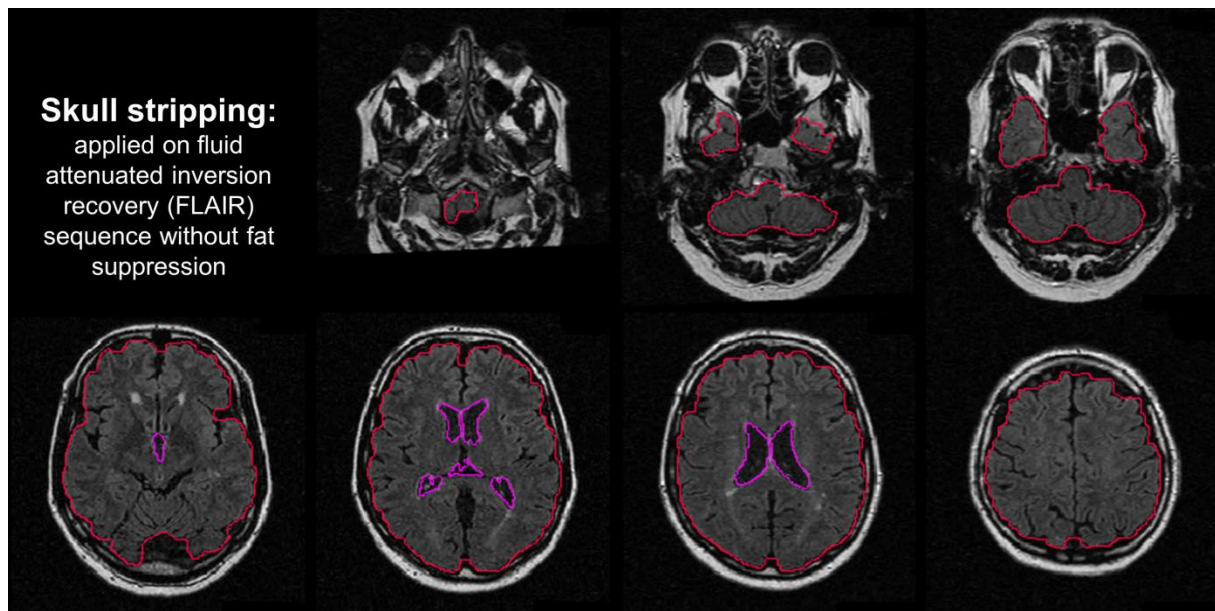
Figure 2. Example of lesion segmentation provided by ScanView and Jim software.

Automated identification of T2-lesions by ScanView software (Prague)

Semi-automated identification of T2-lesions by Jim software (Buffalo)

| # 1 patient | # 2 patient | # 3 patient | # 4 patient |
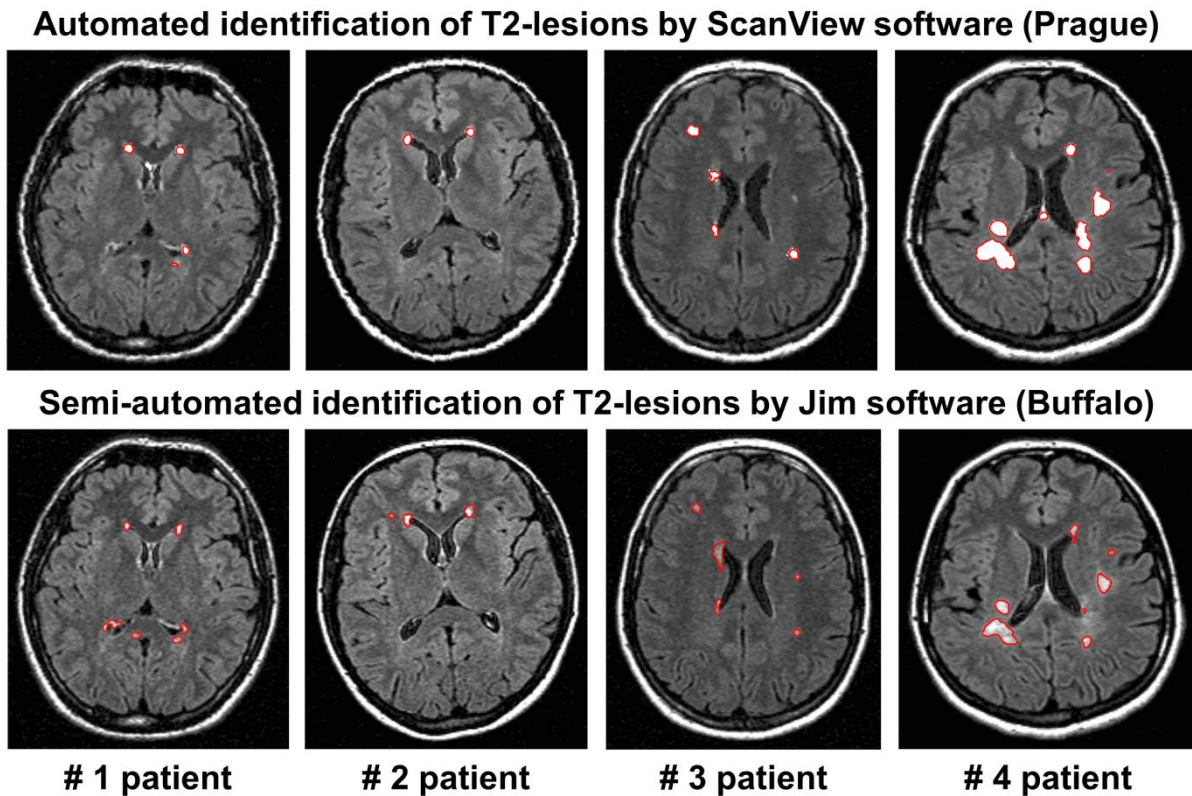
Figure 3. 3D Gaussian filter applied by ScanView software.

3D Gaussian filter

Cube 3x3x3 mm
(27 pixels)

3 pixels (mm)
3 pixels
3 pixels

Intensity of each pixel (1x1x1 mm) is weighet (multiplied by ratio) according its position related to the central pixel:

ratios

1x	2x	1x
2x	4x	2x
1x	2x	1x

2x	4x	2x
4x	8x	4x
2x	4x	2x

1x	2x	1x
2x	4x	2x
1x	2x	1x

The sum of multiplied intensities of the 3x3x3 mm cube is devided by the sum of weightings = The average pixel intensity

Figure 4. Schematic representation of the T2 lesion volume measurement by ScanView software.
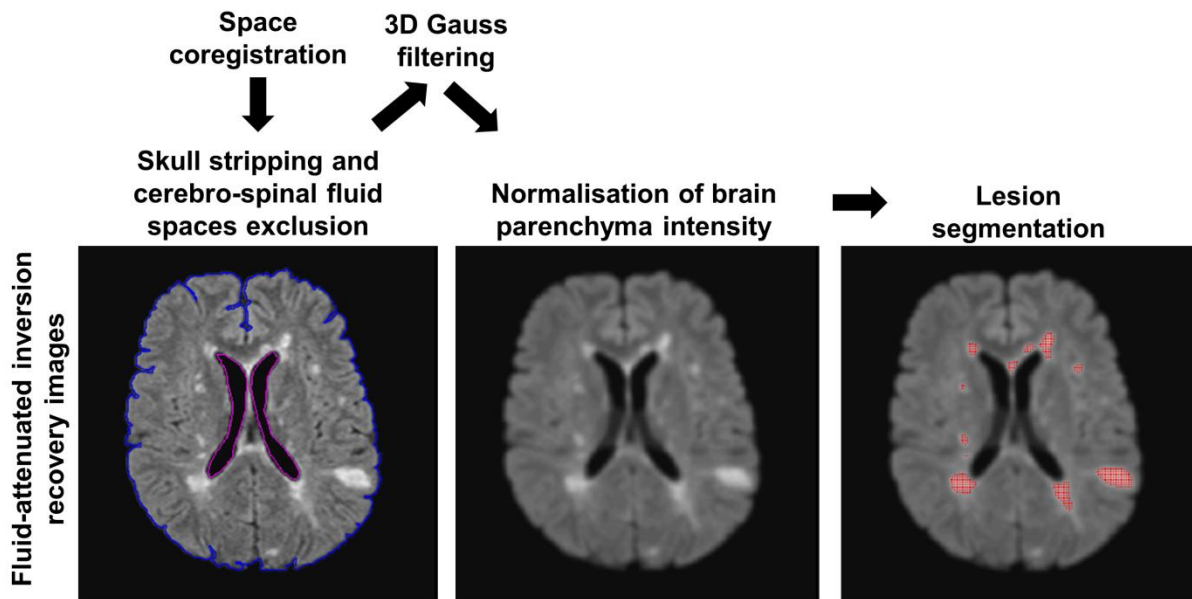
Figure 5. Example of mean squared error estimation in individual subject.

$$MSE_i = [(e_{i1}/SD_i)^2 + (e_{i2}/SD_i)^2 \ldots + (e_{ij}/SD_i)^2] / N_i$$

- i = individual subject
- j = MRI scan rank order (1,2…j)
- e = residual error of single MRI measurement calculated as: observed – estimated MRI value
- SD_i = standard deviation calculated from all MRI scans within one individual subject (i)
- N_i = number of MRI scans within individual (i) subject over follow-up
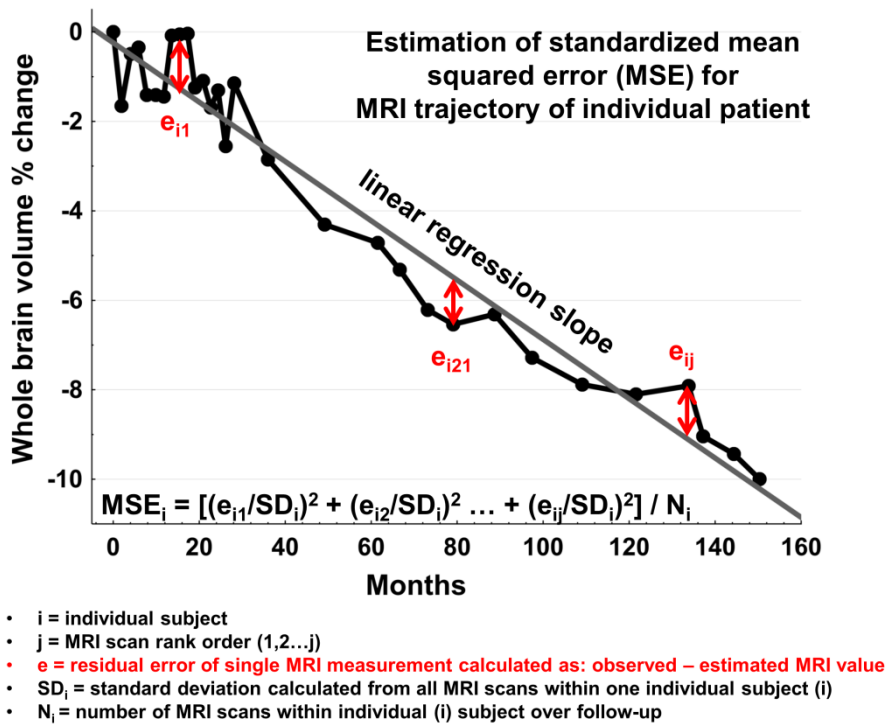
Figure 6. Correlation graphs between MRI measures performed in Prague and Buffalo: A) T2 lesion volume; B) Absolute T2 lesion volume change; C) whole brain volume; D) percent whole brain volume change.

Figure 7. Schematic representation of the homogenization filters applied by ScanView software.

Original MRI image

3D Gaussian filter kernel (3x3x3)

Median filter kernel (3x3x3)

Combined 3D Gausssian filter kernel (5x5x5) and edges-enhancing filter kernel (7x7x7)

5.2 Pathological cut-offs of brain volume loss.

Background: Although a recent long-term study attempted to define a specific cut-off for pathological whole brain volume loss rate,[21] broadly accepted threshold values of regional and global pathological brain atrophy have still not been established. Moreover, it is unclear whether reported cut-offs would be applicable in different MS populations, in short-term studies, or with application of different MRI volumetric software.

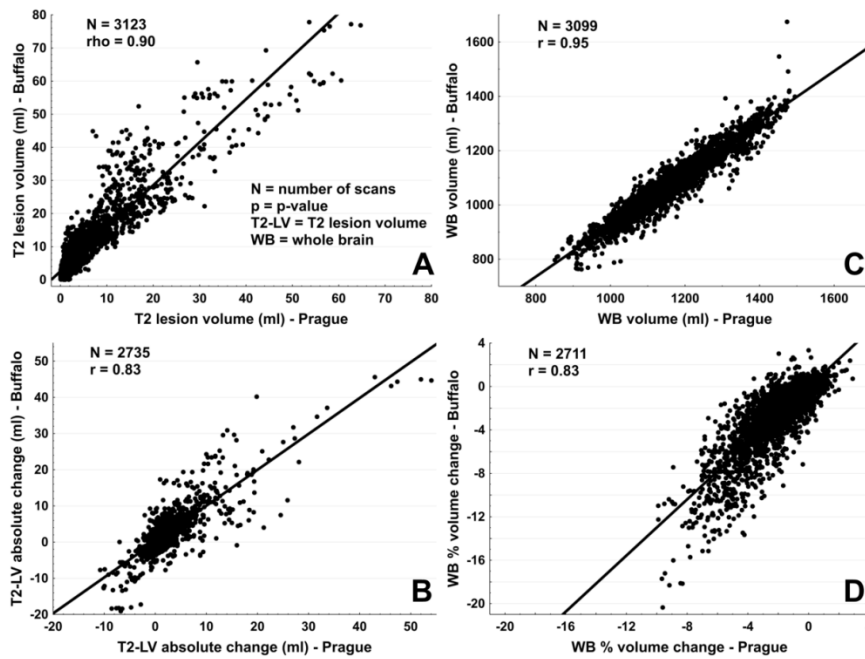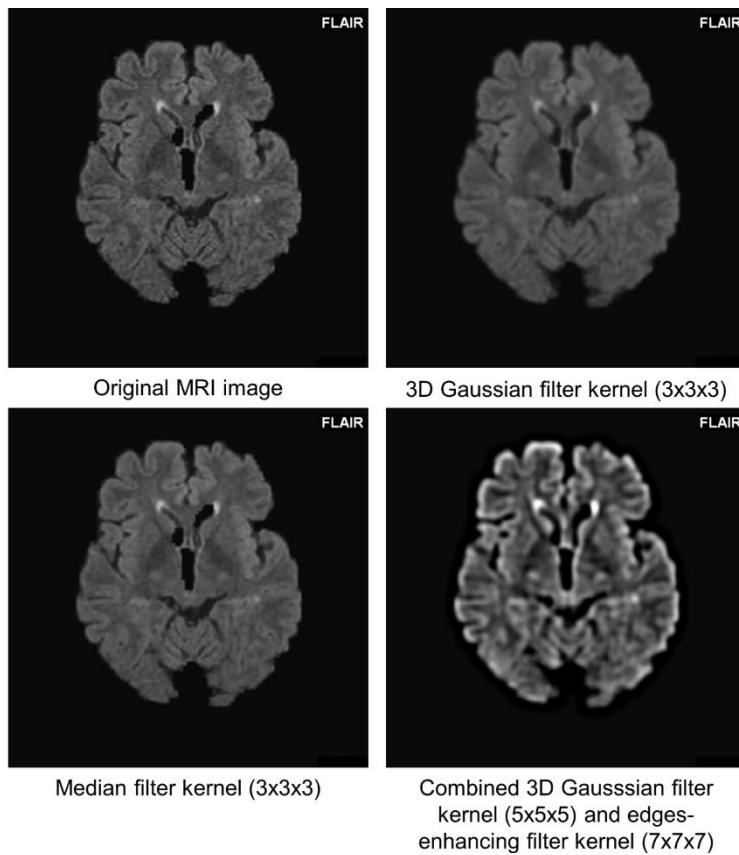**Objective:** In this study we aimed to establish cut-off values of global and regional brain volume loss able to discriminate between healthy controls and MS patients. More specifically, the aim of this study was to discriminate between healthy controls and MS patients by establishing specific cut-offs for whole brain[7-9, 119] gray matter, thalamic[104, 193] and corpus callosum volume loss rates.[149, 174, 194-196]

Methods: The present study was conducted using 4 cohorts: 1) patients after first clinical event suggestive of MS (CIS), who participated in the extension of the original SET trial[2, 11, 149, 197] or in the QMRI programme, 2) patients with relapsing-remitting MS who participated in the extension of the original ASA trial,[1, 179, 188, 189, 197, 198] or in the QMRI programme, 3) patients with secondary-progressive MS from the QMRI programme, and 4), and a group of age-matched healthy controls. Together 386 CIS patients, 964 relapsing-remitting MS patients, 63 secondary-progressive MS patients, and 58 healthy controls were included in this longitudinal study. A total of 11,438 MRI scans performed on the same MRI scanner with the same protocol were analysed. Annualised percentage changes of whole brain, grey matter, thalamus and corpus callosum volumes were estimated. We investigated cut-offs able to discriminate between healthy controls and MS patients.

Results and discussion: Specific cut-offs of brain volume loss rates were identified as possible discriminators between healthy controls and MS patients. Most importantly, cut-offs with a high specificity for the identification of brain volume loss outside the normal range will have the highest clinical relevance. Therefore, the predictive accuracy of cut-offs was investigated using a predefined specificity of 80, 90, and 95%.

At these predefined levels of specificity, highly similar brain, gray matter, thalamic and corpus callosum volume loss cut-offs were determined, regardless of the investigated MS cohort or volumetric software in case of brain volume loss. Generally, the corpus callosum

volume loss cut-offs showed a slightly higher sensitivity to discriminate between healthy controls and MS patients (38-63%), as compared with brain volume loss analysed by ScanView and gray matter or thalamic volume loss cut-offs (17-52%). More specifically, at a predefined specificity of 90%, the annualised percentage change cut-off of corpus callosum volume (-0.57%) was able to distinguish between healthy controls and patients with the highest sensitivity (51% in CIS, 48% in relapsing-remitting MS and 42% in secondary progressive MS patients). Lower sensitivities (22-49%) were found for cut-offs of whole brain, grey matter and thalamic volume loss. It is worth noting that gray matter volume loss cut-offs showed a similar sensitivity compared to corpus callosum volume loss, but only in the CIS and relapsing-remitting MS cohort.

Importantly, due to a relative low accuracy of the proposed cut-offs, any increase of their sensitivity (by increasing of their values) would lead to a large decrease in their specificity. For example, the brain volume loss cut-off value with 90% sensitivity would have only 10% specificity for the identification of brain volume loss outside the normal range as measured by ScanView in the relapsing-remitting MS cohort. This would prevent application of the proposed cut-offs in individual patients. Although, highly specific cut-offs are associated with a high rate of false negative results, we might confidently identify brain atrophy outside the normal range at least in a proportion of individuals.

Taken together, sensitivities of the established cut-offs were approximately 10-20% higher in the subset of relapsing-remitting MS cohort from the ASA study compared to the CIS, or the relapsing-remitting MS patients from the QMRI programme, possibly due to ASA cohort consisting predominantly of patients with higher disease activity and a worse availability of more effective immunomodulatory drugs. These findings suggest that predictive accuracy of the cut-offs may differ in various MS populations. relapsing-remitting MS cohort from the ASA study[179, 189] showed greater global and regional brain volume loss compared to both the CIS[2] and the relapsing-remitting MS patients from the QMRI cohort, which resulted in slightly higher predictive accuracies of cut-offs in this group. A higher brain volume loss in the relapsing-remitting MS patients from the ASA cohort corresponded to higher disease activity during the study and preceding study baseline.[179, 189] Moreover, we suggest that differences in immunomodulatory drugs might also play an important role. There are several studies showing that immunomodulatory drugs, such as alemtuzumab, fingolimod or natalizumab[4, 8, 199-201] are more effective in suppressing accelerated brain atrophy in MS patients compared to interferons or glatiramer acetate.[2, 8, 202] This was also observed in patients from the QMRI cohort, who switched from interferons to natalizumab treatment (data not shown). Additionally, not only

the proportion of patients (SET: 19.2%, ASA: 23.9%, QMRI: 27.8%), but also an average proportion of the follow-up duration spent on natalizumab or fingolimod was substantially higher in the QMRI cohort (12.0%) than in the SET or ASA cohorts (6.0-7.1%). A decreased availability of more effective immunomodulatory drugs in the ASA cohort was caused by the fact that immunomodulatory drugs initiation in this cohort started between 1999 and 2003, which is 5 years (median) earlier than the SET, and 7 years earlier than the QMRI cohort. In this context, we suggest that decreased accuracy of the cut-offs may be expected in MS cohorts treated with immunomodulatory drugs that are more effective in limiting the progression of brain atrophy. Conversely, a higher sensitivity of the cut-offs could be expected in untreated or highly active MS patients with a greater rate of brain atrophy. Although different immunomodulatory drugs show variable effectiveness in supressing pathological brain atrophy, treatment status was not considered in our analyses since we aimed to provide more general and treatment-effect independent cut-offs for brain atrophy rates, as suggested in a recent study.[21]

To improve estimation of annualized percent change over follow-up, we fitted linear regression slopes to all available brain percentage volume change measurements within the individual subject. We hypothesise that overall predictive accuracy of the cut-offs for brain volume change may be lower in clinical practice due to considerable intra-individual fluctuations of brain volume outcomes over follow-up, as a result of various biases.[7, 8, 119, 174] Moreover, biological variability and measurement errors of brain volume assessment could be further magnified in real-world settings and clinical trials where the follow-up time is typically shorter. To evaluate the applicability of the proposed cut-offs in a short-term period, all analyses were re-evaluated using 2-year data. Importantly, we confirmed the discriminative accuracy of established cut-offs. Indeed, the sensitivity of most of the cut-offs using 2-year data was approximately 10-20% higher compared to long-term follow-up, which is likely a result of greater brain volume loss during the first years after immunomodulatory drugs initiation due to pseudo-atrophy effects.[203] Also a lower proportion of patients on more effective disease-modifying treatments during the first years of drug treatment may contribute to the underlying cause of this finding.

A recent study investigating predominantly relapsing-remitting MS patients showed that using a PBVC cut-off of <-0.40%, as measured by SIENA, is able to discriminate between healthy controls and relapsing-remitting MS patients with 80% specificity and 65% sensitivity.[21] In our study, at the predefined level of 80% specificity, we identified a slightly higher cut-off value (-0.34%) of PBVC as measured by SIENA. Interestingly, the PBVC cut-

off and its z-score were comparable with brain volume loss cut-offs identified by ScanView but slightly different compared with cut-offs reported in the previous SIENA study.[21] Hence, cut-offs presented in this study are not prepared yet for extension into clinical practice. Our findings suggest that proposed cut-offs might be largely influenced not only by MRI volumetric software applied but also by characteristics of the healthy controls cohort. Given that the current, as well as the recent study,[21] included relatively small samples of healthy controls, development of a large healthy controls MRI dataset is needed to provide physiological rates of brain volume loss for reference in future clinical studies. Last, but not least, we must not forget that all MRI scans analysed in this study were performed using a single 1.5-Tesla scanner with a standardised protocol. Therefore, the individual accuracy of brain volume loss cut-offs may be substantially affected when using different MRI scanners or protocols when monitoring individual patients.

Global and regional brain volume loss cut-offs were associated with greater accumulation of disability, as assessed by annualised absolute EDSS change in the CIS and the relapsing-remitting MS cohorts, highlighting the clinical relevance of this measure. We hypothesize that the small sample size of the SPMS cohort, in which a relatively high proportion of subjects were on effective immunomodulatory drugs may explain the surprisingly low brain volume loss rates and the limited associations observed between disability accumulation and cut-offs of brain volume loss in this cohort.

Conclusion: Our study suggests that monitoring established global and regional brain atrophy rates may facilitate the identification of patients with disease progression who are at an increased risk of accumulating permanent disability over an extended period.

Limitations: Although, cut-offs measured by SIENA were comparable with ScanView, they were associated with higher sensitivity. This finding is in agreement with the recent study showing lower intra-individual variability (i.e. higher accuracy) of the registration-based SIENA compared with segmentation-based ScanView software.[174] Interestingly, cut-offs measured by SIENA also showed higher sensitivity compared with CC volume loss cut-offs measured by ScanView. Therefore, we hypothesize that an analysis of gray matter volume loss or CC volume loss using a more accurate registration-based method could further improve the sensitivity of proposed cut-offs in future studies. Given that direct comparison of our global and regional cut-offs within the existing literature is not possible, our results need to be confirmed by further studies. Finally, global and regional brain volume loss was analysed using different volumetric software (ScanView for WB and CC; SIENAX for gray matter). Therefore,

direct comparison between MRI cut-offs provided by different software should be taken with caution. Nevertheless, a relatively large representative sample of real-word MS patients, inclusion of patients at very early stages of MS progression, and analysis over a large number (11,438) of MRI scans performed over long follow-up duration, all characterise strengths of this study. Finnaly, potential presence of non-linearity of brain atrophy trajectories over long-term follow-up has not been verified.

Reference:

Uher T, Vaneckova M, Krasensky J, Sobisek L, Tyblova M, Volna J, Seidl Z, Bergsland N, Dwyer MG, Zivadinov R, De Stefano N, Sormani MP, Havrdova EK, Horakova D. Pathological cut-offs of global and regional brain volume loss in multiple sclerosis. Mult Scler. 2017 Nov 1:1352458517742739. doi:10.1177/1352458517742739.[174]

Figure 1. Spaghetti plots and observed frequency distribution of annualised percent changes of whole brain volume loss with a fitted normal distribution overlay, and the optimal cut-offs discriminating healthy controls from MS patients.

Figure 2. Spaghetti plots and observed frequency distribution of annualised percent changes of corpus callosum volume loss with a fitted normal distribution overlay, and the optimal cut-offs discriminating healthy controls from MS patients.

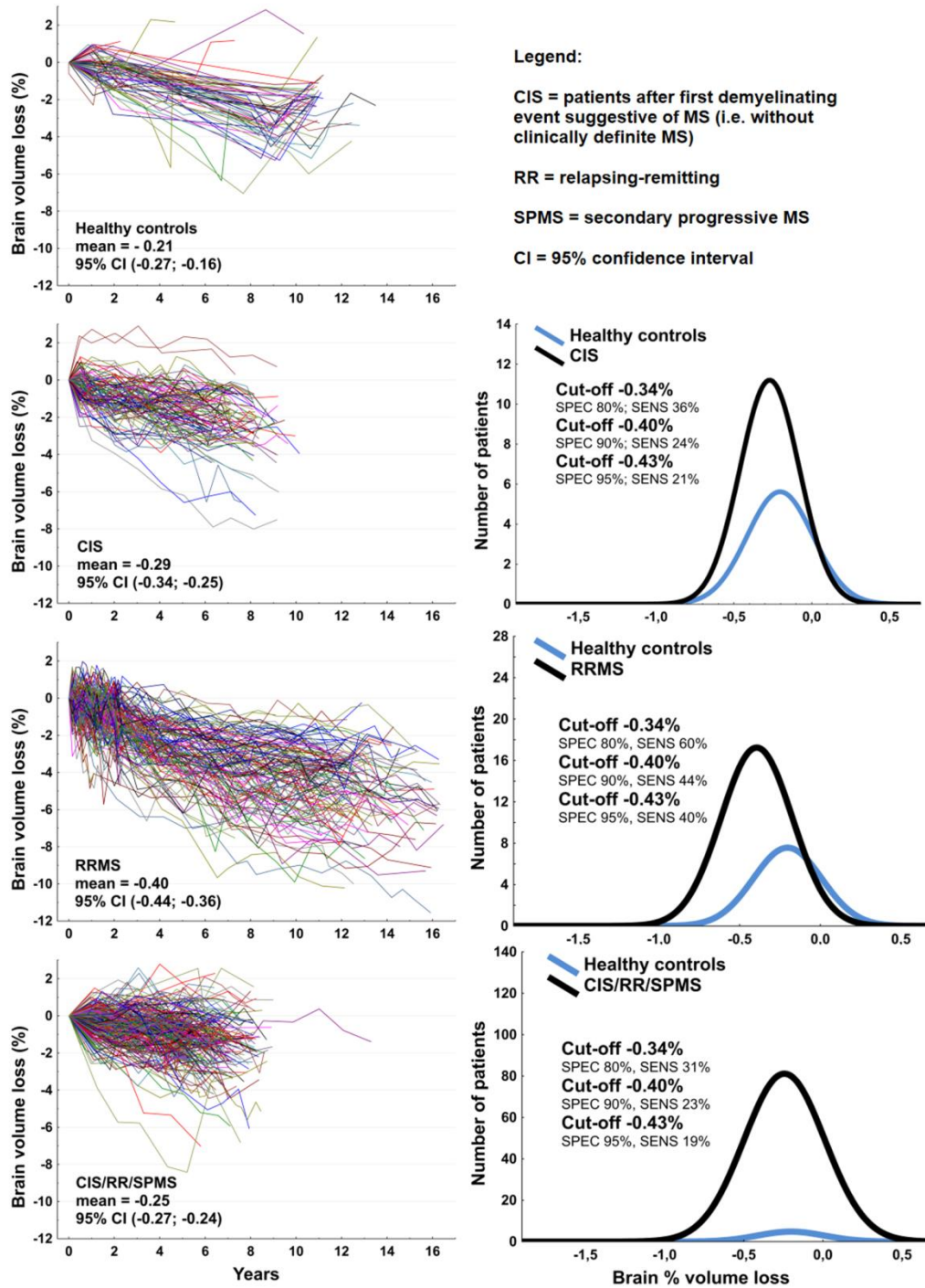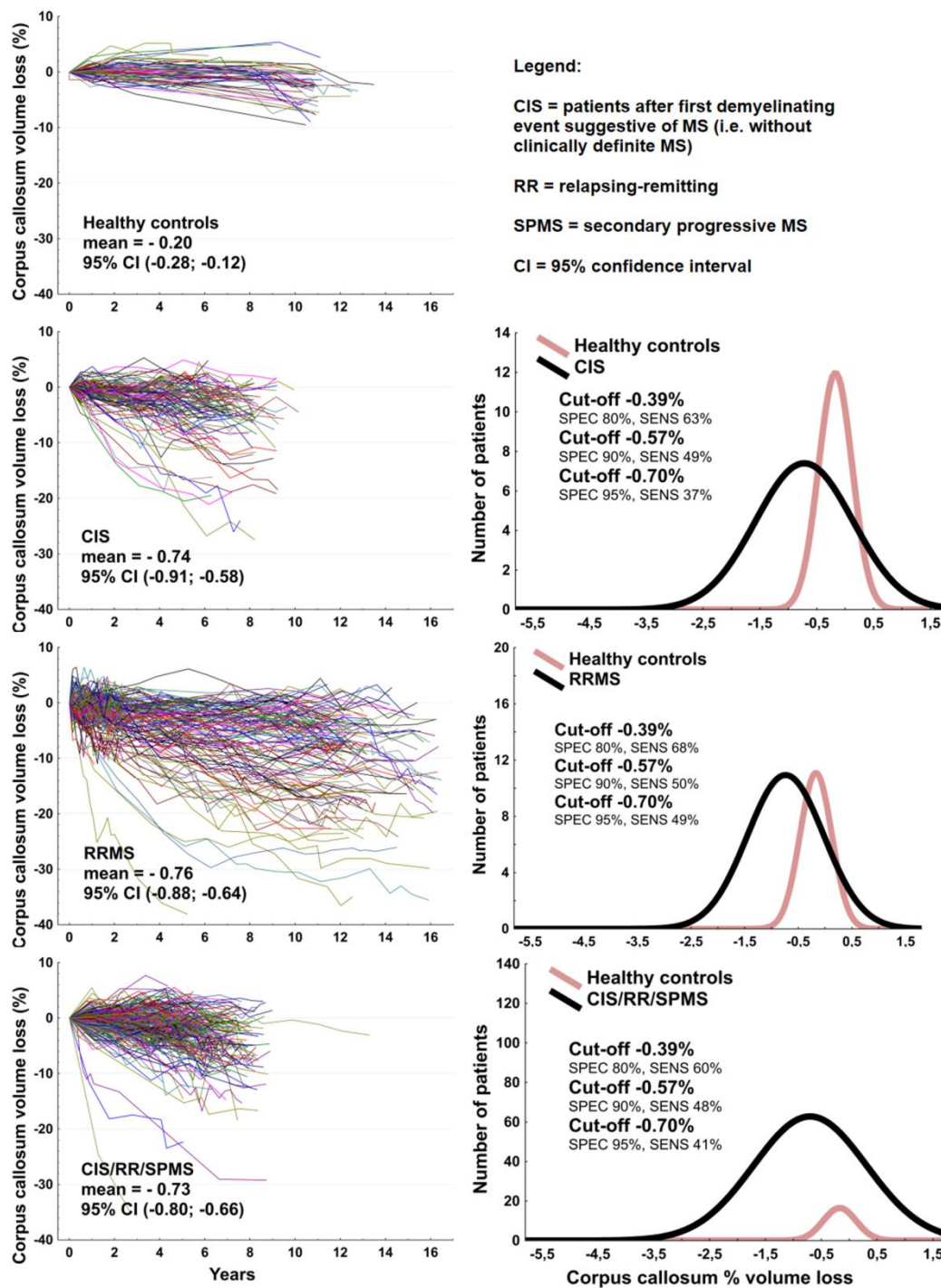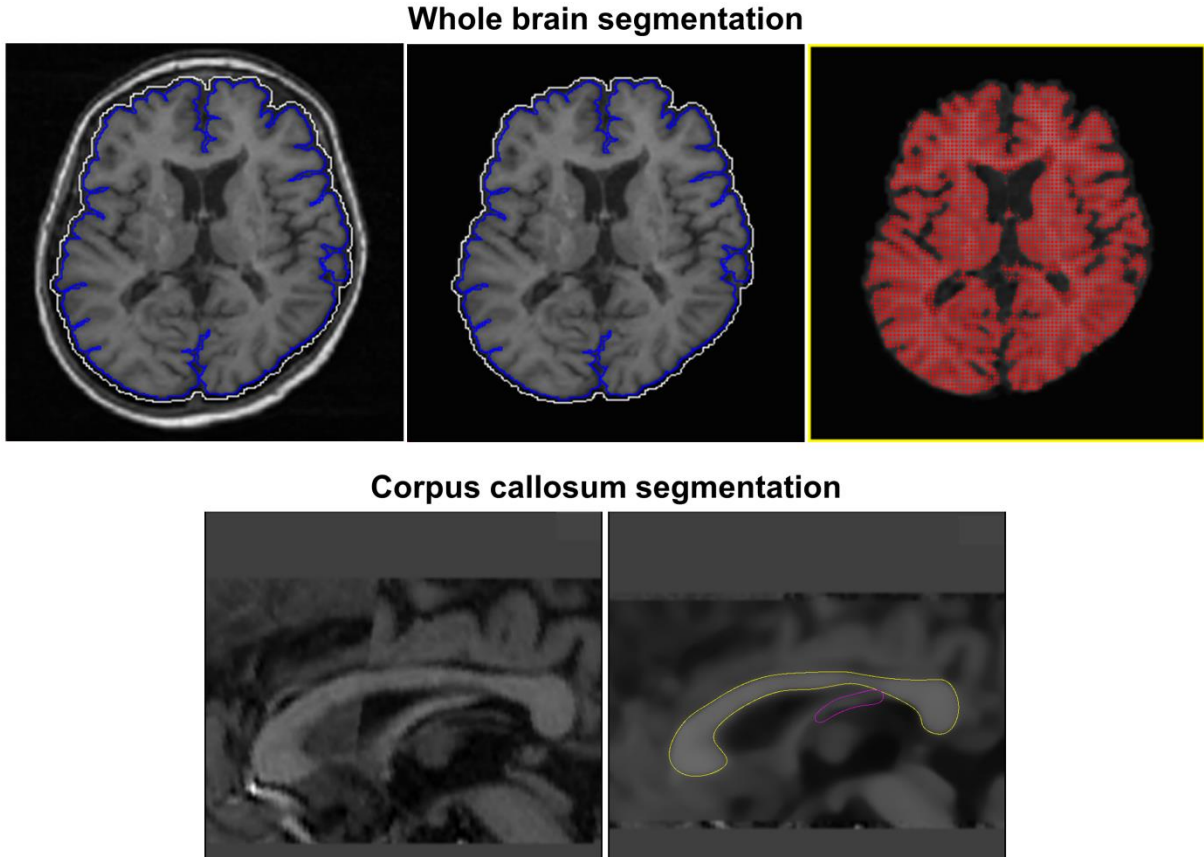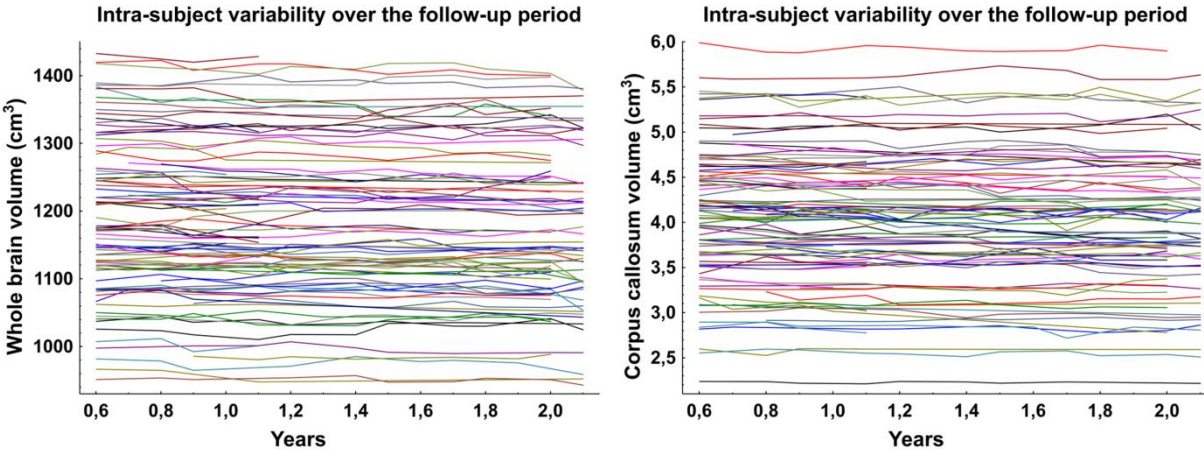Figure 3. Whole brain and corpus callosum segmentation by ScanView software.

Whole brain segmentation

Corpus callosum segmentation

Figure 4. Intra-subject variability of whole brain and corpus callosum volumes analysed by ScanView software over the follow-up period.

5.3 Occurrence of non-linear brain volume loss in multiple sclerosis patients

Background: In recent MS studies with repeated MRI measures, calculation of linear regression slopes for estimation of individual rates of brain volume loss was used. Although, linear course of brain volume loss was assumed, a potential occurrence of non-linear brain volume loss trajectories has not been investigated yet.

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

Methods: We included 1546 MS patients from the QMRI programme with ≥5 MRI scans (mean=9.3, median 7.0 scans) and ≥4 years (mean=7.0, median 6.3 years) follow-up. Majority of patients was treated with disease-modifying treatments. Brain volume loss was measured using ScanView software. We calculated coefficients of determination of individual linear (lin-R^2) and quadratic (quad-R^2) regression models. Non-linear trajectory was assumed, if quadratic model was better fitting the trajectory of brain volume loss compared with linear model (quad-R^2 >5% or >10% higher than lin-R^2; $p<0.01$). Characteristic of patients with linear and non-linear brain volume loss were compared using Mann-Whitney test and adjusted logistic regression.

Results: A total of 98 (6.3%) of patients had non-linear brain volume loss (quad-R^2 >5% higher than lin-R^2). Prevalence of non-linear brain volume loss decreased to 63 (4.0%) patients, if applied more strict definition (>10% higher). Non-linear brain volume loss showed deceleration in 44 (2.8%) and acceleration in 19 (1.2%) of patients. Occurrence of non-linear Brain volume loss was 27.3% (>5% higher) or 11.3% (>10% higher) in patients with ≥10 years follow-up. Occurrence of non-linear brain volume loss was 29.3% (>5% higher) or 12.6% (>10% higher) in patients with ≥15 MRI scans. Patients with non-linear brain volume loss deceleration (>5% higher) had higher brain parenchymal fraction at baseline (p=0.003), higher rate of brain volume loss (p<0.0001), greater T2 lesion volume increase (p<0.001), greater disability progression (p=0.001), younger age (p=0.002) and shorter disease duration (p=0.017) compared with patients with linear brain volume loss. Patients with non-linear brain volume loss acceleration (>5% higher) were similar to those with linear brain volume loss.

Conclusions: The vast majority of MS patients had a linear trajectory of brain volume loss over short-term follow-up. However, considerable proportion of non-linear brain volume loss trajectories was found in patients with longer follow-up and higher number of MRI scans.

Hence, the assumption of linearity of brain volume loss should be verified, especially in long-term MRI studies. Factors associated with occurrence of non-linear brain volume loss need to be investigated.

Reference: Data not published yet.

Figure 1. Brain volume loss trajectories with deceleration and acceleration over follow-up.
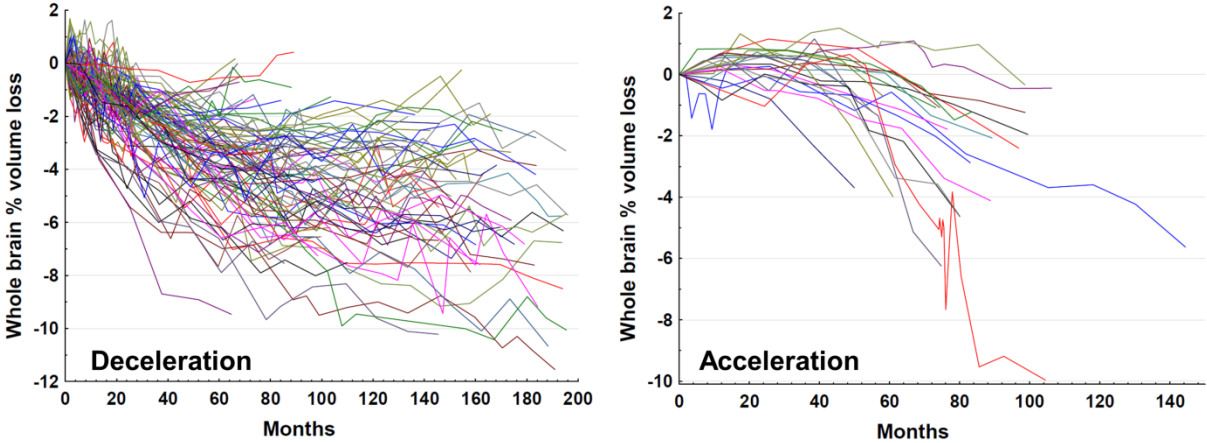
Figure 2. Individual brain volume loss trajectories with deceleration over follow-up.
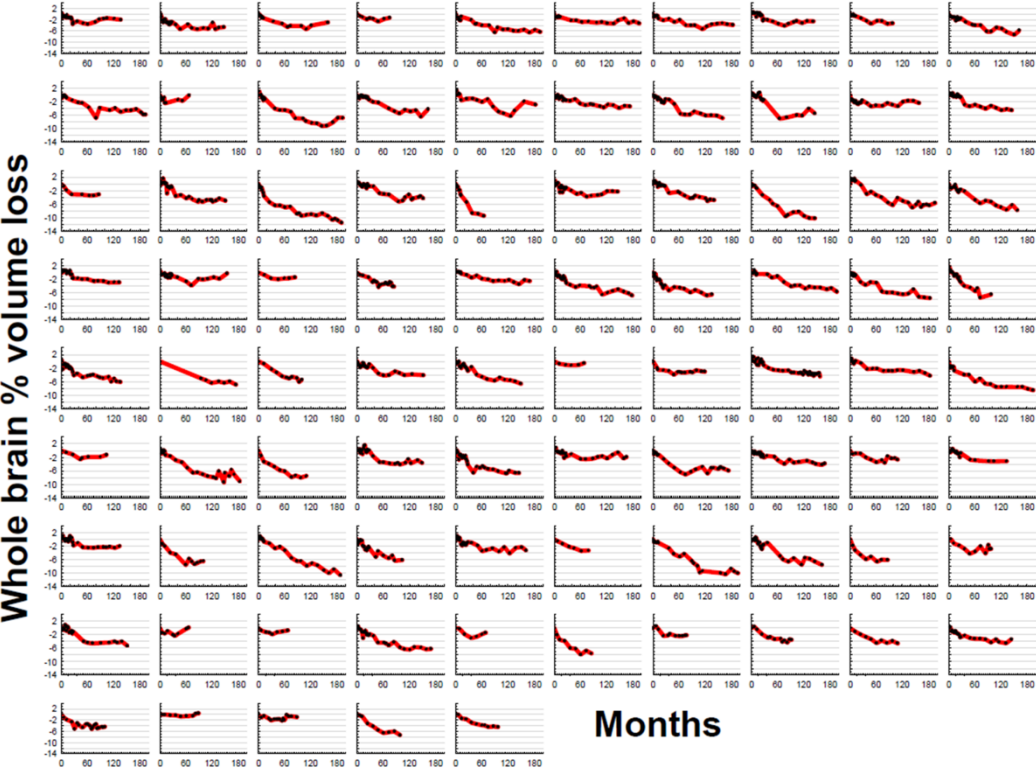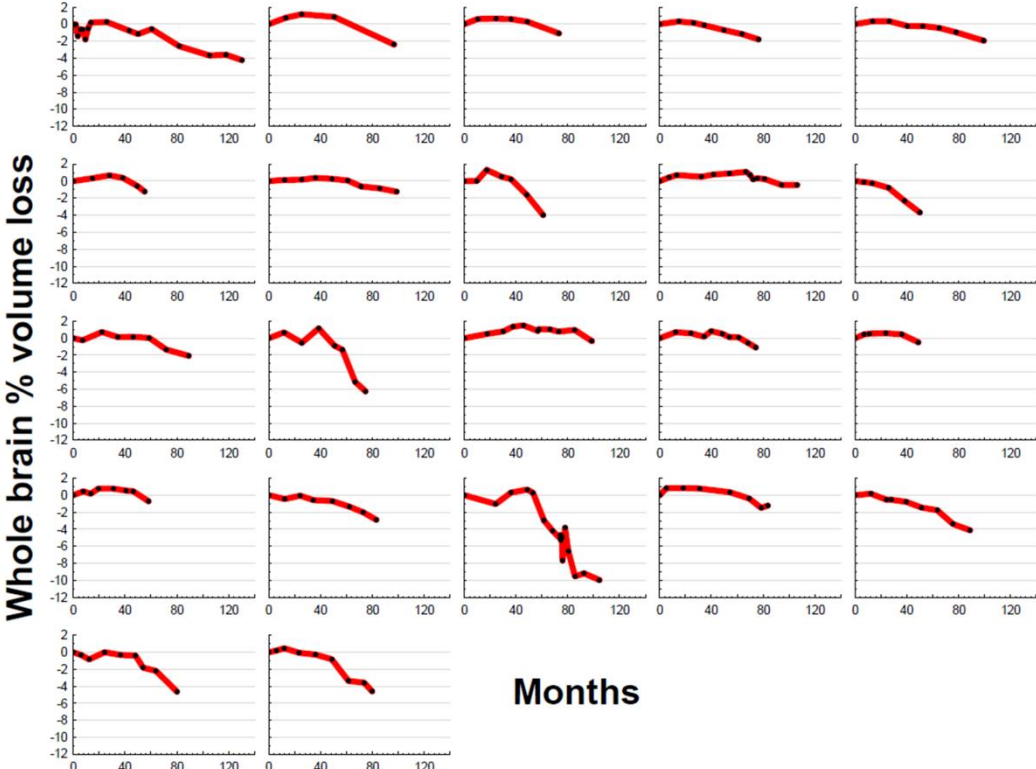

Figure 3. Individual brain volume loss trajectories with acceleration over follow-up.

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

Background: A great effort has been devoted to bring measurements of brain volume loss into clinical practice for decision-making in individual patients in recent years.[9, 21] Assessment of brain volume loss in individual patients could facilitate the identification of those with disease progression and are at high risk of accumulating permanent disability.[12] Unfortunately, a relatively high intra-individual variability of longitudinal brain atrophy measures renders challenging its application in individual MS patients.[7-9, 22] Although, current volumetric methods provide sufficient precision for cohort studies, they may not yield sufficient confidence when assessing brain volume loss in individual patients.[22]

Objective: In this study, we aimed to quantify the degree to which the precision of brain volume loss assessment could be improved with high-frequency brain MRI monitoring over short-term follow-up. We assumed that a higher number of MRI scans would provide a more precise estimate of brain volume loss in individual patients since random errors and biological, as well as technical confounding effects should be averaged out, similarly as in cohort studies.[22] In other words, we hypothesized that a greater frequency of MRI scanning over short-term follow-up would provide a more objective estimation of real brain volume loss compared to an estimate derived from only two MRI scans, as generally done in clinical practice. More specifically, we assumed that the increased precision provided by assessment of 7 MRI scans over 12 months or 13 MRI scans over 24 months may outweigh intra-subject variability of single brain volume loss measurements and provide an estimate more closely correlated with a real brain tissue loss, which is, unfortunately, not measurable in vivo in humans. Moreover, we aimed to investigate if the improved precision of brain volume loss assessment is clinically relevant and affects identification of pathological brain volume loss and "no evidence of disease activity-4" (NEDA-4) status in individual patients. In addition, we assumed that identification of patients with pathological brain volume loss (i.e. brain volume loss under certain cut-off value) may be associated with lower statistical error compared with establishing the absolute value of brain volume loss.

Methods: For this purpose, we investigated a large group of 157 relapsing-remitting MS patients with a total of 1,585 MRI scans performed bi-monthly on the same 1.5-Tesla scanner

using an identical scanning protocol. All 157 relapsing-remitting MS patients had 7 MRI scans over 12-months follow-up. Volumetric analysis was performed by ScanView and SIENA software. Linear regression analysis was used for estimation of annualized brain volume loss, with a cut-off greater than 0.4% defined as pathological. We compared proportions of patients with pathological brain volume loss obtained by analysis of different number of MRI time-points.

Results and discussion: In this study, we aimed to quantify the degree to which the precision of brain volume loss assessment could be improved with high frequency brain MRI monitoring over short-term follow-up. An analysis of 7 MRI scans (month 0 and 12) showed pathological brain volume loss (i.e. brain volume loss <-0.40%)[21, 183, 192] in 65.0% of patients. When 3 MRI scans (month 0, 6 and 12) were analyzed, we found only 12.1% false negatives or false positives (proportion of patient with pathological brain volume loss based on assessment of 7 MRI time-points, who had normal brain volume loss by analysis of a lower number of MRI time-points (or vice versa) compared with the analysis of 7 MRI scans. In addition, an analysis of 2 MRI time-points showed 14.7% false negatives or false positives compared with the analysis of 7 MRI time-points, with an overlap being almost 85%.

The cut-offs of pathological brain volume loss applied in this study were based on the results of our previous large sample study,[183] with results which are in agreement with previous research.[21, 192] We did not find any significant differences among investigated cut-offs. Worth noting is also the fact that the change in mathematical accuracy was similar in clinically stable patients compared with clinically active patients with significantly higher rate of brain volume loss. Also, no significant differences were found, neither among 3 study arms of the original ASA trial, nor between patients with greater or lower brain volume loss rates.

In the further validation analysis performed over longer follow-up, assessment of 13 MRI time-points over 24 months showed 17.1%-19.7% false negatives or false positives compared with the analysis of 2 MRI time-points over 24 months. This change in accuracy was slightly higher but comparable with the 12-months analysis. We hypothesize that this might be caused either by a non-linear brain volume loss trajectory in some patients or better estimation of real brain volume loss by analyzing more MRI scans over longer follow-up. Importantly, our results were also confirmed after re-baselining at 12 months and by using SIENA[204]. In this context, the improved performance of SIENA in all investigated MRI monitoring regimes favors the use of SIENA over ScanView and can be explained by slightly lower intra-individual variability of SIENA for longitudinal measurement of brain volume loss.[19] Given that loss of

NEDA-4 status during the first year was present only in a very small number of patients (5.7%) and was driven mostly by the occurrence of new/enlarging T2 lesions on MRI (in 74.7% of patients) we did not find important differences in proportions of patients with NEDA-4 status when comparing standard and high-frequency MRI monitoring approaches. From this point of view, we hypothesize that, even if the proportion of patients with NEDA-4 status in our study would be higher, modification of MRI monitoring frequency would not have an important effect on assessment of NEDA-4 status in individual patients.

Conclusions: Taken together, our results indicate that an increase of the frequency of MRI monitoring from every 12 months to every 6 months would lead to only a marginal improvement (≤2.6% accuracy change) in estimating brain volume loss in individual patients over short-term follow-up. Therefore, 6-monthly MRI monitoring would probably not provide substantial add-on value to regular annual MRI monitoring.[18] On the other hand high-frequency MRI monitoring (bi-monthly) was associated with 10.5-22.2% accuracy change in identifying pathological brain volume loss at the individual patient level compared with yearly MRI monitoring. Although, a misclassification rate of up to approximately 20% appears to be relatively high, there is no doubt that bi-monthly MRI monitoring would increase both the patient and financial burden and would be not feasible from an organizational point of view. Hence, despite the increased classification accuracy of high- frequency MRI monitoring, this precision improvement would not likely affect clinical management in MS population, and would favor a standard yearly MRI monitoring.[6] In this respect, improved standardization of MRI monitoring[198] and combining different MRI measures may represent future areas of research. The present study adds to the understanding of applying brain atrophy assessment to individual patients, which is still not ready for application in clinical practice mainly due to its high individual variability.

Limitations: The most important limitation of the study represents the fact that we have only indirect statistical evidence supporting the more objective estimation of brain volume loss based on the assessment of a higher number of MRI time-points. If we assume that brain volume loss estimation derived from more frequent MRI scanning is closely correlated with the real brain tissue loss, then our results could increase the overall confidence for assessing pathological brain volume loss in individual patients based on assessment of 2 MRI time-points over short-term follow-up. Moreover, our results showed only a relatively low improvement in precision (increase of area under the curve; a decrease of standard error; increase of specificity and

sensitivity – data not shown) along with increased number of MRI time-points over follow-up. Hence, the aim of the study to define the number of MRI time-points needed to provide reasonable measurement accuracy remained partially unmet and will require investigation in further studies. Another limitation of the statistical strategy may be the fact that MRI scans performed at 0 and 12 months are potentially the most influential points in the regression analysis by virtue of being "in the tail" of the time distribution. Because these two time-points (month 0 and 12) enter data into each of the three sampling strategies, adding interim MRI time points may not be as strongly influential. On the other hand, this is the approach that would most likely be applied in clinical practice when estimating annualized brain volume loss when more than two MRI scans are available. In spite, of MRI scans having been obtained between 1999 and 2005, we suggest that the scans were of sufficient quality. It must be emphasized that all 1,585 MRI scans were performed using the same 1.5T scanner that did not undergo major hardware upgrades over follow-up. Both FLAIR and 3D-T1 sequences were non-gapped and remained unaltered over follow-up. Additionally, MRI analyses underwent quality control and were reviewed by trained operators (Jan Krasensky or Niels Bergsland) at all critical points of segmentation. Hence, changes in MRI scanner or protocol may be associated with increased variability of brain volume loss estimates, rendering challenging the interpretation at the individual or even group levels.

Reference:

Uher T, Krasensky J, Sobisek L, Seidl Z, Bergsland N, Dwyer MG, Kubala Havrdova E, Zivadinov R, Horakova D, Vaneckova M. The Role of High-Frequency MRI Monitoring in the Detection of Brain Atrophy in Multiple Sclerosis. J Neuroimaging. 2018 May;28(3):328-337. doi: 10.1111/jon.12505.[205]

Figure 1. Number of patients with normal and pathological brain volume loss by assessment of a different number of MRI time-points over 12 months follow-up.
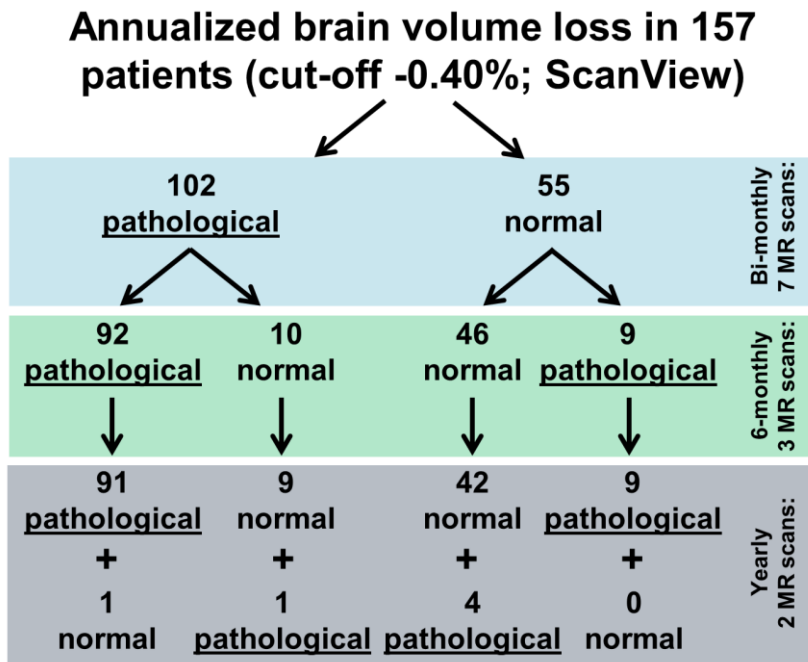
Figure 2. Brain volume loss rates provided by assessment of a different number of MRI time-points using (A) ScanView and (B) SIENA technique. Spaghetti plots of brain volume loss rate in individual patients provided by assessment of a different number of MRI time-points using (C) ScanView and (D) SIENA technique.
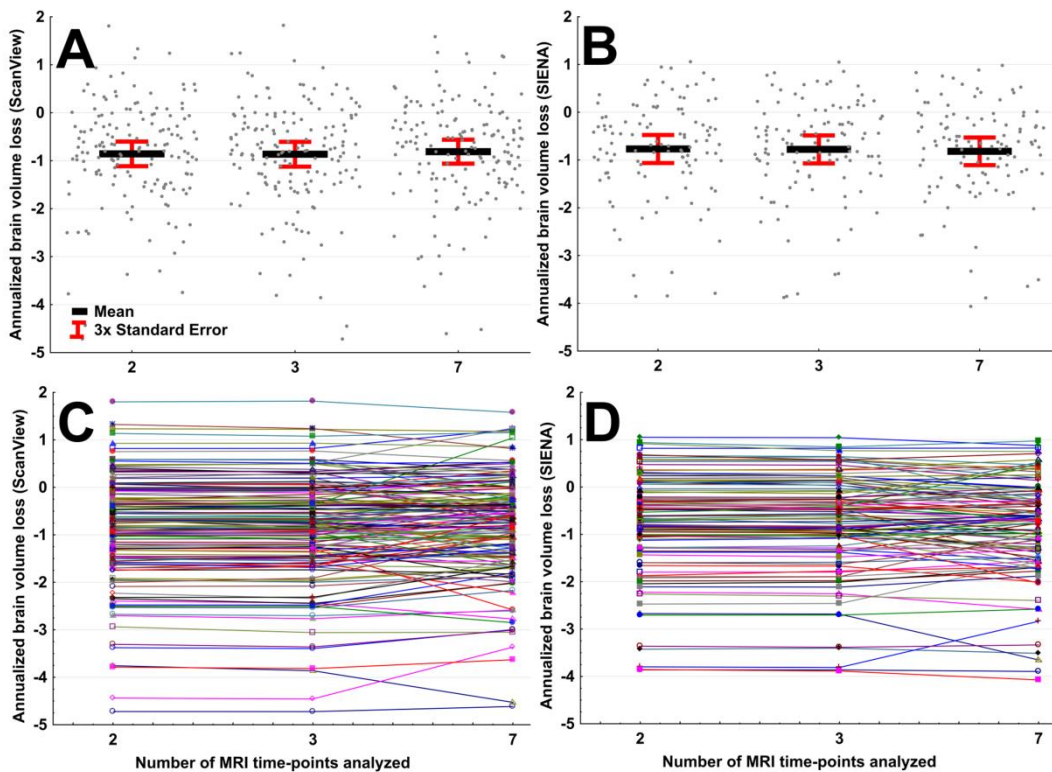
Figure 3. Overview of the statistical approach. Estimation of pathological brain volume loss rate in individual patient using: (A) yearly brain MRI monitoring (clinical practice); and (B) bimonthly MRI monitoring (statistical model).

Estimation of pathological brain volume loss rate in individual patient

A. Clinical practice: *yearly* MRI scan

0 %

% Brain volume loss

Mean = -0.86%

Month 0 — Month 12

13 (8.3%) false positive
10 (6.4%) false negative
92 (58.6%) true positive
42 (26.8%) true negative

Number of patients with pathological % brain volume loss (< -0.40%) based on assessment of 2 MRI scans

B. Statistical model: *bi-monthly* MRI scan

0 %

-0.81%

Month 0 — Month 12

102 (65.0%) pathological brain volume loss
55 (35.0%) normal brain volume loss

Number of patients with pathological % brain volume loss (< -0.40%) based on assessment of 7 MRI scans

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

Background: CIS describes a first clinical episode suggestive of MS. Abnormal MRI findings were shown to be the most informative surrogate markers of conversion to clinically definite multiple sclerosis (CDMS).[2, 104, 149, 206, 157, 207, 208] Particularly, T2 lesion number[207] and volume (T2-LV),[208, 209] together with presence of contrast-enhancing lesions at first clinical event [210, 211] were suggested as the important predictors of new relapse activity. Furthermore, global brain,[2, 104, 208, 212] thalamus[104, 106] and corpus callosum[149, 155] pathology and lateral ventricle volume enlargement [2, 104] over the first months following first clinical event have been robustly associated with ongoing disease activity. However, the majority of studies investigating the predictive value of MRI toward clinical progression in patients after first clinical attack included subjects without disease modifying treatment,[207-209, 213, 214] focused on a limited spectrum of MRI outcome variables[210, 215] or included short-term follow-up.[210, 216] Most importantly, previous studies have not investigated the predictive role of change in MRI outcomes over such a short time as a first 6 months after immunomodulatory drug treatment initialization.

Although the use of intramuscular interferon beta-1a (IFNβ-1a) has been shown to delay confirmed disability progression[217] and conversion to CDMS[16] in a reasonable proportion of CIS patients, there is a subpopulation of patients who continue to show disease activity despite treatment.[2, 218] Moreover, given that irreversible acute axonal damage is most extensive in early disease stages,[148] it is exceedingly important to identify as early as possible those individuals who fail to respond to immunomodulatory drugs, even if the mechanisms by which this occurs remain poorly understood at this time.[2] In this context, an important, yet currently unmet, need of modern MS treatment is to determine reliable, and in routine clinical practice applicable MRI predictors of subsequent disease activity in CIS patients after first clinical attack.

Objective: The aim of this study was to investigate the predictive role of baseline and 6-month changes in MRI outcomes with respect to relapse activity and development of confirmed disability progression in patients after first demyelinating event suggestive of MS treated with weekly intramuscular interferon beta-1a after 48 months. Based on the previous research, showing robust relationships between corpus callosum[149, 155, 214] and thalamic[104, 106] pathology and lateral ventricle volume enlargement[2, 104] with increased disease activity in CIS patients,

we hypothesized that early changes of these brain structures during the first 6 months following first clinical attack may be reliable MRI predictors of relapse activity and development of confirmed disability progression in CIS patients over mid-term. Moreover, we hypothesized that these regional brain atrophy markers have at least comparable predictive value to the presence of T2[207, 213, 219] and contrast-enhancing lesions[210, 211] and their volumes,[208, 209] and may add additional predictive information in selection of patients with higher risk for clinical progression.

Methods: A prospective observational SET study in patients after first clinical attack was originally designed to determine clinical and MRI outcomes associated with the relapse activity and confirmed disability progression after 48 months.[2, 104, 149] This study examined 210 patients. Multivariate Cox proportional hazard models were used to analyze predictors of relapse activity and confirmed disability progression after 48 months. Compared to the previous research,[104, 106, 149, 155] the current study was performed on the same patient cohort, however applied longer follow-up and tested significantly broader spectrum of MRI lesion and brain atrophy predictors. More specifically, analysis of cumulative number of new and new enlarging T2 lesions, number and volume of contrast-enhancing lesions, regional volume changes of cortical gray matter, subcortical deep gray matter, thalamus, hippocampus and lateral ventricle enlargement has been added. Furthermore, we applied different statistical approach and data presentation (evolution of MRI measures over the first 6 months of follow-up), which is more applicable for risk estimation of future disease activity in clinical routine.

Results and discussion: In this prospective, observational study of patients diagnosed with first clinical attack suggestive of MS, we have identified several independent early MRI predictors of new relapse activity and to confirmed disability progression over the 48 months following the initial clinical event.

Approximately half of the patients, who experienced second clinical attack during 48 months, experienced new relapse activity already during the first 6 months of the study. This observation supports previous findings of early occurrence of new relapse activity with subsequent decline in its incidence.[16, 210, 215, 216, 220]

The results of the study indicate a two-fold greater risk of second clinical attack in patients with contrast-enhancing lesions (HR=2.13; p<0.001) as well as with largest T2 lesion volume (HR=1.81; p=0.005) at baseline. Both criteria were highly specific (83%), although were able to capture only about 30-40% of patients with future second clinical attack.

Interestingly, a higher number of contrast-enhancing lesions at baseline were associated with higher risk second clinical attack. Moreover, presence of more than two contrast-enhancing lesions at baseline had very similar individual predictive accuracy than composite criterion containing contrast-enhancing lesions positivity and largest T2 lesion volume at baseline. Taken together, we confirmed that presence of contrast-enhancing lesions at baseline represents robust independent and early MRI predictor of second clinical attack in patients treated with weekly intramuscular IFN-βa which supports findings of previous studies.[2, 208, 210, 215] Although, baseline T2 lesion volume has been already shown as a predictor of second clinical attack,[208] contrary to some recent studies[207, 213, 219] we did not find a strong relationship between relapse activity and baseline T2 lesion number or T2 lesions accumulation over the first 6 months of the study. We hypothesize that this could be attributed to study inclusion criteria resulting in enrolment of patients with only higher number of T2-hyperintense lesions (at least two or more) on diagnostic MRI.

One of the most important results of the study is that patients after first clinical attack with increased corpus callosum volume loss (>0.33%) in the prediction phase had a threefold greater risk (HR=2.74; p=0.001) of second clinical attack over the response phase, and those with greater lateral ventricle volume enlargement (>4.5%) had double the risk (HR=2.43; p=0.002). Interestingly, presence of either increased corpus callosum volume loss or greater lateral ventricle volume enlargement or both criteria, was associated with almost a fourfold higher risk of second clinical attack. On the other hand, absence of any of these two criteria identified approximately half of the patients after first clinical attack, which remained relapse-free over a response phase. Given that absence of any of these two criteria was found in considerable proportion of patients, we suggest that future research should investigate if these brain volume predictors might be applicable in routine clinical practice for early detection of patients with lower clinical activity and a more favourable disease course.

Although the relationship between lateral ventricle volume enlargement and second clinical attack has been reported already,[2, 104] the predictive value of very early lateral ventricle volume enlargement for second clinical attack in a longer follow-up has not been analyzed previously. The predictive role of early pathology of the corpus callosum is in line with recent studies showing that corpus callosum lesions[155, 214] and volume loss[149] are strongly associated with higher risk of second clinical attack. Although, early WB volume loss has been also associated with relapse activity in some previous studies[208, 212] we did not confirm its predictive value in this study. This could be presumably attributed to different sample selection criteria or different methodological approaches. In addition, although we have previously reported an association

between thalamus volume loss and relapse activity over 24 months in the SET study,[104] we did not confirm its early independent predictive value over the first 6 months of follow-up for the 48 months clinical progression in the current study. For the first time, we have directly compared predictive value of early T2 lesion volume, corpus callosum volume, lateral ventricle volume enlargement and global and regional gray matter volume changes that have been shown as potential predictors of relapse activity in previous studies.[106, 149] Although several gray matter variables, including thalamus volume, were significantly related to clinical progression in univariable Cox proportional hazard models, their contribution to the overall prediction relative to other more robust predictors was relatively small in the multivariable models.

Our findings indicate a nearly fivefold increased risk (HR=4.70; p=0.001) of confirmed disability progression in patients with greater lateral ventricle volume enlargement over the first 6 months of the follow-up. Interestingly, this criterion captured 74% of those with confirmed disability progression and absence of this criterion was associated with 92% chance of stable EDSS after 48 months, therefore future research is needed to investigate if the lateral ventricle volume enlargement after first clinical attack is reliable predictor of increased risk of confirmed disability progression. Although previous CIS studies have shown that contrast-enhancing lesions positivity,[208] number of T2 lesions[157, 207] and T2 lesion volume[209] at baseline or over first years following the initial event[208, 219, 221] are associated with mid- and long-term disability accumulation, we did not find relationships between development of confirmed disability progression and these MRI predictors.

In this context, critical need for early identification of patients with poor response to interferon treatment led to development of the Rio score[151] and the modified Rio score.[153] According to the modified Rio scoring system, patients with new relapse activity and greater number of new or new enlarging T2 lesions during first year of the interferon treatment have increased risk of development of confirmed disability progression. The current study employed shorter prediction phase in duration of only 6 months and did not investigate clinical predictors, therefore direct comparison of the Rio score with our data is difficult. Although, proposed MRI volumetric predictors as well as Rio score are relatively specific, there is low sensitivity in the identifying patients at highest risk of disease progression. Therefore, potential predictive value of composite clinical and MRI volumetric predictors warrants further research. This has potential to improve the predictive value of the Rio score in patients who are classified with intermediate Rio scores.

Although, intramuscular IFN-βa has been shown to delay occurrence of second clinical attack,[16] it is known that conventional immunomodulatory drugs in general have only partially

satisfactory efficacy to supress disease activity in specific subpopulations of active MS patients.[217, 222] In this context, the new generation of immunomodulatory drugs have shown greater potential for a decrease of relapse activity.[223-226]

Conclusions: Our study suggests that baseline contrast-enhancing lesions, largest T2 lesion volume, and increased corpus callosum volume loss and greater lateral ventricle volume enlargement early (over the 6 months) after first clinical attack could assist in the identification of patients with highest risk for clinical progression, while being on weekly intramuscular IFN-βa treatment. Hence, controlled or comparative studies are needed to confirm clinical relevance and reliability of MRI surrogate markers of intramuscular interferon-beta1a treatment failure and to investigate effectiveness of new immunomodulatory drugs in the high-risk patients after first demyelinating event suggestive of MS.

Limitations: All patients after first clinical attack entering SET study were treated with immunomodulatory drugs within 4 months after first clinical attack. Hence, the anti-inflammatory effect of immunomodulatory drugs limits generalizibility of our findings to all CIS patients and therefore our results need to be also validated on different patient populations. There were 47% of patients without a second relapse, 19% not fulfilling McDonald criteria and 85% free from disability progression. In fact, because 81% of patients after first clinical attack fulfilled McDonald 2010 criteria at 48 months, this outcome was not included in the analyses of the study. Although a baseline MRI examination was performed at least 30 days after steroid administration, we cannot completely rule out their influence on our measures, particularly on the occurrence of the contrast-enhancing lesions.[227] In the study, we assessed the predictive role of wide range of white matter focal damage and global, tissue specific and regional brain volume changes, while brainstem lesion localisation and spinal imaging analysis were not applied. Hence, further research is needed to clarify the predictive role of spinal and brainstem focal damage in patients after first clinical attack. Given that 23 (11.0%) patients switched to more effective immunomodulatory drugs during follow-up, potential stabilization or improvement of disability status after treatment change might influenced disability outcome of the study. In spite of the fact that second clinical attack occurred within the first 6 months in nearly half of cases, we suggest that risk stratification of patients at disease onset may have its clinical relevance. Considering the fact that effect of majority of immunomodulatory drugs occurs already within the first months of treatment and the observation that irreversible acute axonal damage is most extensive in early disease stages, [148] early risk stratification may help to

identify patients potentially profiting from early initialization of immunomodulatory treatment with greater potential for a decrease of relaps activity and early brain damage.[223-226] Moreover, in health care systems where second clinical event is needed to initiate immunomodulatory drug treatment, the baseline predictors may identify patients profiting from earlier initialization of immunomodulatory drug treatment before any new relapse activity occur. It is worth to mention also the effect of pseudo-atrophy, phenomenon taking place mostly during the first months after immunomodulatory tratment initialization. It is clear, the pseudo-atrophy could strongly influence regional and global brain volume changes over prediction phase of the study. Interestingly, it was shown in previous research that pseudo-atrophy is associated with pre-existing inflammation, assessed by contrast-enhancing lesions positivity.[228] The finding was replicated also in our study where greater volume changes of corpus callosum, lateral ventricle volume and whole brain over prediction phase in patients with contrast-enhancing lesions positivity and largest T2 lesion volume at baseline were found. This relationship emphasizes that our proposed MRI predictors might reflect brain volume loss due to pseudo-atrophy related to anti-inflammatory effect of immunomodulatory drugs rather than neuro-degeneration. Taken together, we hypothesize that level of regional pseudo-atrophy occurring early after immunomodulatory treatment initialization might represent sensitive and very early predictor of further disease activity related to neuro-inflammatory processes.

Reference:

Uher T, Horakova D, Kalincik T, Bergsland N, Tyblova M, Ramasamy DP, Seidl Z, Vaneckova M, Krasensky J, Havrdova E, Zivadinov R. Early magnetic resonance imaging predictors of clinical progression after 48 months in clinically isolated syndrome patients treated with intramuscular interferon β-1a. Eur J Neurol. 2015 Jul;22(7):1113-23. doi: 10.1111/ene.12716.[11]

Figure 1. Cox proportional hazard model estimates of relapse-free survival by number of contrast-enhancing lesions at baseline.

CEL = contrast-enhancing lesions, CIS = clinically-isolated syndrome, CDMS = clinically definite multiple sclerosis, No. = number of patients at risk of conversion to clinically definite multiple sclerosis, pts = patients
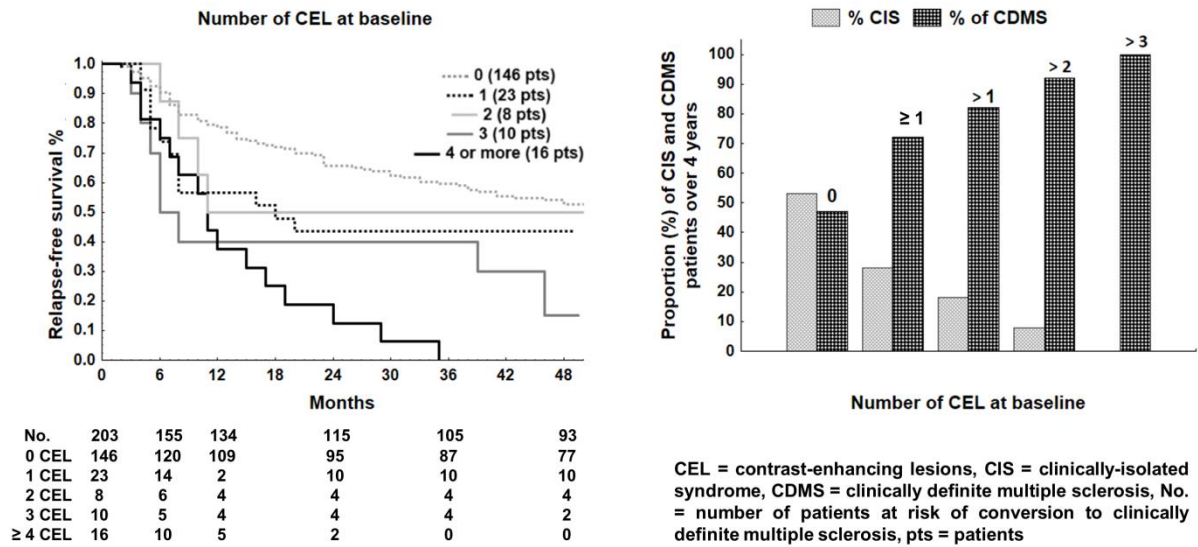
Figure 2. Cox proportional hazard model estimates of relapse-free survival by best MRI predictors of clinically definite MS after prediction phase at 6 months.
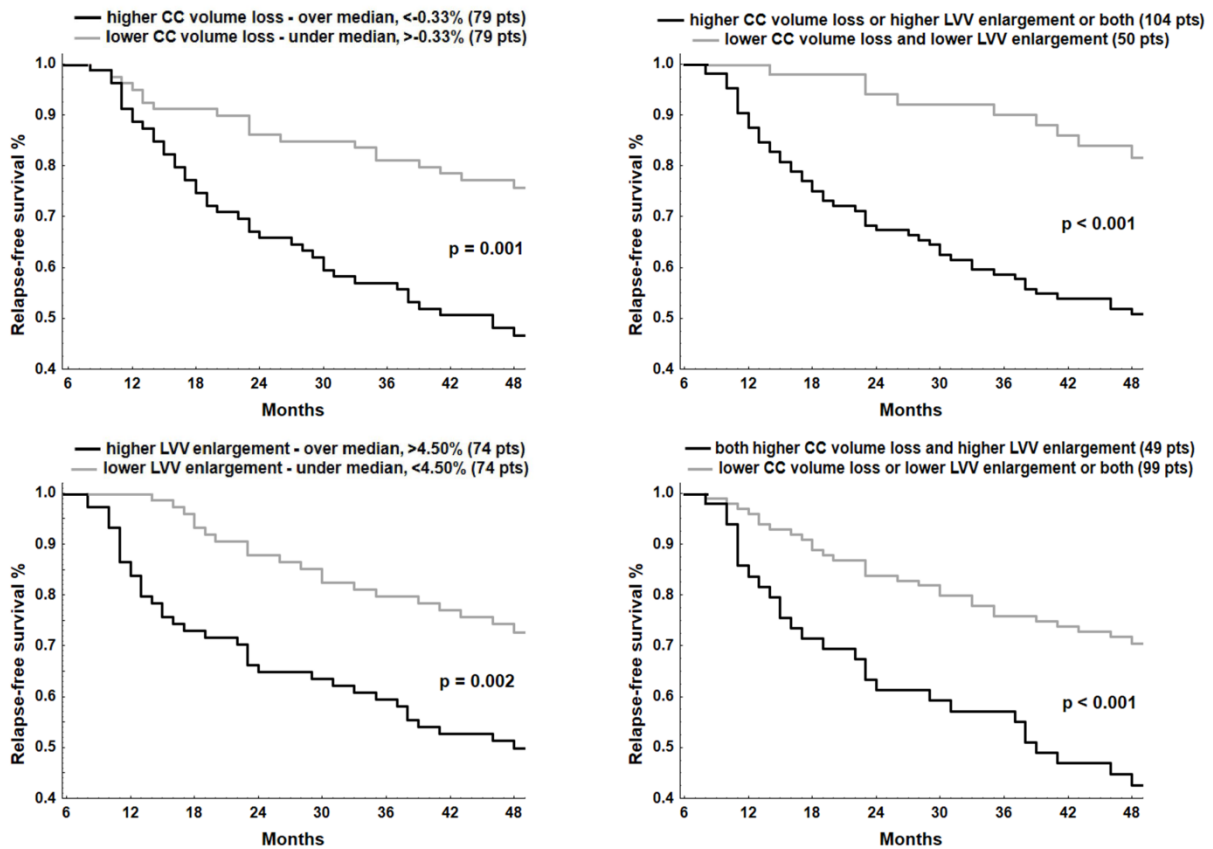
CC = corpus callosum, LVV = lateral ventricle volume, pts = patients

Figure 3. Cox proportional hazard model estimates of relapse-free survival by best MRI predictors of clinically definite MS at baseline.

CEL = contrast-enhancing lesions, T2-LV = T2 lesion volume, pts = patients
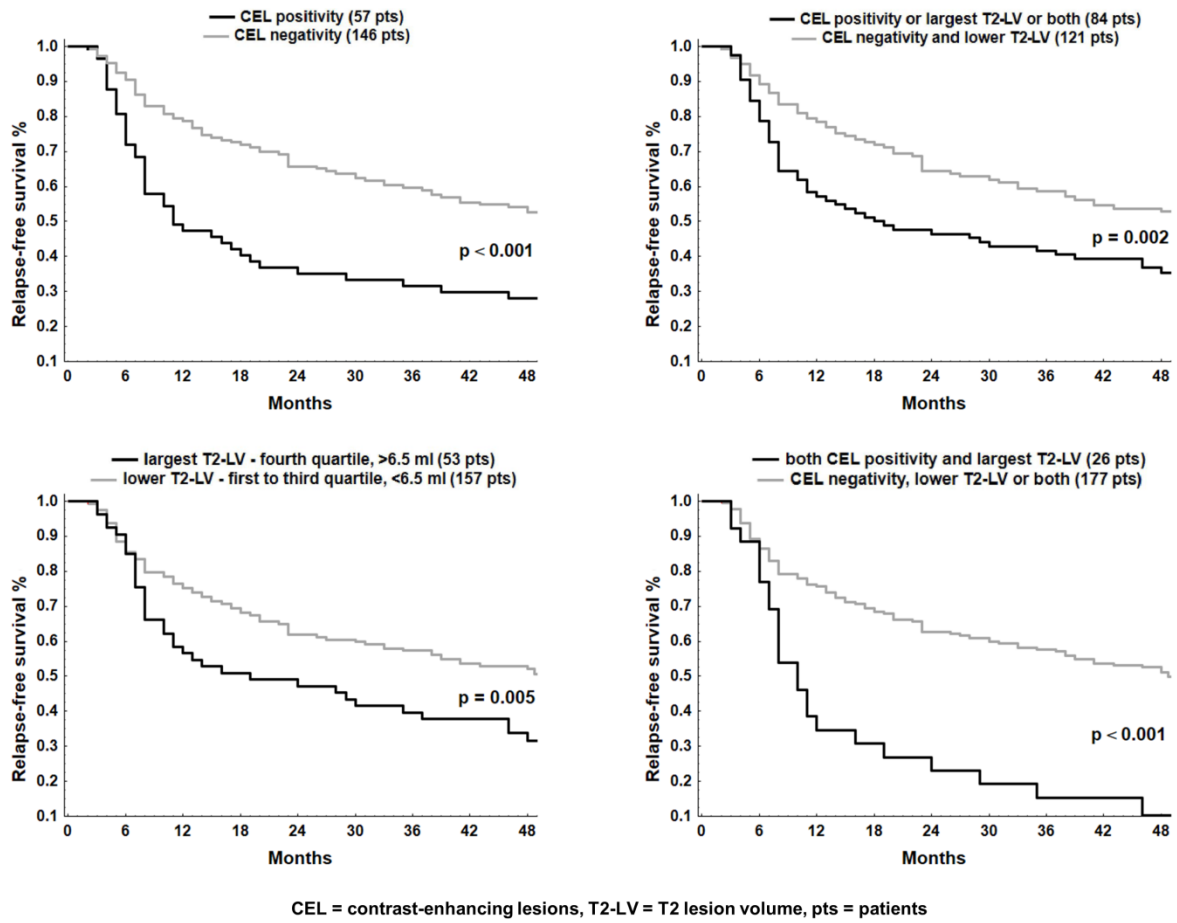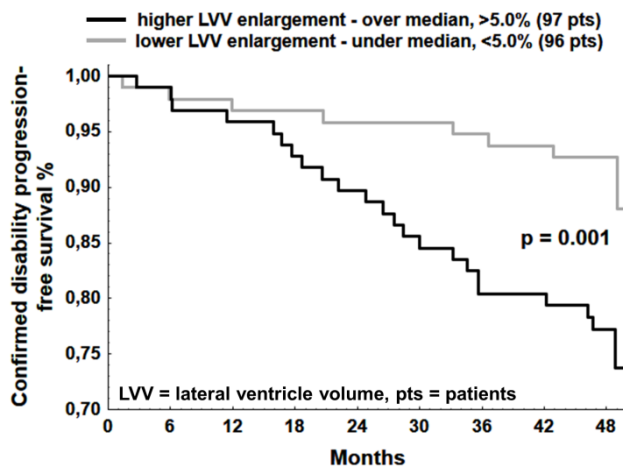
Figure 4. Cox proportional hazard model estimates of confirmed disability progression-free survival by lateral ventricle volume changes during the prediction phase.

LVV = lateral ventricle volume, pts = patients

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

Background: MS has a broad spectrum of phenotypes. Disease progression and treatment efficacy vary among individuals with MS. Because of this, treatment efficacy may be significantly improved by identifying specific MS subpopulations at high risk of disability progression despite immunomodulatory drug treatment. In this context, studies have shown that abnormal MRI findings are the most informative predictors of future disease activity in short-term,[11, 149-151] mid-term,[150, 152, 153] and long-term studies.[150, 154-157]

Particularly, the most important predictors of disability progression in relapsing-remitting (RR) MS have been suggested to be: the occurrence of new T2 lesions,[150-154, 158] accumulation of T2 lesion volume[159, 160] and T1 lesion volume,[161, 162] whole brain[160, 163] and central atrophy[160, 161] as well as grey matter[164] and thalamic volume changes.[165, 166] However, some of these studies focused on a limited spectrum of MRI metrics,[158, 160, 161, 165] investigated heterogeneous patient cohorts regarding immunomodulatory treatments or disease stages[160, 161, 165] and applied different statistical approaches.

Objective: Comprehensive evaluation of predictive accuracy of broad spectrum of early MRI markers in relative homogenous sample of relapsing-remitting MS patients on intramuscular interferon beta-1a treatment was investigated in this long-term study.

Based on previous research showing strong associations between early lesion burden,[11, 159, 161] corpus callosum[11, 149, 155, 214] and thalamic atrophy,[104, 106, 165, 166] and increased disease activity over a short-term follow-up, we hypothesized that these MRI markers and their changes within the first year following immunomodulatory drug treatment initiation, predict the accrual of sustained disability over the 12-years follow-up period. Furthermore, we hypothesized that the combination of volumetric MRI markers with established clinical predictors, such as early relapse activity or changes in disability,[154, 229] may facilitate timely identification of patients with poor long-term disability outcomes.

Methods: 177 patients from the original observational ASA study cohort were included in this study.[179] Participants underwent clinical follow-ups every 3 months. Cox models were used to model the associations between clinical and MRI markers at baseline or after 12 months with sustained disability progression (SDP) over the 12 years observation period.

Results and discussion: Using the observational extension of the ASA trial, we have identified several clinical and MRI predictors of 12 year disability outcomes in relapsing-remitting MS using data within the first year of IFNβ-1a treatment initiation. These predictors were: T2 lesion number and volume, T1 lesion volume, corpus callosum, and thalamus volumes (HR=1.7-4.6; p=<0.001-0.012), and at 12 months, EDSS score and EDSS change, the number of new/enlarging T2 lesions, and the relative change in corpus callosum volume (HR=1.7-3.5; p≤0.001-0.017). Taken together, the results of our study indicate a two- to three-fold greater risk of disability progression (by 1 or 2 EDSS steps) in patients with higher T1 lesion volume and T2 lesion volume, T2 lesion number and lower corpus callosum and thalamus volumes at the time of disease-modifying treatment initiation, and in those with EDSS worsening, occurrence of new/enlarging T2 lesions, and greater corpus callosum % volume loss after the initial 12 months of disease-modifying treatment. Although specificity and sensitivity of these MRI predictors varied, their individual predictive accuracies were similar and ranged between 52% and 68%.

Our results are in agreement with previous studies which have highlighted the role of new T2 lesions,[150, 151, 153, 154, 158] higher T2 lesion volume,[159, 160] and T1 lesion volume,[161] and thalamus atrophy,[165] in the prediction of disability progression in patients with relapsing-remitting MS. However, to the best of our knowledge, there are no previous studies investigating critical cut-off values for dichotomized MRI volumetric predictors of disability, such as thalamus or corpus callosum. Therefore, direct comparison of our findings with the cut-off values within the existing literature was not possible and needs to be confirmed in further studies.

Although MRI predictors reflecting the inflammatory component of MS, such as T2 lesion burden, showed predictive accuracy comparable to volumetric predictors, such as corpus callosum or thalamic volumes, both groups of predictors behaved as independent predictors in the multivariate models. Moreover, early worsening of EDSS was independent from lesion burden and volumetric measures. While the accuracy of these individual predictors may be limited, their combination markedly increased their predictive accuracy, likely even at the individual patient level. The composite predictive score that we propose in this study comprised the strongest identified clinical (worsening of EDSS score over the initial 12 months), MRI-inflammatory (T2 lesion volume at baseline, number of new/enlarging T2 lesions over 12 months) and MRI-volumetric (corpus callosum volume at baseline, corpus callosum volume % change over 12 months) predictors. As such, the predictive composite score markedly enhanced

the predictive accuracy over the individual predictors. Indeed, the risk of the accumulation of permanent disability over the following 12 years was five-fold in patients with 3 positive predictors compared to those with no positive predictors. The seven patients with all 5 positive predictors continued to accumulate further disability over the following years. This improved accuracy of the proposed composite score could potentially be attributed to the fact that it combines markers of various aspects of the MS pathophysiology, including inflammation and neuronal loss.[118] Considering a high inter-correlations among lesion burden variables and between thalamic and corpus callosum volumes, it is not surprising, that overall accuracy of the composite score remained unchanged when baseline T2 lesion volume was replaced with T2 lesion number or T1 lesion volume or when assessment of baseline corpus callosum volume was replaced by thalamic volume. Clinical relevance of this increased risk was reflected by the greater accumulation of disability over the follow-up period, as reflected in the significantly higher EDSS scores after 10 years from treatment onset.

Interestingly, regional predictors, such as thalamus and corpus callosum may provide improved predictive information when compared to global brain volumetry over the first 12 months of follow-up. This notion was supported by our present study as well as a number of previously published works.[11, 104, 106, 149, 164-166] Nowadays, there are software providing automatic, not time-consuming and easy reproducible volumetric assessment of thalamic and corpus callosum volumes. Hence brain region-specific abnormalities on MRI volumetry may represent promising markers for clinical practice. However, future studies are needed to confirm its clinical relevance in patients on different disease-modifying treatments and at different disease stages.

EDSS change over the first 12 months of disease-modifying treatment was included in the composite predictive score. Although, overlap between early EDSS worsening and SDP1 or SDP2 was obvious, there is still considerable proportion of patients who did not reach SDP over the follow-up. Only 6% patients reached SDP1 and only 5% patients reached SDP2 during the first 12 months of the study. From 40 patients with unconfirmed EDSS worsening over the 12 months, 87.5% patients reached SDP1 and only 57.5% reached SDP2 over the 12 year observation period. Therefore, in clinical practice, an evaluation of patients with an early EDSS worsening as those who will experience SDP would lead to approximately 15% overestimation of number of patients who reach SDP1 and 45% overestimation of number of patients who reach SDP2 over long-term follow-up. On the other hand, absence of early EDSS worsening in the composite predictive score would result in its decreased overall predictive accuracy.

Importantly, EDSS change was also a significant predictor in the confirmatory analysis with an alternate baseline defined as 12 months after disease-modifying treatment initiation.

Conclusions: Our study suggests that a combination of established clinical and para-clinical predictors with novel regional volumetric markers (such as corpus callosum and thalamic volumes) facilitates the identification of patients who are at high risk of accumulating permanent disability over a long period of time. Therefore, the combination of established MRI and clinical indices with MRI volumetric predictors improves the prediction of SDP over long-term follow-up and may provide valuable information for therapeutic decisions. With the growing recognition of the relevance of the early disease-modifying treatment and increasing number of highly effective disease-modifying treatments,[225, 226, 230, 231] stratification of patients at a highest risk of disability progression may identify patients benefiting from early treatment escalation.

Limitations: All patients enrolled in the ASA study were treated with IFNß-1a during the first 12 months, had ≥2 T2 lesions, ≥2 oligoclonal bands in the cerebrospinal fluid and high relapse activity preceding study baseline. Hence, the anti-inflammatory effect of disease-modifying treatments and inclusion criteria limits the generalizability of our findings to the whole population of relapsing-remitting MS patients. Therefore, our findings require validation in independent patient cohorts. Furthermore, the value of the contrast-enhancement, topography of brainstem lesions, and spinal volumetry were not investigated due to the lack of the respective data. It is worth noting that the cut-off value for the T2 lesion number was relatively high (25 lesions) and this clearly limits its sensitivity and utility in routine clinical practice. The applicability of the lesion count in clinical practice is further limited by its variability in relation to the used MRI protocol and inter-rater error. Given that 44 (25%) patients switched to more effective disease-modifying treatments during the follow-up, potential stabilization or improvement of disability status after treatment change might have influenced disability outcomes in the study. On the other hand, only 5 (5%) patients switched to a more effective disease-modifying treatment before reaching SDP1 and only 4 (4%) before reaching SDP2, hence the potential for confounding originating from disease-modifying treatment switching does not appear to be an important factor. In this study, only patients with high relapse activity preceding the study baseline were enrolled. In this context, we gather that the decreased variability of relapse activity across our patient sample may explain why weaker associations

between the early relapse activity and disability outcomes were found, as compared to several previous studies.[151, 153, 154]

Reference:

Uher T, Vaneckova M, Sobisek L, Tyblova M, Seidl Z, Krasensky J, Ramasamy D, Zivadinov R, Havrdova E, Kalincik T, Horakova D. Combining clinical and magnetic resonance imaging markers enhances prediction of 12-year disability in multiple sclerosis. Mult Scler. 2017 Jan;23(1):51-61. doi: 10.1177/1352458516642314.[1]

Figure 1. Unadjusted Kaplan-Meier curves of sustained disability progression by the dichotomized clinical and MRI predictors.
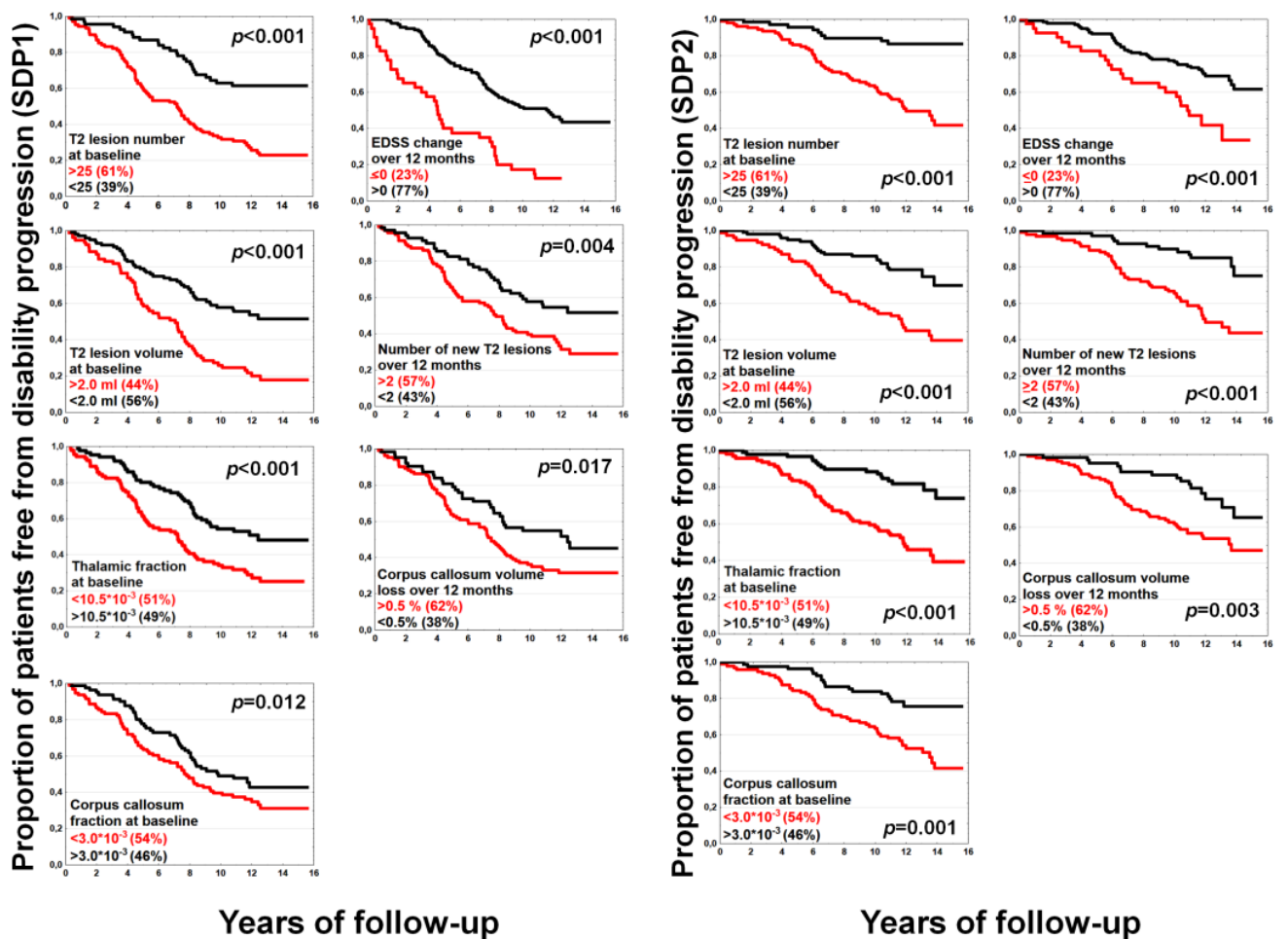
Figure 2. Unadjusted Kaplan-Meier curves of sustained disability progression based on individual composite prediction scores (A-B). Box plots corresponding to EDSS at baseline (C), EDSS at 10 years (D) and EDSS absolute change over the 10 years (E) stratified by the individual composite prediction scores. Bars indicate means, error bars indicate 95% confidence intervals and horizontal lines indicate medians.
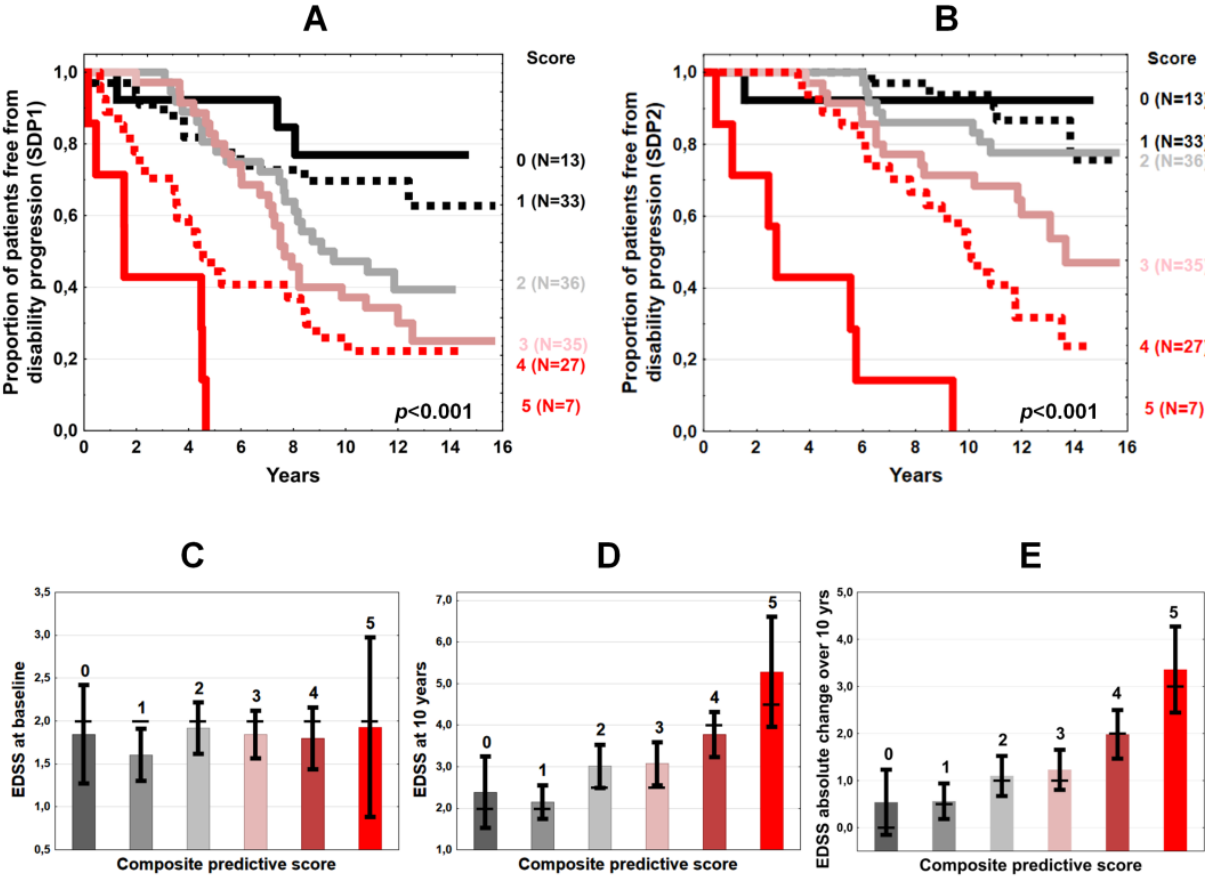
Figure 3. Specificity and sensitivity of the individual clinical and MRI predictors. (A) Predictors at baseline of 10-year sustained disability progression by 1 EDSS step (SDP1), (B) predictors at baseline of 10-year sustained disability progression by 2 EDSS steps (SDP2), (C) predictors at 12 months of 10-year SDP1, (D) predictors at 12 months of 10-year SDP2.

Figure 4. EDSS at 10 years in subgroups defined by the dichotomized clinical and MRI predictors. Bars indicate means, error bars indicate 95% confidence intervals and horizontal lines indicate medians.

5.7 Identification of MS patients at highest risk of cognitive impairment using integrated brain MRI assessment approach
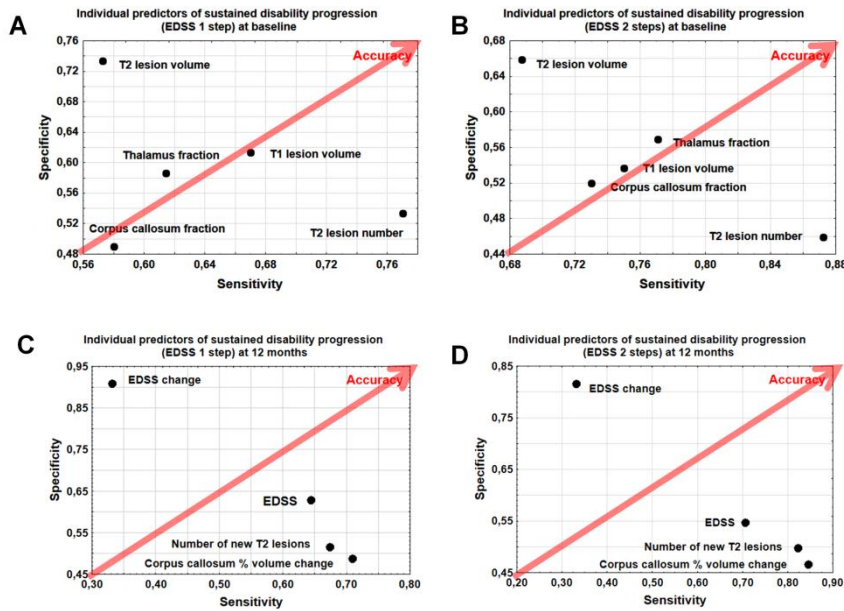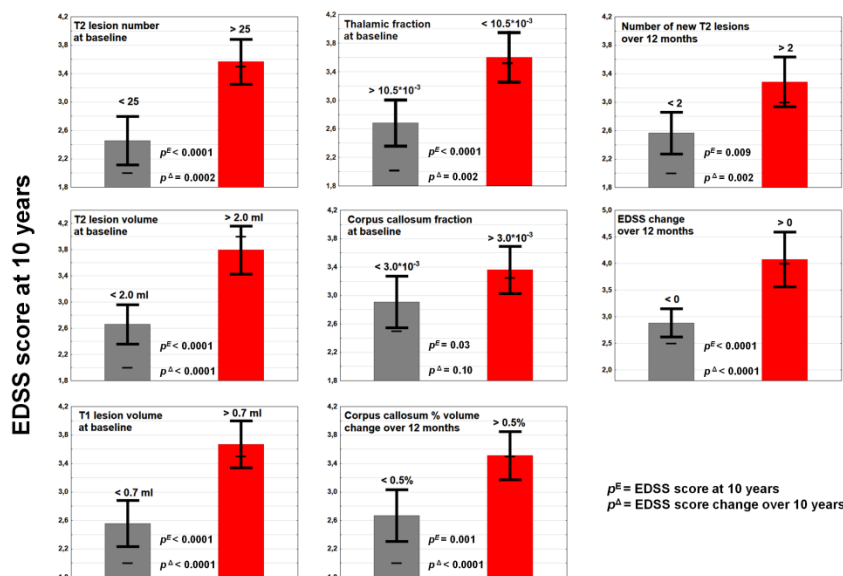
Background: Cognitive impairment is increasingly recognized as an important determinant of employment status and associated societal costs,[84, 85] adversely affects social functioning, coping, quality of life and treatment adherence among MS patients.[86, 87] It is recognised that patient´s cognitive performance should be assessed routinely.[14, 15] Even though abbreviated neuropsychological batteries, such as the BICAMS,[101, 175] were proposed for clinical use, brief cognitive assessment is still not accessible to a large proportion of patients seen by MS specialists. As a result, a considerable number of MS patients with cognitive impairment remain largely undiagnosed.

The association between brain MRI and cognitive performance is well established in MS.[14, 15, 232] It was suggested that T1 and T2 lesion volumes[91, 94, 233] may provide prognostic information regarding the risk of cognitive impairment. However, we know that measures of T1 and T2 lesion volumes or other single brain imaging predictors are necessary but not sufficient to fully characterize the neuropathologic underpinnings of MS associated cognitive impairment.[15] Therefore, it remains to be investigated if intergrated approaches, including also MRI measures reflecting rather neurodegenerative processes would improve our ability to identify MS patients with cognitive impairment.

Objective: The aim of this study was to generate an MRI-based algorithm that allows clinicians to identify the MS patients in the need of neuropsychological assessment and those at highest risk of cognitive decline over short-term follow-up period. More specifically, to examine, if assessment of lesion burden together with whole brain atrophy on MRI improve our ability to identify cognitively impaired MS patients.

Methods: Of the 1253 patients enrolled in the study, 1052 patients with all cognitive, volumetric MRI and clinical data available were included in the analysis. Brain MRI and neuropsychological assessment with BICAMS were performed. It is well known that focal lesions of white matter are pathologically unspecific and play only a partial or complementary role in cognitive impairment compared with damage to microstructural integrity of white matter.[15, 128, 234, 235] In addition, deep gray matter atrophy may represent stronger and more specific MRI correlate of cognitive impairment compared to whole brain atrophy.[15, 97, 128] Based

on potential availability of an established MRI volumetric assessments in common clinical practice, lesion burden measured by T1 and T2 lesion volumes together with whole brain atrophy measured by brain parenchymal fraction were examined in this study.[3, 236-239] 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 timepoints (months 12 and 24).

Results and discussion: This study, investigating a large cohort of actively treated MS patients identified T1 and T2 lesion load together with brain atrophy measured by brain parenchymal fraction as predictors of cognitive functioning, is in agreement with previous research.[14, 15, 91, 94, 233, 240, 241]

In this study, T1 and T2 lesion volume, showed comparable correlations with cognitive performance to brain parenchymal fraction. Importantly, both lesion load and whole brain atrophy were independent markers in the multivariate models. The proportion of the variability explained by the multivariable models was greater than that explained by the univariable models, which suggests that combined MRI measures are to a certain extent complementary. T1 and T2 lesion burden and brain parenchymal fraction explained 16-17% of variability of SDMT and 8-16% of variability of BICAMS. The multivariable models containing T1 lesion volume and brain parenchymal fraction or T2 lesion volume and brain parenchymal fraction, explained 21% of the variability of SDMT and 15-17% of the variability of BICAMS.

Additive effect of the combination of MRI markers was supported in the individual prediction analysis. Increase of T2 lesion volume (>3.5 ml) was associated with the 3-fold greater prevalence of CI in patients with high brain parenchymal fraction (>0.85), but with the almost 6-fold prevalence of CI in patients with low brain parenchymal fraction (<0.85). Here, similar results were found when T2 lesion volume was replaced by T1 lesion volume. In addition, the risk of cognitive impairment was 3-fold greater in patients with high brain parenchymal fraction and high T2 lesion volume than in patients with high brain parenchymal fraction and low T2 lesion volume. Importantly, the risk of cognitive impairment was 6.5-fold greater (OR=6.5 [95% CI 4.4-9.5]) in patients with low brain parenchymal fraction and high T2 lesion volume than in patients with high brain parenchymal fraction and low T2 lesion volume. Low brain parenchymal fraction (<0.85) together with high T2 lesion volume (>3.5 ml) identified in 270 (25.7%) patients predicted cognitive impairment with 83% specificity, 82% negative predictive value, 51% sensitivity and 75% overall accuracy. Overall, the same predictive accuracy of combination of T2 lesion volume and brain parenchymal fraction for

identification of patients with cognitive impairment was also found in consecutive time-points at 12 and 24 months of the study, with predictive accuracy being 73 and 71% respectively. Considering a good negative predictive value (82%) but relatively poor positive predictive value (53%) of the combined MRI markers, this MRI algorithm may help to identify especially those individuals who are unlikely to be cognitive impairment.

We have also shown that MRI markers of lesion burden and brain atrophy were not associated only with greater prevalence of cognitive impairment cross-sectionally, but also with greater risk of cognitive decline. The risk of confirmed cognitive decline over the follow-up was greater in patients with high T2 lesion volume (OR=2.1; 95% CI 1.1-3.8) and low brain parenchymal fraction (OR=2.6; 95%CI 1.4-4.7). Higher lesion burden and more advanced brain atrophy were associated with approximately 2-fold greater risk and combined MRI predictors with approximately 3-fold greater risk of confirmed cognitive decline over short-term follow-up period.

Although white matter lesions represent histo-pathologically heterogeneous and dynamic group of focal brain pathology, ranging from oedema and inflammation to demyelinitation and axonal loss, their neuro-inflammatory origin is well accepted.[42] Brain parenchymal fraction is a well known marker of whole brain atrophy, which is known to be accelerated in MS and may reflect tissue loss due to neuroinflammation and neurodegeneration.[3, 236-239] Therefore, we suggest that an integrated approach covering presumably various aspects of MS pathophysiology may improve our ability to explain contribution of MRI pathology to cognitive functioning in MS. This is also supported by other studies showing complementary effect between lesion accumulation and brain atrophy to predict disability progression.[19] In this context it is worth noting that lesion accumulation and whole brain atrophy driven predminantly by gray matter volume loss occur simultaneously.[242] On the other hand in approximately 25% of MS patients lesion burden and whole brain atrophy do not seem to be associated.[238] Therefore, it is feasible to expect that lesion burden and global brain atrophy may contribute to cognitive impairment independently.

It is known that neuroinflammatory and neurodegenerative processes associated with irreversible axonal damage and brain tissue loss may accumulate in relatively asymptomatic patients. However, once the brain reserve is surpassed, compensatory mechanisms may fail and neurologic and cognitive disability may occur.[239] Based on our results, we hypothesize that brain tissue reserve, estimated by brain parenchymal fraction, may explain why patients with advanced brain atrophy are relatively resistant to the increase in cerebral lesional injury before developing measurable cognitive impairment. In other words, both inflammation and

neurodegeneration need to be substantially present in an individual patient to exceed brain tissue reserve and thus cause clinical impairment, as suggested by the „two-hit" hypothesis.[238]

Conclusions: Our study suggests that a combination of MRI assessment of lesion burden and whole brain atrophy facilitates identification of the MS patients with cognitive impairment. In a routine practice where more advanced volumetric MRI assessment is not available, measurement of T2 lesion volume and brain parenchymal fraction may provide clinicians with a tool used for evaluation of individual risk of cognitive impairment. With the growing recognition of the clinical relevance of cognitive impairment in MS, advances in neuro-rehabilitation and increasing number of highly effective disease-modifying treatments, early identification of those at a highest risk of cognitive impairment may be of great importance for both patients and physicians.

Limitations: Cognitive functioning in this study included assessment of BICAMS battery. BICAMS is brief cognitive assessment tool that is not intended to replace comprehensive evaluation of the cognitive functioning in MS patients (such as that provided by a more extensive specialised neuropsychological batteries). On the other hand, its accuracy and reliability are comparable to those of the comprehensive MACFIMS and thus was recommended as a cognitive screening tool in MS.[14, 15, 100] Majority of the patients enrolled in the study were relapsing-remitting MS patients treated with disease-modifying treatment, with average disease duration 10 years and median EDSS 2.5. This may limit generalizability of our findings to the whole population of MS patients, especially those with primary and secondary progressive MS. On the other hand, we suggest that our results may be generalizable to the populations of patients on disease-modifying treatments (i.e., the populations, most often seen by MS specialists in clinical practice). It is also worth noting that the number of lesions; value of the contrast-enhancement; regional brain volume changes such as corpus callosum and thalamic volumes; topography of brainstem lesions and advanced non-conventional MRI methods (such as assessment of normal-appearing white matter, diffusion tensor MRI, voxel-wise analysis or identification of cortical lesions) were not investigated in this study. It has to be acknowledged that especially assessment of diffuse white matter damage and deep gray matter atrophy represent stronger and more specific MRI markers of cognitive impairment in MS.[15, 97, 128, 234] On the other hand most of these MRI techniques may by difficult to implement in routine clinical practice. Therefore, T2 lesion volume and normalized whole brain volume measured by brain parenchymal fraction as one of the most available and established volumetric

MRI markers of disease burden were analyzed in this study.[3, 236-239] Even then, provided cut-off values for lesion volumes and brain parenchymal fraction has to be validated on other MRI scanners and with different volumetric software. In this context, we provided the z-scores corresponding to our cut-off values that may be compared more easily across MS centers. Indeed, brief cognitive testing using BICAMS is much easier and less expensive tool for identification of cognitive impairment compared with brain MRI volumetry. Therefore, brain MRI as a screening tool for identification of cognitive impairment patients appears to be counter-intuitive. However, brief cognitive assessment is still not accessible to a large proportion of patients seen by MS specialists. Until brief cognitive assessment become routine and accessible for all MS patients, MRI volumetry may provide add-on value of cognitive screening in the face of objective measures of MS-related brain damage.

Reference:

Uher T, Vaneckova M, Sormani MP, Krasensky J, Sobisek L, Dusankova JB, Seidl Z, Havrdova E, Kalincik T, Benedict RH, Horakova D. Identification of multiple sclerosis patients at highest risk of cognitive impairment using an integrated brain magnetic resonance imaging assessment approach. Eur J Neurol. 2017 Feb;24(2):292-301. doi: 10.1111/ene.13200.[20]

Figure 1. The trial profile.
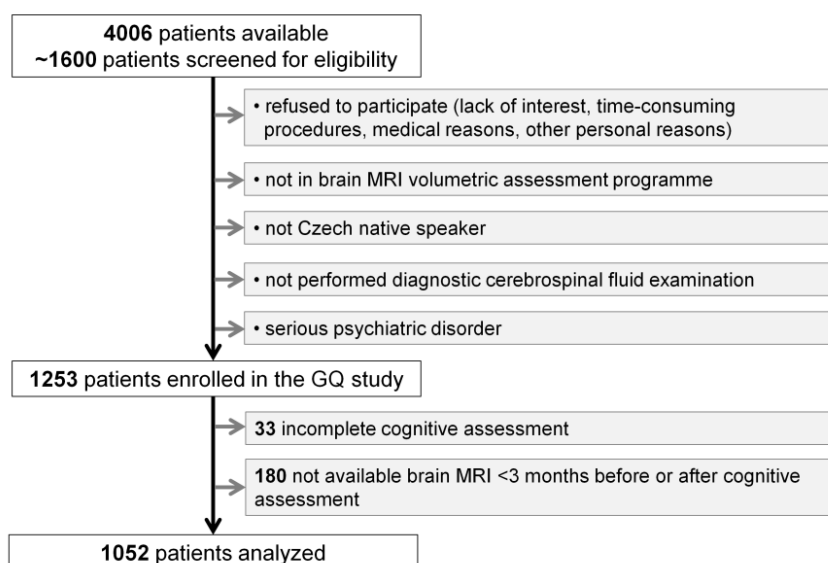
Figure 2. Associations between: (a-c) T2 lesion volume or (d-f) Brain Parenchymal Fraction and (a, d) Symbol Digit Modalities Test (SDMT); (b, e) Brief Visuospatial Memory Test-Revised (BVMTR); (c, f) California Verbal Learning Test (CVLT2). Here, Spearman´s correlation coefficients (rho) and standardized coefficients beta (β) of adjusted linear regression analysis are reported. Full line is regression line representing the best-fitting line a simple linear regression analysis using the least squares method.
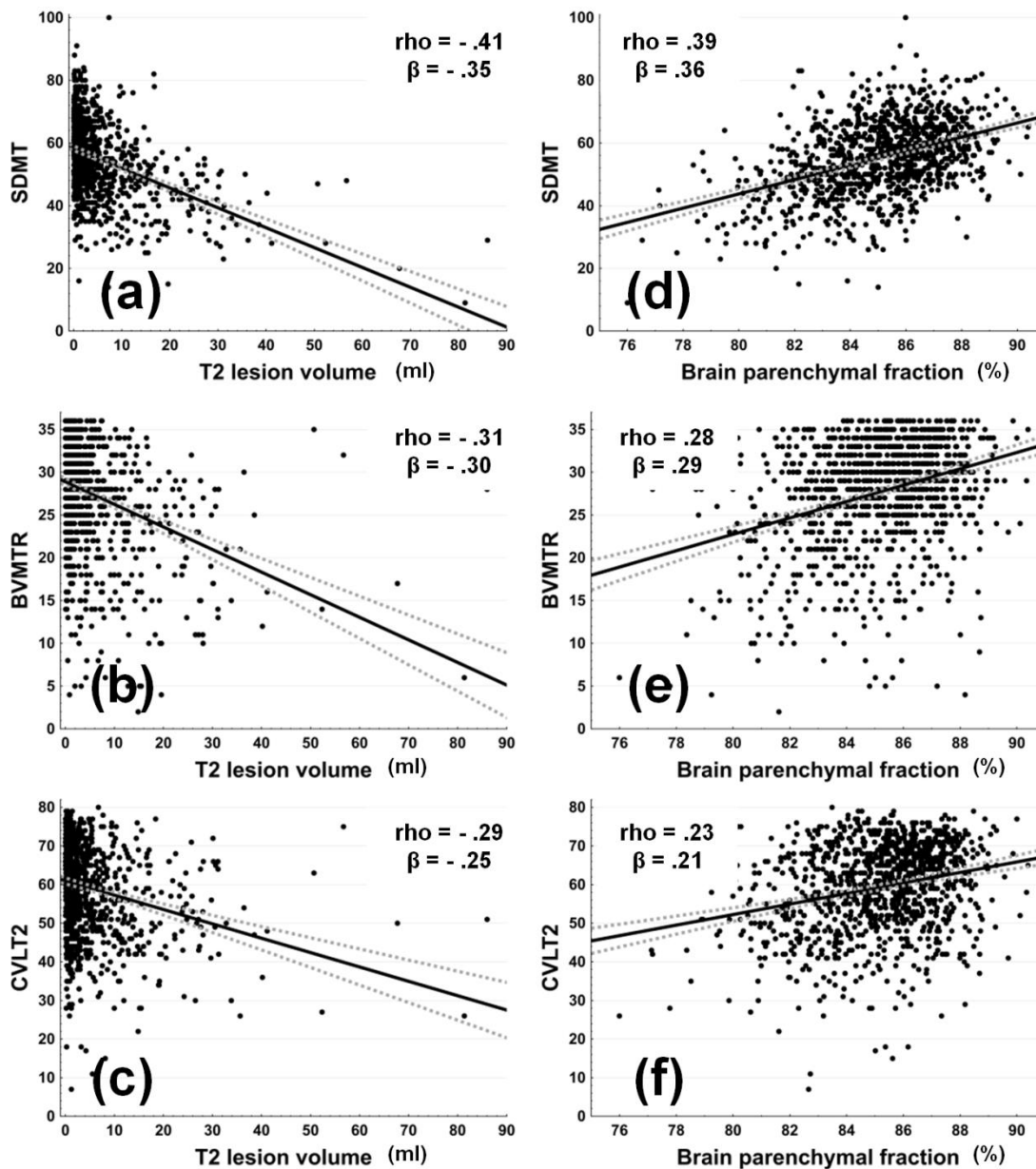
Figure 3. Proportions of cognitively impaired patients (i.e., abnormal outcome of BICAMS battery) in subgroups of patients defined by dichotomized MRI predictors (T2-LV = T2 lesion volume and BPF = Brain Parenchymal Fraction) at baseline of the study. Means and 95% confidence intervals are shown.
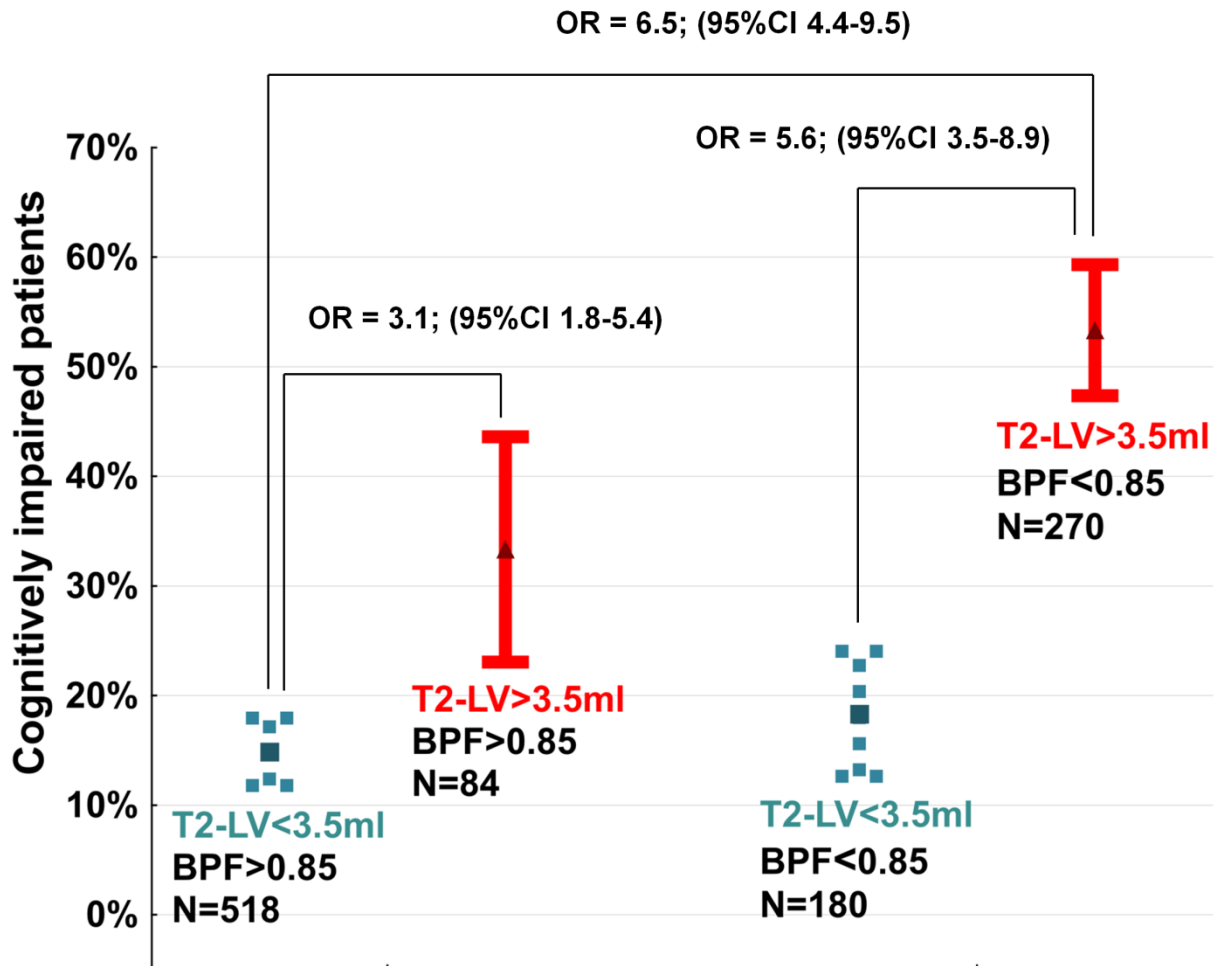
Figure 4. Proportions of cognitively impaired patients (i.e., abnormal outcome of the BICAMS battery) in the subgroups of patients defined by dichotomized MRI predictors (T2-LV = T2 lesion volume and BPF = Brain Parenchymal Fraction) at 12 and 24 months of the study. Means and 95% confidence intervals are shown.

Figure 5. Prediction of cognitive decline (i.e., newly occurred abnormal outcome of the BICAMS battery) in the subgroups of patients defined by dichotomized MRI predictors (T2-LV = T2 lesion volume and BPF = Brain Parenchymal Fraction) at baseline of the study. Means and 95% confidence intervals are shown.
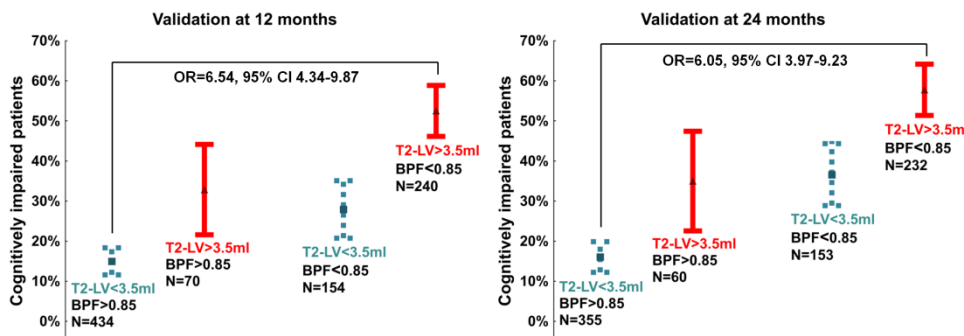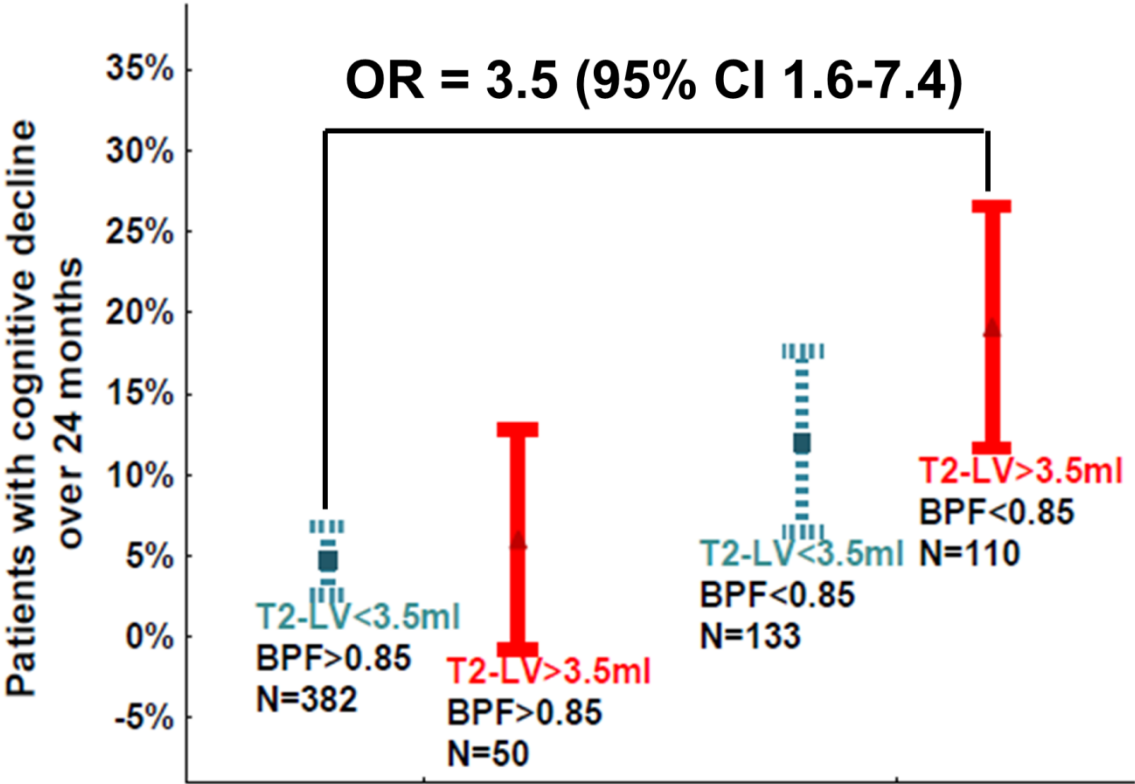
5.8 Cognitive clinico-radiological paradox in early stages of multiple sclerosis

Background: Associations between brain imaging measures and cognitive functioning have been observed in MS patients.[14, 15] Particularly, T1 lesion volume and T2 lesion volume,[91, 233] damage of normal-appearing white matter,[127, 128] occurrence of cortical lesions,[243, 244] and grey matter,[193, 245] or thalamic atrophy[95, 128] have been suggested as important brain imaging correlates of cognitive impairment. Even though most previous studies have shown a relationship between lesion burden or brain atrophy on MRI and cognitive impairment, there are still a number of studies that did not report such associations.[81, 234, 241, 246-253] In addition, the magnitude of reported associations vary wildly between studies.[14, 15, 254] By further examining these inconsistencies, the interpretation of associations between structural and cognitive metrics can possibly be improved by identifying contextual determinants of the strength of these associations. Most previous studies investigating MRI correlates of cognitive impairment included small samples or heterogeneous MS populations. We hypothesized that the strength of the association between brain MRI and cognitive measures in MS varies as a function of disease stage, cumulative disease burden and patient characteristics.

Objective: The aim of this study was to investigate whether the strength of the association between MRI metrics and cognitive outcomes differs between various MS subpopulations.

Methods: The present study was conducted using a large and clinically well described observational cohort of 1052 patients with predominantly relapsing-remitting MS, mostly on disease-modifying treatments. Brain MRI (T1 and T2 lesion volume and brain parenchymal fraction) and neuropsychological assessment (BICAMS and PASAT) were performed.

Results and discussion: Although most previous MS studies have observed associations between brain atrophy or MRI lesion measures and cognitive performance, the magnitude of these associations were highly variable between studies.[14, 233, 254] It has been suggested that heterogeneity of the utilized imaging and cognitive metrics are primarily responsible for this variability.[254] It is, however, not yet clear whether this association varies with respect to patient characteristics as well.

In this cross-sectional study of 1052 well-defined patients with MS, we found that the strength of associations between volumetric brain MRI and cognitive measures increases with clinically and radiologically more advanced disease. Correlations between brain MRI and cognitive metrics were relatively low in patients with a low disease burden (i.e. short disease duration or young age, low EDSS, low lesion load and low brain atrophy). Weak correlations between cognitive domains and MRI measures were observed in younger patients (age≤30 years; absolute Spearman ' s rho=0.05-0.21), with short disease duration (<2 years; rho=0.01-0.21), low EDSS (≤1.5; rho=0.08-0.18), low T2 lesion volume (lowest quartile; <0.59 ml; rho=0.01-0.20) and high brain parenchymal fraction (highest quartile; >86.66; rho=0.01-0.16).

In contrast, considerably stronger correlations between brain MRI and cognitive measures were found in patients with a high disease burden (i.e. long disease duration or older age, high EDSS, high lesion load or high brain atrophy). Stronger correlations between cognitive domains and MRI measures were observed in older patients (age>50 years; rho=0.24-0.50), with longer disease duration (>15 years; rho=0.26-0.53), higher EDSS (≥5.0; rho=0.23-0.39), greater T2 lesion volume (highest quartile; >5.33 ml; rho=0.16-0.32) and lower brain parenchymal fraction (lowest quartile; <83.71; rho=0.13-0.46).

The observed trends of increasing radiological burden were confirmed by interaction terms with quantitative MRI metrics (T1 lesion volume, T2 lesion volume and brain parenchymal fraction) versus cognitive tests. Even though patients with older age, longer disease duration, or higher EDSS also showed more pronounced correlations between MRI and cognitive metrics, majority of interactions between age, disease duration, or EDSS and MRI measures in multivariable models predicting cognitive performance were not significant after correction for false discovery rate. We hypothesize this may be explained by the fact that associations between age or disease duration and cumulative disease burden are only indirect, and that EDSS scale (as a measure of clinical disease burden), is less objective and a relatively inaccurate measure of disease burden,[72] as compared to volumetric MRI measures.[255]

Taken together, the strong associations between MRI and cognitive metrics in more advanced disease imply there is a greater sensitivity of cognitive performance to structural changes in patients with already greater cumulative structural brain damage. We suggest that cognitive decline does not become clinically apparent until a certain threshold of substantial structural brain changes has been reached. The concept that a threshold for brain pathology needs to be met before cognitive decline becomes clinically apparent means that irreversible axonal damage and brain tissue loss under a certain threshold may accumulate in relatively asymptomatic patients. Once the cognitive brain capacity has been surpassed and functional

compensatory mechanisms fail, cognitive disability may become clinically apparent.[239, 256, 257] This could explain why patients with some but minor brain atrophy or with a small lesion burden often show only limited cognitive deterioration relative to brain pathology[20]. In contrast, patients with significant pre-existing brain damage are relatively sensitive to any additional structural brain damage.

It has been established that the sensitivity of cognitive and MRI measures to minor changes of cognitive performance or brain pathology is limited due to a number of factors influencing measurement accuracy. For example, actual lesion or brain volume differences between two subjects with a low radiological disease burden might be observed as comparable due to the fact, that lesion or brain volume measurement can be error influenced by biological and technical biases. The same could also be a factor in cognitive measures in patients with a relative preserved cognitive performance. Here, an actual cognitive difference between two subjects might be observed as comparable due to cognitive performance measurement error or physiological fluctuations influenced by variety of factors such as motivation and other personality factors, time of day, and fatigue level. Hence, it is not particularly surprising that the accuracy of correlations between MRI and cognitive measures in patients with low radiological and cognitive disease burden may be affected substantially.

Findings from this study could help elucidate the reason for the lack of associations found in the literature between brain MRI and cognitive metrics in MS cohorts with a low disease burden. For example, in five cross-sectional[241, 250-253] and two longitudinal studies,[81, 92] which included between 43 and 81 patients in very early stages of MS, no significant associations between MRI lesion burden and cognitive measures were reported. Four of these studies also examined the relationships between global or regional brain atrophy and cognitive measures but found no associations.[81, 251-253] Other studies did not find associations between MRI and cognitive measures presumably due to the small sample size and heterogeneity of studied cohorts.[234, 246-248]

Conclusions: Our study suggests that greater structural brain damage corresponds to higher cognitive impairment, especially in patients with a greater pre-existing cumulative disease burden, disease duration, or age. Although our results may be not surprising, they have several practical implications that have not been considered in previous research. Firstly, patients' MRI and clinical characteristics should be taken into consideration when interpreting associations between structural and cognitive changes in clinical trials, research studies and clinical practice. Our results also emphasise the need for balanced recruitment of participants into clinical trials

in terms of radiological disease burden. Finally, in clinical settings, the accumulation of subclinical brain damage in MS cannot be interpreted as 'benign disease' since the clinical impact of quantitative brain damage may be delayed. With an increasing number of highly effective disease-modifying treatments,[201, 258] the identification of patients at highest risk of developing clinically apparent cognitive deterioration, with the aim of preserving their cognitive capacity, belongs among the top priorities of effective MS management.

Limitations: Cognitive function was assessed by the BICAMS battery and PASAT. While these tests evaluate a number of cognitive domains, including rapid information processing, visuospatial learning and memory, visual scanning, verbal learning and memory, working memory, attention switching and calculation, several domains such as higher executive functions, visual-spatial ability or phonemic fluency were not tested.[100] However, the domains tested in the present study are know to be most commonly impaired in MS.[175] A relatively low proportion of patients had abnormal BICAMS outcome (27%). This could be a result of our sample consisting of predominantly patients with a short disease duration and low disease burden. Another limitation is that focal MRI lesions of white matter are only partially reflective of the disseminated pathology in MS.[15, 127, 128] Their specific topography rather than volume may play a role in the pathogenesis of cognitive impairment. More sophisticated non-conventional MRI techniques, such as magnetisation transfer ratio, diffusion tensor imaging, proton MRI spectroscopy, and functional MRI measuring various aspects of MS pathology,[15] are likely to further improve our understanding of the associations between MRI and cognitive function at different stages of MS. Finally, sufficiently powered longitudinal studies are warranted to investigate the long-term associations between MRI and cognitive changes.

Reference:

Uher T, Krasensky J, Sobisek L, Blahova Dusankova J, Seidl Z, Kubala Havrdova E, Sormani MP, Horakova D, Kalincik T, Vaneckova M. Cognitive clinico-radiological paradox in early stages of multiple sclerosis. Ann Clin Transl Neurol. 2017 Dec 15;5(1):81-91. doi: 10.1002/acn3.512. eCollection 2018 Jan.

Figure 1. The strength of associations between brain MRI (Brain Parenchymal Fraction, T1 and T2 lesion volume) and cognitive measures (Symbol Digit Modalities Test, three-second interval Paced Auditory Serial Addition Test, Brief Visuospatial Memory Test Revised, California Verbal Learning Test Second Edition) in MS subpopulations. Subgroups of patients stratified by (A) age, (B) disease duration, (C) EDSS, (D) T2 lesion volume or (E) Brain Parenchymal Fraction were used for graphical purposes (the primary analysis of interaction models was performed using continuous variables).
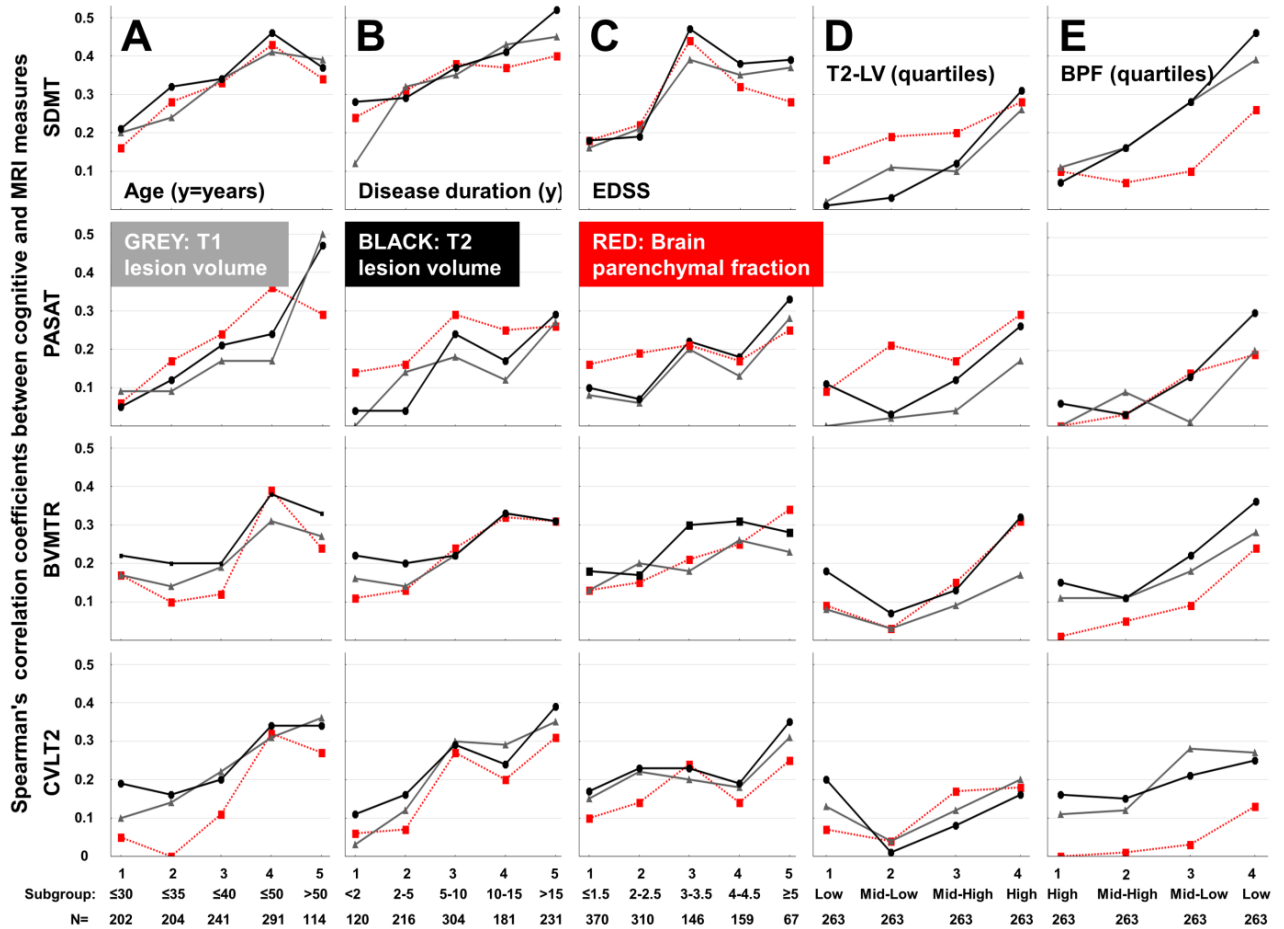
Figure 2. (A) The strength of associations between brain T2 lesion volume (cox-box transformed) and Symbol Digit Modalities Test scores in four MS subpopulations according their Brain Parenchymal Fraction quartiles. (B) Example of the graphical presentation.

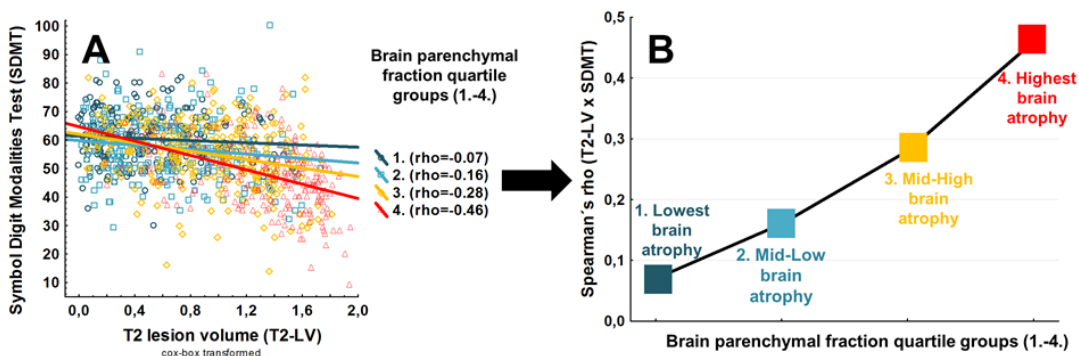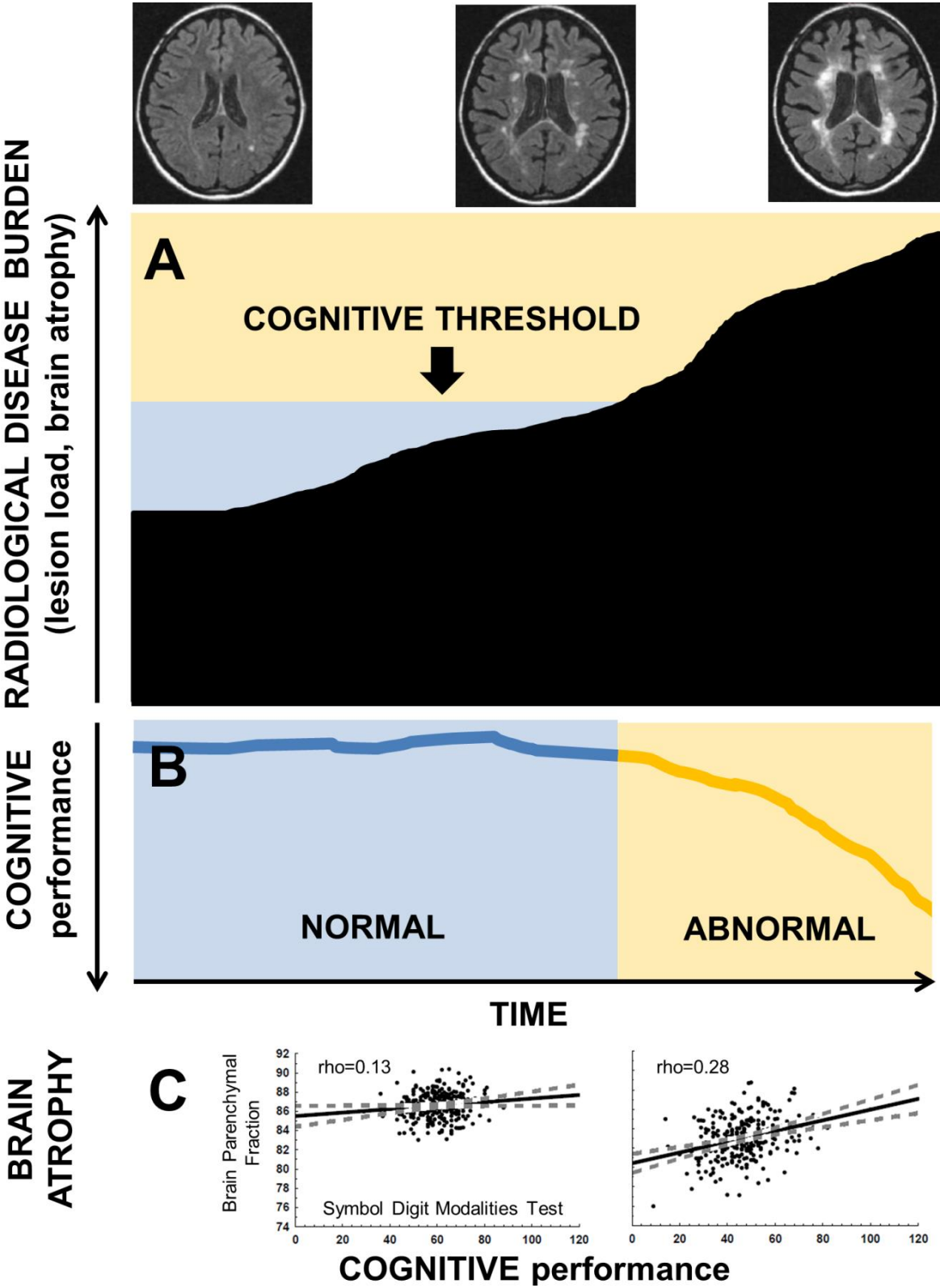Figure 3. (A-B) The threshold concept of brain pathology for the manifestation of a cognitive decline in MS patients. (C) The strength of associations between Brain Parenchymal Fraction and Symbol Digit Modalities Test scores in patients within the lowest and the highest quartile of T2 lesion volume.

6. Summary and future directions

6.1 Time course and stability of brain atrophy

There is a relative high intra-subject variability of global and regional MRI volumetric change measures. However, inter-subject variability differed substantially among MRI markers. For example, compared to whole brain atrophy rate, the percent volume loss of gray matter and thalamus was approximately two-fold. The variability in the corpus callosum volume loss was even three-fold higher. Considering the high measurement error of MRI volumetric measures, we hypothesize that specifically the MRI markers with high inter-subject variability (such as corpus callosum or lateral ventricle) could represent the most sensitive markers for monitoring of disease activity.[174, 183]

The vast majority of patients had a linear time-course of global and regional brain volume loss and lesion burden accumulation. Although, a non-linear time-course of brain volume loss occurred in proportion of patients with high rate of brain volume loss, long follow-up duration, and a high number of MRI scans over time. Additional factors associated with non-linear brain volume loss needs to be addressed in further analyses. It is possible that in datasets with longer follow-up and a higher number of MRI scans, the proportion of individuals with non-linear MRI trajectories is higher. Hence, the assumption of linearity of brain volume loss should be verified, especially in long-term MRI studies.

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

We identified cut-offs of annualized global and regional brain volume loss rates able to discriminate between physiological and pathological brain atrophy. Our findings suggest that the predictive accuracy of proposed volumetric cut-offs for the discrimination of pathological brain volume loss may differ in various MS populations. In general, the overall accuracy of cut-offs was greater in MS subgroups with higher rates of brain atrophy, such as in highly active relapsing-remitting MS, or in patients on disease-modifying treatment with a lower efficacy, such as interferons (80% specificity and 60% sensitivity). Conversely, the overall accuracy of

the established cut-offs was relatively low in clinically stable patients after first demyelinating event suggestive of MS, and in patients on natalizumab treatment with a mild rate of brain volume loss (80% specificity and 30% sensitivity).[183]

6.2 Relationship between brain atrophy and lesion burden accumulation

We found a strong association between lesion burden accumulation and brain atrophy progression. Only in a small proportion (5-20% depending on definition) of MS patients showed a weak association between lesion volume accumulation and brain atrophy over long-term follow-up. Given that there is no definition of this dissociation, several types of arbitrarily defined thresholds (median, tercile, quartile, percentile, parametric and nonparametric z-score) were tested.

Disproportionally higher brain atrophy, as compared to relatively low lesion burden accumulation, was only observed in a very small proportion of mostly young patients, in early disease stages, with a lack of advanced brain atrophy and low lesion burden. Whether this finding can be explained by a delay between lesion burden accumulation and brain atrophy, or pseudoatrophy effects, remains to be elucidated in further research. A disproportionately higher rate of atrophy vs. lesion accumulation was also present in patients with a very high lesion load at baseline and in patients with early massive decrease of lesion volume due to treatment interventions.

On the other hand, a disproportionately higher lesion burden accumulation, as compared to relatively low brain atrophy, was observed mainly in older patients, in advanced disease stages with an advanced brain atrophy at baseline, and high lesion burden at baseline (data not published). We speculate that this may be due an exhausted brain tissue reserve or the result of partially different pathological processes responsible for volume enlargement of large or confluent T2 lesions.

6.3 Prognostic role of MRI atrophy measures

MS patients with 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-beta1a) were at the highest risk of disability accumulation over long-term follow-up. The statistical predictive ability of these early MRI phenotypes was improved by their combination with early clinical outcomes. A composite score was generated from a subset of the best MRI and clinical predictors of disability progression, and we suggest that this composite score may provide valuable information for therapeutic decisions, even in individual patients.[1]

Specific cross-sectional MRI phenotypes (high lesion load and high brain atrophy assessed by brain parenchymal fraction, and their combinations) were also able to identify patient subgroups at highest risk of cognitive impairment and cognitive deterioration over short-term follow-up. Importantly, the statistical approach was based on the evaluation of cross-sectional MRI measures with have higher inter-subject variability and lower measuring error compared to longitudinal MRI markers. Therefore, we suggest that this integrated brain MRI-using assessment approach also has the potential to be used for risk estimation of cognitive impairment, also in individual patients.[20]

Finally, we hypothesize that high brain reserve, estimated here 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 increase of brain injury. On the contrary, patients with high pre-existing brain damage (a high degree of brain atrophy and lesion load) are relatively sensitive to any additional structural brain damage, a sign that is indicative of exhausted brain reserve.[182]

6.4 Recommendations for use of brain atrophy measures in clinical practice

Currently, it is broadly accepted that high intra-subject variability of longitudinal MRI measures resulting from biological and technical biases does not allow for confident estimation of brain atrophy rates in individual patients.[174] Moreover, we observed an unsatisfactory accuracy of

established brain atrophy cut-offs attempting to discriminate between physiological and pathological brain atrophy, even in models where linear regression slopes were fitted to all available brain percentage volume change measurements within the individual subject. Hence, overall predictive accuracy of the suggested cut-offs may be even lower in clinical practice due to considerable intra-individual fluctuations of brain atrophy outcomes occurring in patients due to various biases. In spite of this, we suggest that the evaluation of complex brain MRI phenotype monitoring could still be feasible in individual MS patients, if the following points are considered:

1) The application of single longitudinal volumetric measures of global or regional brain atrophy for individual prediction, 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,[183] may have the potential to outweigh high intra-subject variability of single imaging markers, and provide high measurement accuracy enabling its application in individual patients.

2) The accuracy of brain volume loss measures may be improved in individual patients when instead of a single scan, a series of consecutive MRI scans over a short-term follow-up are conducted. This approach could represent another option to minimize the high intra-subject variablity 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 cut-offs of brain atrophy, remains to be confirmed with further research. Preliminary findings have shown that longer follow-up ($\geq$2 years) rather then a higher number of MRI scans may provide better precision of measurement of brain volume loss in individual patients.

3) Cross-sectional measures, such as absolute T2 lesion volume or brain parenchymal fraction, have substantially lower measurement errors and higher inter-subject variability compared to longitudinal outcomes. Therefore, we suggest that these cross-sectional MRI markers could be applied for the prediction or risk estimation of disability progression, also in individual patients. However, due to the cross-sectional nature of the brain parenchymal fraction measure it cannot be used for longitudinal monitoring of brain atrophy.[1, 20]

6.5 MRI phenotypes

Identification of specific MS phenotypes may be of great importance for the risk estimation of disability progression and individually tailored treatment. Determination of the specific disease patterns may also improve our understanding of disease mechanisms of MS and it would contribute to the current research of genetic associations with disease progression.[259] Finally, identification of specific MS subgroups would also influence future research through the possibility to test new relevant hypotheses, to re-evaluate findings of previous research and to improve patient recruitment into clinical trials.

However, currently used MS classification reflects purely clinical course and comprise only limited number of clinical subtypes.[77] The narrowed spectrum of clinical subtypes is however not only limitation. There is also great heterogeneity of clinical course, treatment response and objective biological markers within established clinical subgroups. As the results, translation of the clinical classification into clinical practice for treatment decision-making and prognosis estimation in individual patient is limited. In this context reliable imaging and laboratory surrogate markers that would potentially provide objective criteria for identification of specific disease patterns are lacking.

Several previous studies suggested that brain imaging markers may potentially modify or complement the current clinical MS classification.[77] Especially MRI which clinical importance is growing rapidly is considered more accurate, more pathologically representative and more objective tool for description of disease patterns as compared to clinical assessment confused by several biases. It was hypothesised that MRI is able to identify MS phenotypes that are more directly influenced by pathophysiological mechanisms.[259] Additionally, combination of MRI imaging together with clinical outcomes was also suggested to improve identification of MS phenotypes.

To the best of our knowledge, only few attempts have been made to investigate specific MRI phenotypes in MS systematically. Moreover, previous studies were cross-sectional, employed small sample sizes and investigated only conventional or global MRI volumetric measures.[238, 260, 261] For example, recent cross-sectional studies described four MRI phenotypes based on brain imaging measures of inflammation (assessed by T2 lesions volume or occurrence of contrast-enhancing lesions) and brain imaging measures of axonal/tissue loss (assessed by brain parenchymal fraction). Although, in most of the patients higher T2 lesion burden was associated with greater brain atrophy, considerable proportion of patients had either high T2 lesion load or high brain atrophy. It was suggested that different pathophysiological

mechanisms, individual regeneration and repair capacity, neuronal and axonal integrity or dominating gray matter pathology may explain occurrence of different MRI patterns. Notably, correlation between disability and MRI outcomes was different in MRI subgroups indicating potential prognostic role of MRI phenotypes.[238] It is also worth noting that MRI phenotypes were not related to current clinical MS classification. This is in an agreement with some other studies showing MRI variability among individuals within established clinical MS subtypes.

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

6.6 Summary of the main findings

- A high intra-individual variability of longitudinal brain MRI volumetric measures is present in the majority of patients.

- Established cut-offs of pathological brain atrophy have a relatively low accuracy.

- Meassurement error of brain atrophy estimates is comparable with suggested cut-offs of pathological brain atrophy rate.

- 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 potentially allow us to use volumetric measures in individual patients in future.

- Some regional brain atrophy measures (corpus callosum, lateral ventricle) may be more suitable for disease monitoring due to their greater disease specificity, higher inter-individual variability, but similar intra-individual variability as compared to whole brain atrophy measures.

- Cross-sectional volumetric measures (for example lesion volume or brain parenchymal fraction) have higher inter-individual variability and lower intra-individual variability compared to longitudinal volumetric measures. Therefore, it can reliably be applied for the prediction or risk estimation of disability progression, also in individual patients.

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