Behavioral and imaging markers of multiple sclerosis

Ph.D. Thesis summary

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Introduction

Multiple sclerosis (MS) is a devastating disease which mostly affects young adults and it is more common in females. The focal and diffuse inflammation and consequential changes cause the various clinical symptoms. While the white matter lesions are characteristic for the disease, diffuse microstructural and neurodegenerative alteration occur as well. A variety of symptoms presents such as optic neuritis, sensory and motor disturbances. The expanded disability status scale (EDSS) is an established tool to evaluate clinical severity. It is easy to administer, widely used clinically and requires no additional equipment. Besides the many advantages, there are few limitations of the scale. The scale is insensitive to small changes and certain functions are underrepresented such as cognition and oculomotor deficits.

Magnetic resonance imaging (MRI) is used in the diagnostic evaluation, monitoring and differential diagnosis of MS, and a great number of research investigated the potential role of MRI markers in MS biomarker research. A biomarker is a trait, which is measured objectively and assessed as an indicator of physiological and pathological processes or the response to a therapeutic intervention. For example, a new lesion in FLAIR/T2 weighted image in clinically isolated syndrome means disease conversion to relapsing remitting MS. However, its relation with the clinical status is ambiguous. Gadolinium (Gd) enhancing lesions are found in areas where blood-brain barrier breakdown and inflammation are described histopathologically in MS patients. Moreover, it is a weak predictor for long term, cumulative disability and impairment. The fluid attenuated inversion recovery (FLAIR) and T_2-weighted sequences are used to identify hyperintense lesions. The hyperintense lesions in general only modestly reflect the clinical disability in MS, therefore, other measures are necessary to attain a more comprehensive description of clinical impairment. Some of the hyperintense lesions in FLAIR/T_2W images are hypointense in the T_1-weighted images, which refers to the widely used “black hole” phenomenon. The clinical condition is in modest-to-strong association with the black hole status, and it is better than the T_2 hyperintense lesions. Brain and spinal cord atrophy turned out to be a good biomarker of MS. Microstructural alterations of the normal appearing white matter could be observed with special MRI sequences such as diffusion tensor imaging and magnetic transfer imaging. Several measurable MRI parameters are identified which represent the inflammatory or the neurodegenerative nature of the disease, or both. It is not clear that neurodegeneration or inflammation or both together cause clinical disability, but the combination of imaging markers could predict the clinical status. Moreover, to elucidate which mechanism in what extent is in the background of certain clinical symptoms such as oculomotor deficit may aid the selection of future neuroprotective or remyelinating therapies.

Diffusion is the non-bulk motion of molecules which occur at any temperature. The displacement of a particle over a period of time is proportional with the diffusion coefficient. The simplest form of diffusion is isotropic which means that particles move in any directions with equal probability. In contrary, in anisotropic medium, there are preferred directions of diffusion. In biological tissues, obstacles such as membranes restrict diffusion of water in directions perpendicular to them and diffusion is preferred in the parallel direction. Highly organized structures such as nerve fiber tracts could be found in the human central nervous system (CNS). For the description of direction dependent diffusion coefficient in anisotropic media a tensor model is introduced. At least the six non-collinear diffusion directions are sufficient to determine the tensor, however, more directions used in practice to increase signal to noise ratio (SNR). From the tensor model, several derived parameters are available to characterize white matter changes qualitatively. Fractional anisotropy (FA) describes the diffusion asymmetry within a voxel. In the geometrical representation, FA describes the prolongation in the main diffusivity direction and its relation to the perpendicular direction. Mean diffusivity (MD) and the main eigenvectors ($\lambda 1$, $\lambda 2$, $\lambda 3$) represent the average diffusivity within the voxel and the diffusion along the main diffusion directions.

Diffusion tensor imaging (DTI) uses the previously mentioned tensor model to characterize tissue microstructure in vivo. Since diffusion weighted measurement is typically characterized by relatively small SNR, increasing the number of diffusion encoding directions (NDED) is necessary. More directions increase SNR but prolong the measurement. In addition, special analytical methods could be sensitive to the bias of the tensor value. The widely used tract based spatial statistics (TBSS) is an easy to use and robust method to analyze DTI images. The algorithm is a semi-automatic way to detect group differences of microstructural DTI parameters within the main white matter tracts or test if there is any correlation between these parameters and clinical or behavioural data. NDED might have specific implications for this analysis technique.

Oculomotor deficit is common in MS among the many other symptoms and reported to occur in 57–70% of all patients. However, eye movement deficits are underrepresented in EDSS. Its significance lies in the observation that the presence of eye movement abnormality is associated with greater disability and greater disability progression. Subtle alterations might remain undetected with bedside oculomotor investigation. Eye tracker devices are suitable for objective and quantitative measurements of eye movements and are more sensitive to detecting subclinical abnormalities.

The aim of eye movements is to keep the object of interest on the fovea. These voluntary and reflexive movements are the rapid jerky saccades, the smooth pursuit and vergence movements. These intricate ocular movements are accomplished by six extraocular muscles, the movement of which is coordinated by a complex network of cortical and subcortical neuronal elements. The cortical signals for voluntary eye movements are generated in the frontal eye field (FEF) in close interaction with other centers such as supplementary and pre-supplementary eye fields, the dorsolateral prefrontal cortex and parietal cortex. From the cortical centers the information is conveyed via the superior colliculus to the nuclei of the oculomotor nerves directly and indirectly as well. Over the course of information flow various subcortical, brainstem and cerebellar centers are modulating the process. The structural background of subtle eye movement deficits in MS is not well-understood. The widespread extent of the oculomotor system and close relation with other systems makes it suitable to investigate its alterations, and test its role as a potential biomarker in MS.

Aims and objectives

The aims of our studies were to investigate oculomotor deficit in MS patients. The extended network which is responsible for the precise guidance of gaze could be impaired in the early stage of the disease. In addition, the underlying pathology is not well understood. Therefore, we tested MS patients in an in-house built prosaccade and antisaccade task. The higher order pathological background of such abnormal eye movements was investigated by correlating the behavioral measures with MRI parameters such as lesion location and gray matter atrophy.

In order to extend our study to identify the white matter tracts responsible for the altered eye movements, as a first step, we conducted another study to optimize our DTI sequence. The available DTI sequences are frequently time consuming and optimal parameters are necessary to reduce scan time but detect biologically important differences. While NDED is directly proportional to scan time, we decreased it and tested if it changed the applied statistical test result. Optimal protocols are necessary to translate research sequences into clinical practice. Our aim was to evaluate the effect of the NDED on the performance of TBSS in a real world patient population. Before the TBSS approach enters the clinical routine, one needs to understand the effect of basic acquisition parameters on the analysis results.

Methods

Participants

Thirty-nine MS patients and 34 healthy control participated in study #1 and 78 MS patients and 126 healthy individuals in study #2. Clinical data were collected for all the MS participants. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

Study #1

Visuo-motor task

The subjects completed an in-house built prosaccade and an antisaccade task. Eye movement recording was carried out with a Tobii TX300 eyetracker. Data from both eyes were recorded simultaneously. The recorded data was processed offline and saccades were detected automatically. For all trials the position-time diagram was re-checked visually and inadequate trials were excluded. Saccade's latency, peak velocity, amplitude, gain and duration were assessed. Antisaccade's latency, gain, peak velocity were determined similarly in the correctly performed trials and antisaccade performance from all trials. Moreover, a dysconjugacy index (DI) was calculated in the saccade task from both eyes as the ratio of the abducting and adducting eye's velocity.

Magnetic resonance imaging

In study #1, magnetic resonance imaging was performed with a 3 T GE Discovery 750w MR Scanner. High resolution T1 weighted anatomical images, CUBE T2 FLAIR for lesion detection, CUBE double inversion recovery (DIR) and spin echo (SE) T1 weighted images were acquired.

Image analysis and statistics

Lesion load was determined in the periventricular, infratentorial and juxtacortical regions on the FLAIR and DIR images manually and was correlated with the behaviour parameters. The SE T1 images were used to determine black hole burden. The high resolution T1 weighted images were used for voxel-based morphometry analysis. We looked for group differences within the measured parameters in a mixed model ANOVA. Pearson or Spearman correlation between MRI markers and behavior parameters were calculated in separate analyses.

Study #2

Magnetic resonance imaging

In study #2, imaging was performed on a 1.5 T GE Signa Excite HDxt MR Scanner. T1-weighted images and 60 direction diffusion-weighted images with 6 non-diffusion-weighted reference volumes were recorded.

Image analysis and direction reduction

The analyses of MR images were carried out with the tools from the FMRIB Software Library (FSL, FMRIB) and in-house built MATLAB scripts. From the original data (60 diffusion encoding directions) the diffusion encoding directions were reduced to n=10, 15, 20, 25, 30, 35, 40, 45, 50, 55. We used a reduction procedure to keep the uniform distribution via maximizing the total angular distribution energy. The generated subset of diffusion encoding directions was selected and fed into the DTI analysis. Individual white matter masks were used as region-of-interests (ROIs) to evaluate parameters under the white matter area. The mean and standard deviation of FA, MD, axial diffusivity (AD) and radial diffusivity (RD) within the white matter mask were calculated for each subject. For every subject we calculated the bias from its original 60-directions image for each subsampled data set, that is the 60 directions image was considered as a reference. The FA, MD, RD and AD bias was also calculated within different white matter regions, by dividing the white matter mask into separate bins, namely areas with 0.2-0.4, 0.4-0.6, 0.6-0.8, 0.8-1 FA values in the reference (60-dir) image. To evaluate the effects of the number of diffusion directions on TBSS results we performed a TBSS analysis separately on each DTI parameter as proposed in the FSL guideline. The analysis was performed in the following 4 subgroups of reduced directions: DTI parameter images of 15, 30, 45, 60 directions comparing the two groups (126 HC and 78 MS). In a second analysis, the threshold in the last step of the TBSS pipeline was chosen to keep skeleton size similar. The number of voxels in the skeleton and the number of significant voxels were calculated. Also, the mean and standard deviation of the FA, MD, RD and AD values in the significant voxels were investigated. Mean group differences were calculated in the significant voxels.

Results

Oculomotor alterations in MS

Saccade latency was significantly prolonged in MS patients. Prosaccade peak velocity was slightly smaller in the MS group but there was no significant difference between the two groups. However, a significant interaction effect could be observed in two conditions: (i) group

x movement type, which means that the higher velocity of adduction compared to abduction in HC was reversed in MS patients, resulted in a higher peak velocity in abduction compared to adduction and (ii) group x distance, which means that the slower peak velocity in the closer cue condition in HC group was slightly smaller in the MS group. There was a marked difference between the two groups in the anti-saccade performance. The HC group reached more than 80% (±12.2%) accuracy while the MS group obtained only 64% (±22.5%). Based on the dysconjugacy index five patients were classified as having subclinical internuclear ophthalmoparesis (INO).

T2 lesion burden or lesion location did not show significant correlation with any of the measured MRI parameters. A positive correlation were detected between anti-saccade latency and the number of black-holes (Spearman's rho: 0.45, $p = 0.011$) and negative correlation between the anti-saccade peak velocity and the number of black-holes (Spearman's rho: −0.47, $p < 0.01$) The VBM analysis revealed that anti-saccade peak velocity correlated with gray matter density in parietal areas. That is, smaller anti-saccade peak velocity was associated with lower gray matter densities in the left parietal areas.

Effects of NDED on TBSS analysis

We considered the value of the '60 directions' group as the reference value. A decreasing trend of AD and FA could be observed with increasing NDED. The RD had an increasing trend and the MD was constant. Moreover, the 40, 35, 35, 20 directions did not significantly differ from the 60 directions based on post-hoc analysis in AD, FA, MD and RD, respectively. Lower FA bins had a greater overestimation with decreasing directions and this effect was not seen in MD. The TBSS analysis between 78 MS patients and 126 healthy controls revealed slightly different results as a function of NDED when threshold values were kept constant. The total size of the skeleton was reduced with increasing NDED. Similarly, the number of significantly different voxels was reduced. However, when the skeleton size was constant, the number of significant voxels was similar in FA and RD but there was a drop at 15 directions in MD and AD. Regarding the group differences, the mean FA value within the significant voxels of the skeleton showed a decreasing trend with the elevation of the encoding directions. This was more pronounced in AD but absent in MD and RD. In addition, the MS group had smaller values in any number of diffusion directions for FA and higher values for AD, MD and RD on a group level. There was a statistical significant interaction between the effect of disease group and NDED in the equal and unequal skeleton size conditions for all

parameters. This means that the mean differences of the groups change differently as a function of direction.

Discussion

In our study we investigated visually guided prosaccade and antisaccade task performance in MS patients and their possible association with focal brain alterations. Out of several eye movement parameters, we found significantly increased latency in the prosaccade task and significantly worse performance in the antisaccade task in MS patients. The detailed examination of conjugated eye movements revealed 5 subclinical INO cases. As regarding the MRI parameters, the peak velocity and latency of the antisaccade movement correlated with the number of black-holes, but none of the eye movement parameters were associated with the T2 lesion burden or location. Most importantly, local gray matter atrophy in the left inferio-parietal lobule and temporo-parietal junction correlated with antisaccade peak velocity.

Oculomotor alterations found with various paradigms are common in MS. Previous studies showed that the performance is deteriorated mainly in more cognitively demanding saccade tasks. Antisaccade performance is deteriorated and associated with cognitive performance. In our investigation the antisaccade peak velocity, but not that of the parameters of the saccade task correlated with number of black-holes and focal gray matter atrophy in the temporo-parietal region. Prolonged latency of prosaccade could mirror the delayed initiation of saccades. The prolonged latency of antisaccades, however, might reflect a prolonged volitional decision process or a delayed initiation of saccade in the opposite direction or both. The difference might relates to the time, which is not necessary for the reflexive part such as inhibition or vector transformation. Hence the correlations we have found are mainly reflecting the higher order cognitive processes of eye movements rather the reflexive parts. The non-reflexive part of the anti-saccades might be dysfunctional, leading to an error. While if it is delayed but to a level that is not sufficient to make an error it could only be investigated via its delayed latency. This could especially be important in multiple sclerosis, in which demyelination, and slowed conduction is a key feature of the disease.

Saccade peak velocity is affected by multiple cognitive functions (arousal and mental workload). In our study, focal gray matter volume variability showed correlation with antisaccade peak velocity in the left inferior parietal lobule, left temporo-parietal junction and in the putative left V5/MT motion sensitive visual region. These parietal regions are identical to those frequently implicated in attention tasks. In their seminal paper, Corbetta and co-workers in a remarkably similar paradigm found activation in the intraparietal sulcus during sustained attention and in the right temporo-parietal junction when a target was detected,

particularly at an unattended location. These two conditions correspond to the top-down and bottom-up attentional subsystems. Moreover, the parietal cortex has its direct connection to the superior colliculus, and the pontine nuclei as well. Alternatively, parietal region has a potential role in saccades. The parietal eye field and posterior parietal cortex are involved in saccade generation and visuospatial attention. Moreover, human and animal studies suggested that this region has a role in the vector inversion process, which is a crucial step in antisaccade.

The joint damage of gray and white matter explain cognitive disturbance in MS, which is in close relationship with eye movements. To further elucidate the structural background of the observed oculomotor alterations, white matter microstructure should be investigated. The optimal choice of diffusion tensor imaging parameters is crucial to spare scan time but detect pathological alterations. As NDED is directly proportional to scan time, we investigated its effects on detection of disease related alterations, with a special emphasis on detecting differences in a patient population with the commonly used TBSS approach. We found an overestimation of FA and AD, if the NDED was below 30 directions. Areas with low FA values seemed to be more prone to the overestimation. When considering the TBSS analysis, the overestimation of FA leads to an enlarged skeleton and within that skeleton more significantly different voxels can be found at low NDED.

The decrease of the encoding directions leads to an overestimation of the FA and AD and an underestimation of RD but does not affect MD. The overestimation seems to be more severe if the uniform spherical distribution of directions has not been kept. As it was emphasized, the relationship of the fiber orientation and the encoding direction has a major effect on the tensor, especially in low NDEDs. The investigation of the performance of different schemes on real-life data is more complicated because of the spatial and tissue dependent SNR and other imaging related artifacts. An increment in NDED was reported to lead to an elevation in SNR and to reach its plateau at 53 directions for FA and 51 directions for MD in the white matter. Moreover, FA and AD seem to be affected more profoundly by NDED in general than “isotropic indices” (MD, RD) which averages information from more directions. A skeleton is defined in the TBSS approach by the relatively high FA values (usually higher than 0.2-0.3), which by itself reduces the bias, since areas with low FA values are more prone to the overestimation of FA. Here, we found extensive alteration of DTI parameters in the white matter in MS patients compared to HCs. The reason of this broad difference could be the high number of participants in the study, which may lead to the detection of smaller differences. Furthermore, the higher number of MS patients may also contribute because their inclusion leads to an increased percent of focal demyelinating lesions in the skeleton, which have reduced

FA and increased MD. The mean difference in the significant voxels of FA and AD decreased with the NDED, that is, the two groups mean values depended on NDED in different way. In the lowest NDED, the group difference was the highest. One possible explanation could be the underlying pathology in MS. The white matter FA value is decreased throughout the brain, which is in turn, more prone to overestimation. Moreover, voxels included in the skeleton in the TBSS approach are more ambiguous. However, the significant change of group differences between the directions is still very low. Regarding the two skeleton sizes, the group differences of the parameters followed a similar trend. The number of significant voxels and the skeleton size were different between different schemes and decreased with the NDED. The overestimation of skeleton size could be the consequence of the overestimation of FA within the small FA areas. In addition, the elevated number of significant voxels of FA simply resulted from the higher number of voxels. This effect could be reversed because correcting for skeleton size, the number of significant voxels of FA was equal as well. Conversely, the number of significant voxels of MD and AD was smaller at 15 directions compared to higher directions.

Conclusion

In conclusion, eye movements are substantially affected in MS patients, which reflected in several behavior parameters. Moreover, subclinical involvement of the oculomotor system is detected in several cases and highlights the possible use of eye tracker systems to adequately quantify disease burden. The global and focal gray matter alterations are associated with brain areas important in cognitive functions, such as attention. To correctly test white matter integrity, the optimization of diffusion tensor imaging was evaluated considering the number of diffusion encoding directions. Bias caused by directions is more pronounced for areas with small FA values and seems to be constant above 30 directions. Moreover, NDED has a slight effect on TBSS, which makes it reasonable to cautiously compare results from different TBSS studies with different NDED. Regarding all the derived DTI parameters 30 directions might be enough to compare healthy and multiple sclerosis patients with TBSS analysis. Importantly, our results indicate that higher FA threshold of the skeleton should be used with lower NDED to avoid false positive results. White matter integrity could be tested with the optimized sequence and its association with oculomotor deficits as well. As eye tracker systems are promising tools to monitor disease progression the follow-up of our cohort could aid our understanding of eye movements as a predicting biomarker.

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