The Relationship of the Various MRI parameters and their contribution with Clinical and Cognitive Disability in Multiple Sclerosis

Ph.D. Thesis

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Multiple Sclerosis

The worldwide incidence of multiple sclerosis is increasing. In 2001 it was estimated to be 3.6/100000 person-years in women and 2.0/100.000 person-years in men. The prevalence of MS has an uneven distribution worldwide, as it varies from high levels in temperate areas to low rates in tropical areas. The exact underlying factors in the development of MS are still not clear. However, some predisposing factors are already known, such as smoking, EBV infection, sunshine (UVB) exposure and also the level of vitamin D. There is an assumption about the genetic background of the disease.

The typical pathological findings in MS are demyelinating plaques caused by perivenular inflammatory lesions containing T-lymphocytes, mainly MHC class I restricted CD8+ T-cells and also a smaller number of B-cells and plasma cells. This inflammatory process leads to damage of oligodendrocytes and the development of demyelination. Although in the early stage of the disease axons are relatively spared, later in a more progressive phase, irreversible axonal damage develops. The classical 'active lesion', characterized by profound lymphocytic inflammation.

The diagnosis of MS is based on the clinical appearance, MRI examinations, the exclusion of other possible diagnoses, and sometimes other paraclinical tests, such as the examination of CSF, or evoked potentials. MS is characterized by clinical episodes (so-called "attacks" or "relapses") of neurological dysfunction. During the relapsing-remitting phase of the disease, between the relapses, the clinical condition of the patients is stable. Clinical disability is determined by the Expanded Disability Status Scale (EDSS). EDSS is based on the measurement of impairment of 8 functional systems (pyramidal, cerebellar, brain stem, sensory, bowel and bladder, visual and cerebral (or mental)), the ability to walk and mobility. The EDSS scale ranges from 0 to 10 in 0.5 unit increments.

In 1996, 4 clinical subtypes of multiple sclerosis were defined: relapsing-remitting (RR), secondary progressive (SP), primary progressive (PP) and progressive relapsing (PR). These descriptions were limited to relapsing (RR, SP, and PR) and progressive (PP, SP, and PR) forms, according to the differentiation, whether the course of the disease was predominantly relapsing or progressing. In October 2012 the Committee recommended changes to the course descriptions and also installed new disease courses, clinically isolated syndrome (CIS) and radiologically isolated syndrome (RIS).

In 2001, the McDonald criteria were developed. The Panel on MS Diagnosis (Panel) modified the McDonald criteria three times (2005, 2010 and 2017) to accelerate and ease the diagnostic process. According to the latest guideline, if at least two MS typical lesions should be present in at least two out of the four locations typical of MS (periventricular, subcortical, infratentorial and spinal cord) in the MRI the criteria of DIS is fulfilled. The coexistence of gadolinium-enhancing and non-enhancing lesions (both in the brain or the spinal cord) was considered sufficient proof for DIT. A new T2 and/or Gd+ lesion(s) on follow-up MRI, with reference to a baseline scan can still prove DIT, independently of the time of the baseline MRI. In 2016, the MAGNIMS made further improvements on the 2010 modified McDonald criteria and summarised them in a stand alone panel.

The treatment of MS is a complex process, including MS-specific disease-modifying therapies, symptomatic therapies and handling symptoms that develop due to the neurological dysfunctions. Treatment of acute relapses includes steroid treatment, plasmapheresis and IVIg. Immunosuppressants (fingolimod, natalizumab, ocrelizumab) or immunomodulatory treatment (interferon-beta, glatiramer acetate) aim to suppress inflammation and also disease activity. The presently available immune reconstitution therapies (alemtuzumab, cladribine) aim to produce long-term immunological processes. The ECTRIMS/EAN guideline on the pharmacological treatment of people with MS was published in 2017, in which questions about treatment efficacy, suboptimal response, response criteria, safety concerns and treatment of MS in pregnancy have been dealt with, and a total of 20 recommendations were proposed.

Since more and more effective DMTs are developed, the actual expectation in MS therapy is to reach no evidence of disease activity (NEDA-4): no relapses, no disability progression, no MRI activity, no brain volume loss.

MR Imaging of multiple sclerosis

While the demyelinating lesions are the diagnostic cornerstones of the disease, thus the most well-known MRI finding in MS, recent studies highlight that the grey matter and the white matter microstructure are also affected.

Conventional MRI is a sensitive technique to detect T2 hyperintense MS lesions, primarily appear in the periventricular WM, the corpus callosum, brain stem, subcortical regions, U-fibers and optic nerves. The focal demyelinating lesions next to the corpus callosum can be best detected by sagittal fluid-attenuated inversion recovery imaging. Besides the white matter lesions, lesions in the gray matter can also appear in MS. Optic neuritis, can be detected by

long-echo short-tau inversion recovery imaging, or a fat-suppression technique combined with contrast-enhanced imaging. In T1-weighted imaging, MS lesions in the acute phase appear isointense compared to the surrounding, while in the chronic phase, or in case of serious inflammatory oedema, they can be hypointense (black holes). In the acute inflammatory stage, the lesion may degrade the blood-brain barrier, causing gadolinium enhancement, which may last from days to weeks. Brain atrophy, presumably a net accumulative disease burden, is also a significant MR hallmark of MS, which can occur in every stage of the disease, even in the very early phase.

Grey matter atrophy

There are several available approaches that can define GM atrophy. The GM volume (cortical with or without subcortical) can be defined

- manually,
- by automatic segmentation of brain tissue types, measuring the gross volume (SIENAX).
- by measuring cortical thickness (VBM).

Diffusion Tensor Imaging

Magnetic resonance Diffusion Tensor Imaging (DTI) is a sensitive method for detecting water diffusion characteristics (for example the primary diffusion direction and diffusion anisotropy) and therefore can be used to investigate white matter tracts and other local properties of brain tissues. DTI can non-invasively portray the diffusion of water in biological tissues. With multiple measurements the diffusion can be described as an elliptoid, of which the three main axes are identified by singular value decomposition. Diffusion properties, which correlate with the tissue microstructure, can be described with the combination of these three axes. FA ($\sqrt{\frac{3}{2}}\frac{\sqrt{(\lambda_1-(\lambda))^2+(\lambda_2-(\lambda))^2+(\lambda_3-(\lambda))^2}}{\sqrt{\lambda_1^2+\lambda_2^2+\lambda_3^2}}$), MD ($\lambda_1+\lambda_2+\lambda_3$)/3, Axial diffusivity ($\lambda_1$), Radial diffusivity ($\lambda_2+\lambda_3$)/2.

Cross-subject comparison of DTI parameters is an inherently difficult task, mainly because of misalignment issues. Tract-Based Spatial Statistics (TBSS) provide a useful approach for localised statistical testing of diffusion parameters solving the registration issues.

Objectives

The identification of a reliable MRI marker and the structural background of clinical and cognitive symptoms in MS are still hot topics. While lesions in the white matter are remarkable MRI markers in MS and counted as one of the diagnostic cornerstones of the disease, the lesion load only modestly correlates with clinical and cognitive decline. This phenomenon is known as the clinico-radiological paradox. Although the Gd-enhancing lesions are slightly more specific MRI markers during the acute phase of the disease, they can rarely be seen on the MRI. It can happen, even in cases of relapse, that no Gd-enhancement appears. Since the Gd-enhancement can only be monitored for 2 or 3 weeks, detection is barely possible during a routine MRI. GM atrophy (cortical with or without subcortical structures) can be detected by several approaches.

Diffusion is the movement in the material, which is not accompanied by huge molar motions. It can be non-invasively portray by DTI. Cellular elements (membranes) inhibit molecular diffusion, thus the architecture of the tissues can be detected by the diffusion profile of water. Tract-Based Spatial Statistics (TBSS) and the manual labeling of the regions-of-interest (ROIs) are useful approaches for localised statistical testing of FA (and other diffusion-related) data.

In our first study we aimed to determine the relationship between cortical atrophy and WM pathology in MS. Within this, we studied whether the pathology of the focal lesions or the diffuse NAWM has a more significant role in the evolution of GM atrophy. Based on Jenha's study, we were also interested in the connection between the desintegration of the periventricular WM and the conception of GM atrophy. Our aim was to test two hypotheses. According to the first, GM atrophy is defined by demyelination-like diffusion features that suggest there is a common root in the development of demyelination in WM and GM atrophy (maybe a common pathological process mediated by the CSF). The second hypothesis proposed that GM atrophy is more connected to axon loss-like diffusion pattern, which points to a role of remote axonal transection in GM loss.

In our second study, we meant to define the pattern of the MRI parameters best predicting clinical and cognitive disability in patients with MS.

Since MRI parameters are strongly related, the conventional linear regression analysis is not an appropriate choice for the statistics. In our studies, we used the model-free partial least square

(PLS) approach, which can detect the design of those parameters that best predicts the questionable parameters, besides dealing with the problem of collinearity.

Methods

Subjects

The first and second studies were conducted on 52 and 53 patients, respectively, with a diagnosis of relapsing-remitting multiple sclerosis, and 50 and 53 healthy, age-matched volunteer controls with no history of any neurological or psychiatric diseases. Patients were recruited from the Multiple Sclerosis Outpatient Clinic at the Department of Neurology. The diagnosis was based on the 2005 revision of the McDonald criteria. The clinical disability of the patients was measured on the Kurtzke expanded disability status scale (EDSS). All patients were on disease-modifying therapy. The cognitive performance of the patients was measured by Brief International Assessment for MS (BICAMS)**.** All patients were in stable clinical condition, no relapses and no EDSS progression had occurred in the preceding six months.

The studies were approved by the ethics committee of the National Institute of Pharmacy and Nutrition and the Regional Human Biomedical Research Ethics Committee and all study participants gave their written informed consent in accordance with the Helsinki Declaration (Ref. No.: 000002/2016/OTIG).

Cognitive assessment of the patients

The Brief International Cognitive Assessment for MS (BICAMS) test is a short form that is a fast, sensitive and specific tool for the determination of the most frequently affected cognitive domains of the patient. The BICAMS test involves three separate tests: the symbol digit modalities test (SDMT), the first five recall trials of the California verbal learning test II. (CVLT-II) and the first three recall trials of the brief visuospatial memory test revised (BVMT-R). In our study, we used the validated Hungarian version of the BICAMS test. For all subtests of BICAMS, the patients' results were compared to the age-matched control group of healthy individuals from our earlier validation study. A difference of more than two standard deviations when compared to the control database was considered as abnormal.

Image acquisition

MR imaging was carried out on a 1.5T GE Signa Excite HDxt MR scanner. 3D spoiled gradient echo (FSPGR: TE: 4.1 ms, TR: 10.276 ms, matrix: 256x256, FOV: 25x25 cm, Flip angle: 15 degrees, in-plane resolution: 1x1 mm, slice thickness: 1 mm), FLAIR (TE: 4.1 ms, TR: 10.276

ms, matrix: 256x256, FOV: 25x25 cm, Flip angle: 15 degrees, in-plane resolution: 1x1 mm, slice thickness: 1 mm) and 60 direction diffusion-weighted images with 6 non-diffusion-weighted reference volumes (TE: 93.8 ms, TR: 16000 ms, matrix: 96x96, FOV: 23x23 cm, Flip angle: 90 degrees, in-plane resolution: 2.4x2.4 mm slice thickness: 2.4 mm, b: 1000 s/mm^2, NEX: 2, ASSET) were acquired for all subjects.

Evaluation of lesion load

Lesions were manually segmented on the FLAIR images by ET, and rechecked by ZTK, having considerable experience in MS neuroradiology.

Evaluation of Global Atrophy

We calculated the total brain volume with SIENAX, part of FSL. SIENAX started by extracting brain and skull images from the single whole-head input data.

Volumetric analysis of the subcortical structures

Image analysis was carried out using tools of FSL (FMRIB Software Library, http://www.fmrib.ox.ac.uk/fsl). To automatically segment the subcortical structures, FIRST, a deformable-model-based segmentation/registration tool was used that uses a Bayesian Appearance Model (FMRIB's Integrated Registration Segmentation Toolkit).

Microstructural alterations of the white matter

Diffusion data were corrected for Eddy currents and movement artefacts by a 12 degree-of-freedom affine linear registration to the first non-diffusion-weighted reference image. Diffusion images were processed using FDT (FMRIB's Diffusion Toolbox part of FSL: www.fmrib.ox.ac.uk/fsl/fdt/)). Fractional anisotropy, Mean diffusivity (($\lambda_1 + \lambda_2 + \lambda_3$)/3), Axial diffusivity (λ_1) and Radial diffusivity (($\lambda_2 + \lambda_3$)/2) to the principal diffusion direction were computed for the whole brain.

A voxel-wise alteration of the diffusion parameters, (microstructural integrity index - MII), was calculated for each patient, by comparing the value of every voxel with the distribution from the normal subjects in the spatially matching voxel (z-score).

Analysis of the connection between the MR parameters and clinical and cognitive status and between Brain Atrophy and the Compartmental Diffusion Metrics

We used partial least squares (PLS) regression analysis to estimate the contributions of the various MRI parameters (partial brain volume, normalised gray matter and white matter

volume, volume of the subcortical structures, lesion load and the diffusion parameters of the lesions and the normal appearing white matter) to the EDSS and the subscores of the BICAMS test. Using this analysis, we also determined the contributions of the calculated compartmental diffusion parameters to the EDSS, gray matter and global brain atrophy.

We performed volumetric comparison across groups and the correlation tests with the Statistical Package for Social Sciences (SPSS 17 for OS X, SPSS Inc., http://www.spss.com).

Results

Clinical, cognitive and imaging parameters of the patients

Despite the comparatively long disease duration, the patients who took part in our studies have only mild to moderate disability as measured by EDSS. In our first study the SIENAX analysis revealed reduction in total brain volume (patients: 718.764 ± 14.968 cm3, controls: 791.772 ± 22.692; mean $\pm$ SE), total WM (patients: 323.237 ± 7.246 cm3, controls: 355.350 ± 10.929; mean $\pm$ SE), and total GM (patients: 395.527 ± 8.050 cm3, controls: 436.422 ± 12.011; mean $\pm$ SE) volume in the MS patients compared to controls. Compared to the healthy controls, TBSS showed significant decrease in the FA ($p < 0.0002$), increase of MD and RD ($p < 0.0002$) of the MS patients in essentially all the WM fiber bundles. Importantly, the WM, diffusion alterations (FA, MD and RD) were distributed in the NAWM and also in the periventricular WM, where lesions appeared with high probability. In contrast, an increase in AD was only found in the more central fibers ($p < 0.0002$). In the second study, out of the 53 patients, 18 had cognitive dysfunction on one cognitive test, 8 on two tests and 5 on all three tests.

Lesion Probability Distribution

In our first study we determined the distribution of the white matter lesions and their connection with the total brain and gray matter atrophy. The average native space lesion load was 12.328 ± 16.100 cm3 (mean $\pm$ SD) and the lesion load normalized to the intracranial volume (v scaling factor) was 17.087 ± 22.509 cm3 (mean $\pm$ SD). The normalized lesion volume showed a negative correlation with the normalized GM volume ($R = -0.32$, $p < 0.021$), but there was no correlation with the normalized brain volume. The lesion load did not correlate with the EDSS of the patients either. The lesions were distributed across widespread WM regions, but the lesion probability was highest in the periventricular WM.

The Connection between the Brain Atrophy and the Compartmental White Matter Pathology

In our first study our main interest was to the determine those compartmental diffusion parameters (periventricular and non-periventricular $\overline{Z}_{FA}$, $\overline{Z}_{MD}$, $\overline{Z}_{AD}$ and $\overline{Z}_{RD}$) which best predict brain atrophy. In the first PLS analysis the normalized GM volume was the dependent variable. Only the first latent variable was evaluated because the second latent variable explained only a small part of the variance of the dependent measure (<5%) and the permutation test showed a non-significant latent variable. The permutation test revealed that the first latent variable was significant (p < 0.001) and accounted for 47.3% of the variation of the dependent variable and 76.5% of the predictors. The X loadings and the corresponding Variable Importance in the Projection scores indicated that the MD and RD of the lesioned and non-lesioned periventricular and the non-periventricular lesioned WM contributed significantly to GM atrophy. Similar results were found in the case of normalized brain volume: only the first latent variable was significant according to the permutation test. The first latent variable accounted for 24.9% of the variation of the dependent variable and 76.7% of the predictors. X loadings coding the optimum contrast of the predictors exhibited a similar pattern to that of GM volume.

The imaging parameters influencing clinical and cognitive functions

We examined the MRI markers that best predict clinical disablity. In the PLS analysis in which the EDSS was the dependent variable, the first latent variable was significant according to the permutation test (p < 0.001). It was responsible for 50.67% of the variation of the dependent variable and 27.08% of the predictors. Age (VIP score: 1.72) and the AD of the NAWM contributed most to clinical disability (VIP score: 1.979). While far less, the MD of the NAWM (VIP score: 1.169) and the demyelination features of the lesions (VIP$_{FA}$: 1.17, VIP$_{RD}$: 1.08) were still significant contributors.

In our second study we primarily focused on the determination of those MRI structures that best predict the alteration of different cognitive domains. In these analyses, the raw scores from the three subtests of the BICAMS test were used as dependent variables. The first latent variable was estimated, as the second latent variable was responsible for just a small fraction of the variance of the dependent measure (<5% in case of BVMT and CVLT and 10% in case of SDMT) and the permutation tests indicated non-significant latent variables. The permutation test showed that the first latent variable was significant (p<0.001 for each subtest) and explained 50.99% of the variation of the dependent variable and 23.89% of the predictors in the case of

BVMT, as well as 50.93% of the variation of the dependent variable and 22.24% of the predictors in the case of CVLT, and 50.67% of the variation of the dependent variable and 22.43% of the predictors in the case of SDMT. Age contributed significantly to all cognitive tests (VIP score: 1.538, 1.127 and 1.296 for BVMT, CVLT, and SDMT respectively). Gender was a significant contributor to CVLT and SDMT (VIP score: 1.356 and 1.345 respectively).

As regarding the **visuo-spatial working memory**, the most critical contributor was the size of the bilateral hippocampi (VIP scores: 1.183 and 1.2 left and right, respectively) and the demyelination features of the lesions (VIP_{FA} score: 1.257, VIP_{MD} score: 1.008, VIP_{RD} score: 1.158) and axon loss diffusion features of NAWM (VIP_{FA} score: 1.125, VIP_{L1} score: 1.232). Lesion load was also a marginally significant contributor (VIP score: 1.031). For **verbal memory,** the best predictor was the size of the right hippocampus (VIP score :1.972), the lesion load (VIP score: 1.274), the partial brain volume (VIP score: 1.119) the total white matter volume (VIP score: 1.008), the total grey matter volume (VIP score: 1.058), the size of the right caudate (VIP score: 1.152) and the FA of the NAWM (VIP score: 1.012). In the case of the **SDMT test,** the most significant contribution was from the demyelination-like diffusion parameters of the NAWM (fractional anisotropy and radial diffusivity VIP scores: 1.615, 321 respectively). The FA, mean and radial diffusivity of the lesions (VIP scores: 1.289, 1.082 and 1.271 respectively) and the size of the right hippocampus (VIP score: 1.101) also contributed significantly to performance.

Discussion

In our MRI studies, we explored the connection between GM atrophy and the microstructure of the WM, and also between the clinical and cognitive disability and various MRI parameters in RRMS patients. Our model-free, PLS analysis confirmed that the demyelination-like diffusion parameters, the increased MD and RD in the lesions and the periventricular non-lesioned WM were related primarily to GM atrophy.

In the second study it was the AD of the NAWM that best influenced clinical disability. The PLS analysis revealed a complex interaction between cognitive disability and the multiparametric MRI data. The different cognitive domains were predicted by several MRI parameters. The visuo-spatial working memory was affected by the size of the bilateral hippocampi and the demyelination-like diffusion profile of the lesions, and the axon loss of the NAWM. The size of the brain, white and grey matter and the right hippocampus were the best predictors of the verbal memory. The information processing speed was primarily influenced by alterations in the demyelination-like diffusion parameters of the WM.

According to earlier studies, the pattern of diffusion parameter changes refer to pathological changes in the WM. The alterations of AD and RD allude to damage of the axons or myelin, respectively. Besides, FA and AD correlated with the total axon number. According to this, the alterations of diffusion parameters we found in MS patients suggest widespread demyelination in the WM. We also detected widespread demyelination in the NAWM, which was only barely reviewed in former DTI studies. The sensitivity of our investigation was most probably increased by the high number of diffusion directions we applied in our study. More importantly, we found a pattern of demyelination-like diffusion parameter alterations in the periventricular WM (lesions and also non-lesioned areas) which best corresponded to the development of GM atrophy. This finding supports the hypothesis of Jehna about a CSF-mediated common process of cortical atrophy and periventricular demyelination. Subpial demyelination and cortical atrophy were associated with meningeal inflammation, infiltration of B-cell follicle-like structures, and CD3+, and CD8+ T-cells. Besides the demyelination in the cortex, there is a gradient of neuronal and astrocyte loss toward the pial surface and microglia activation in the opposite gradient. These findings are concordant with the results of *in vitro* studies and suggest the role of cytotoxic tissue damage due to a B- and CD8+ T-cell-mediated non-targeted general immunopathological response or microglial activation in the development of the cortical pathology. The spatial location of the abnormalities also strengthens the theory of a common pathomechanism behind the periventricular lesions and cortical demyelination. Subpial lesions are often located around deep sulci, with extended Virchow-Robin space, which contains many immune cells. Likewise, the periventricular lesions evolve quite often around the venules and have also been observed in MS-enlarged Virchow-Robin spaces.

It has long been known that not only the lesion load but the localisation of the lesions show weak correlation with clinical and cognitive disability. While the connection between clinical disability and the brain, predominantly the GM, appears to be stronger, several earlier studies, using voxel-wise morphometry failed to reveal a connection between the disability and the focal GM atrophy.

According to former papers, the volumetry of the thalamus correlated with information processing speed, verbal memory and the attention/executive function, while putaminal atrophy was associated with the information processing speed. Several investigations proved the connection between cognitive decline and hippocampal atrophy. The thinning of different cortical areas appears to be connected to different cognitive domains. Cortical thinning in the insula and the parietal region were connected to verbal and visual memory performance,

respectively. Left-sided cortical thinning of the anterior cingulate area was associated with decreased verbal fluency, while right-sided was related to reduced figural fluency. The thinning of the orbito-frontal cortex was connected to the speed of auditory information processing.

There are also contradictory results about the relationship of the diffusion metrics with disability. While Griffin did not find either diffusion parameter alteration in the NAWM or a correlation with disability, Ciccarelli revealed a connection between the clinical disability and the FA of the supra and infratentorial NAWM, in particularly FA and MD of the cerebral peduncles. Onu et al., in a whole brain TBSS analysis, described widespread differences between MS patients and controls and also found a correlation between FA, EDSS scores, ambulation, and hand function. Filippi et al. revealed a modest connection between disability and the MD of lesions.

The above-mentioned studies have their limitations, as most of them focus their analyses only on the FA and also the extent of the reported microstructural damage was usually far less. It was previously reported that alterations of RD and AD suggest demyelination and axonal loss, respectively. It is also important to use a high number of diffusion directions, thus the alterations of the demyelination-like diffusion parameters can be shown widespread in the WM skeleton.

As the summary of the above-mentioned studies shows, there is only a limited number of investigations that examine the pattern of various structural MRI markers contributing to a set of clinically relevant cognitive tests. According to our results, we suggest that the three subtests of the BICAMS are associated with alterations of different brain structures.

Atrophy of the bilateral hippocampi was the most critical contributor to **the visuo-spatial working memory**. Earlier studies, examining the structural background of visuo-spatial abilities reported controversial results. While Kern et al. found the uncinate fasciculus connecting the mediotemporal structures to the frontal cortex as the best predictor, Koenig and Dineen declared that the visuospatial memory correlated with the diffusion parameters of the fornix, the primary hippocampal efferent, but not the atrophy of the hippocampus itself. Importantly, the structural abnormality of one structure is usually associated with the other, functionally or structurally, connected structures.

The **verbal memory** test performance was defined by the volume of the total GM and right hippocampus. We should highlight the interesting fact that Dineen, in the study mentioned above did, not find a correlation between hippocampal atrophy and verbal or visuo-spatial memory scores, however, he used a smaller group of MS patients. According to the study of Kiy et al., the consolidation scores of the CVLT test were associated with the volume of the right temporal horn, which indirectly measures hippocampal atrophy. A recent study discovered

that hippocampal viscoelasticity, induced by physical activity, improves the performance on CVLT-II test in MS patients. While the CVLT long delay recall was correlated with global brain atrophy, the learning score of the CVLT-II was associated with the volume of the thalamus, amygdala, hippocampus and caudate, but not with the lesion load or the brain parenchymal fraction. A linear regression analysis declared the size of the caudate as the best predictor of the verbal learning ability.

It is interesting to note the relationship between the laterality of hippocampal atrophy and cognitive decline. The volume of both hippocampi predicted the BVMT similarly, while only the right hippocampus was a significant predictor of the CVLT. This latter finding is especially remarkable since, according to earlier studies, the volume of the right hippocampus is related to the visuospatial memory, while verbal memory is best defined by the left hippocampal volume.

We should highlight the fact that in our study, out of the three cognitive tests, **SDMT** showed a connection with the largest area. Yu et al. recently found widespread alterations of the demyelination-like diffusion parameters in the NAWM of MS patients, which showed a significant connection with the performance on the SDMT. According to another study the SDMT performance was related to the GM fraction and also the diffusion parameters of the brain parenchyma. In this case, similarly to our study, the authors used summary statistics for the diffusion parameters.

Conclusions

In our studies, we detected widespread diffusion parameter alterations in MS patients that suggested demyelination, both in the high lesion loading periventricular WM and the NAWM. Significant brain, WM and GM atrophy was found in the patients. According to the PLS analysis, the GM atrophy was best predicted by a pattern of demyelination-like diffusion parameters in the periventricular WM. Our findings also showed that the various MRI markers affect the development of the clinical disability and the decline of several cognitive domains.

While the pathology of MS is quite diverse, according to our results, even those processes that are spatially distant from each other may have common origins. The CSF is an assumed substance, mediating between such processes, but further investigations are needed to clarify those factors which are accountable for the demyelination in the GM and WM. Our results show that the atrophy of the cortical and subcortical structures and the diffusion measurements of the WM are crucial to understanding the disability progression. Accordingly, these measurements should be taken into account in clinical trials and highlighted in everyday clinical practice.

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