SUMMARY

The overall aim of this thesis was to evaluate the clinical significance of magnetic resonance imaging and body fluid biomarkers for disease progression in multiple sclerosis (MS) with a focus on the earliest stage of the disease, i.e. in clinically isolated syndrome (CIS).

The aim of Chapter 2 was to investigate regional brain iron levels in patients with CIS and relapsing remitting MS (RRMS) and to explore their relation with demographical, clinical, and conventional MRI parameters, including T2 lesion load, and normalized brain volumes.

Increased iron deposition is supposed to play a role in the pathophysiology of MS. However, previous reports on iron deposition in MS were mainly based on visual analysis of signal reduction on T2-weighted images. R2* relaxometry allows to assess brain iron accumulation quantitatively. We thus used this method for brain iron quantitation. Thirty-two patients with CIS and 37 patients with RRMS underwent 3T MRI and we assessed R2* relaxation rates in deep gray matter structures (thalamus, caudate nuclei, putamen, pallidum, hippocampus, amygdale, and nucleus accumbens) and the brain-stem. Basal ganglia R2* rates correlated with increasing age (r = 0.3–0.6; p < 0.01). RRMS patients had significantly higher basal ganglia iron levels compared to CIS (p < 0.05). By applying multivariate linear regression analysis, we identified the patients' age, disease duration, and gray matter atrophy as independent predictors of putaminal iron deposition.

It was concluded that quantitative assessment by R2* relaxometry indicates increased iron deposition in the basal ganglia of MS patients, which is associated with disease duration and brain atrophy. Assessment of brain iron levels using R2* relaxometry combined with long-term clinical and radiological follow-up appears suited to clarify if brain iron accumulation is implicated in MS pathology.

In extension the above-mentioned study, in Chapter 3 we investigated brain iron levels using R2* relaxometry in a larger series of MS patients and compared R2* rates in defined cerebral structures between patients with different stages of the disease and age matched healthy controls (HC). Apart from detailed neurologic examination, patients also underwent neuropsychological testing to allow for a more comprehensive assessment of the clinical impact of increased brain iron deposition in MS.

We analyzed 113 patients (35 CIS, 78 MS) and 35 HC with 3T MRI and iron levels in subcortical gray matter structures were assessed by automated, regional calculation of R2* rates. Patients underwent detailed clinical and neuropsychological examination. We found significantly increased basal ganglia iron deposition in MS compared to CIS (p < 0.001) and HC (p < 0.005). R2* rates were correlated with age (r = 0.5, p < 0.001), disease duration (r = 0.5, p < 0.001), the EDSS (r = 0.3, p < 0.005) and with z-values of mental processing speed (r = –0.3; p < 0.01). We then applied step-wise linear regression analysis to identify factors associated to R2* levels. Strongest independent predictor of BG R2* rates was gray matter atrophy (p < 0.001), followed by age (p < 0.001), and T2-lesion load (p < 0.005) in the final step. The model excluded the variables disease duration, EDSS, and z-values of mental processing speed.

It was concluded that basal ganglia iron accumulation in MS occurs only with advancing disease and is related to the extent of morphologic brain damage, which argues for iron deposition as an epiphenomenon. The absence of increased iron levels in CIS patients indicates that iron accumulation does not precede the development of MS.

In Chapter 4 we explored the extent of cognitive impairment and its relation to MRI metrics including MT imaging in patients with CIS compared to RRMS. Cognitive deficits can be
frequently observed in the course of MS and they are related to morphologic brain changes. However, up to now only little information existed on the extent of cognitive deficits in the earliest phase of the disease, e.g. in CIS. It was also unclear whether subtle structural changes as detected by MT imaging, was a biological correlate of impaired cognitive performance. We included 44 CIS and 80 RRMS patients. All patients underwent the Brief Repeatable Battery of Neuropsychological Tests (BRB-N) and a 3T MRI scan. We found comparable BRB-N subtest results in CIS and RRMS, with impaired mental processing speed being most prevalent in both groups (CIS 13.6%; RRMS 16.3%). Conversely, when using stepwise linear regression analyses to identify independent predictors for decreased mental processing speed, different results emerged comparing CIS and RRMS. While in RRMS, strongest predictors for decreased mental processing speed were normalized cortex volume (p < 0.001) followed by T2-lesion load (p < 0.05), in CIS cortical MT ratio was the only MRI parameter associated with decreased mental processing speed (p < 0.005).

We could conclude that cognitive dysfunction is present already in CIS in a pattern similar to RRMS, with impaired mental processing speed being most prevalent. Structural changes within the cortex as detected by MT imaging could be an early biological correlate of impaired mental processing speed in CIS, while cortical atrophy and lesion load are the biological correlate in RRMS.

In Chapter 5 we aimed to explore the epitope specificity of serum antibodies directed against the extracellular domain of myelin oligodendrocyte glycoprotein (MOG), a potential target antigen for autoreactive antibodies, in patients with RRMS. The second aim was to determine whether immunomodulatory treatment and/or clinical exacerbation influenced anti-MOG peptide reactivities. We analyzed anti-MOG peptide serum antibodies, including IgG, IgM and IgA immunoglobulin isotypes, in 28 RRMS patients before and after one year of immunomodulatory treatment. We found two immunodominant overlapping linear epitopes encompassing amino acid (aa)37–48 and aa42–53. Recently experienced clinical exacerbations were associated with a significantly increased frequency of positive immunoreactivity against MOG peptides. One year of immunomodulatory treatment had no significant impact on anti-MOG peptide immunoreactivity.

The study presented in Chapter 6 aimed to investigate the predictive value of serum anti-MOG antibodies to indicate treatment response of acute MS relapses. We developed an ELISA test system for detection of serum IgG and IgM Abs directed against native refolded MOG. Serum samples were analyzed in remission before relapse (baseline), during acute relapse before corticoid treatment and 6 weeks after corticoid therapy. Upon corticoid treatment anti-MOG IgG but not anti-MOG IgM Ab levels were significantly reduced. Patients whose clinical symptoms completely remitted after an acute MS relapse had a significant increase of anti-MOG IgM Ab levels comparing baseline and acute relapse values. It was concluded that measurement of anti-native MOG antibodies might be important for decisions in patients with acute relapses, in particular regarding either dose and/or duration of corticoid or escalating therapies.

The aim of Chapter 7 was to validate the potential novel serum biomarker, secretogranin III (SGIII) fragment, as indicator for disease progression in MS. This candidate marker has recently been identified by a high-throughput MALDI-TOF-based mass spectrometry biomarker discovery study. We have analysed serum samples from 90 MS patients (RRMS n = 50, primary progressive (PPMS) n = 50) and 24 healthy volunteers (HC) served as controls. As detection antibody we used polyclonal anti sera obtained from rabbits previously immunized with the
SGIII peptide Cys-KPGGSQDKSLHNRLEASAERPLNEQIAEED-A. All analyses were performed by Western Blot. Using a reference sample, the presence and intensity of the different bands were rated in a semi-quantitative and blinded manner. For quantitative analyses of Western Blot band intensities we used Odyssey infrared imaging software.

We found several SGIII isoforms discriminating MS patients from controls, including Western Blot bands at 12 kD, 23 kD, 37 kD and 100 kD (p < 0.01). Compared to RRMS, patients with PPMS had significantly higher band intensities at 37 kD (p < 0.05). When correlating Western Blot results with clinical data we found that the band intensities of 37 kD correlated with physical disability (r=0.238, p<0.05). Higher band intensities at 23 kD and 100 kD were observed in untreated MS patients compared to those receiving immunomodulatory therapy (p < 0.05). From these first validation results we could conclude that specific isoforms of SGIII might be useful for MS subtyping, particularly to define PPMS and to monitor disease progression and treatment effects.

In Chapter 8 we aimed to explore the value of CSF markers for axonal damage in patients with a CIS and their relation with accumulating tissue damage as indicated by MRI. CSF neurofilaments and N-acetylaspartatic acid (NAA) are among the most promising ones to indicate the extent of axonal loss. We thus assessed CSF levels of neurofilament heavy (NfH), neurofilament light (NF-L) and NAA in 67 CIS patients and 18 controls with neurological diseases of non-inflammatory aetiology (NC). CIS patients also underwent a baseline MRI at 3T, including sequences for assessing T2 lesion load, and normalized regional and whole brain volumes and after one year a follow-up MRI was obtained in 28 of them. NfL (p < 0.001) and NfH (p = 0.05) levels were significantly increased compared to NC. We found a strong correlation of NfH (r = 0.713, p < 0.005) and NF-L (r = 0.929, p < 0.001) levels with age in NC. This correlation was less strong in CIS for NfH (r = 0.325, p < 0.01) and absent for NF-L levels. In CIS patients, NfH correlated with physical disability (r = 0.304, p < 0.05) and with brain volume loss over time (r = −0.518, p < 0.01) but not with the change in T2 lesion load. It was concluded that our findings provide evidence for increased neurofilaments present already in the earliest stage of MS. The association of NfH levels with physical disability and brain volume but not lesional changes supports the association of these markers with axonal damage.

The aim of Chapter 9 was to explore the value of CSF neural cell adhesion molecule (NCAM) as a biomarker for use in clinical practice in patients with CIS and MS. NCAM plays a central role in neuronal repair and regeneration. In MS, reduced CSF NCAM levels have been observed and a continuous increase of CSF NCAM has been described in a limited number of acute MS patients who clinically improved after treatment. However, up to now it was unclear whether soluble NCAM correlates with clinical and MRI findings in CIS and MS. We therefore analyzed CSF NCAM in 85 patients (CIS n = 66; MS n = 19). Patients underwent detailed clinical examination and 3T MRI. In 32 patients a follow-up MRI was performed after an average one year. CSF samples from 17 patients with other neurological diseases served as controls. No differences in CIS and MS compared to the controls were observed. We found significantly reduced CSF NCAM levels in MS compared to CIS (p = 0.05) and in clinically active compared to non-active patients (p < 0.05). Higher CSF NCAM levels correlated with tissue loss of whole brain, gray matter and cortex over time (r = −0.4— −0.6, p < 0.05 — p < 0.005). It was concluded that CSF NCAM is altered in MS dependent on disease stage and activity. CSF NCAM could increase upon regenerative processes, which however may limit brain tissue damage for only a short interval in view of the positive correlation of NCAM with long-term brain tissue loss. Indirectly, however, this could
be evidence for a more active disease process. Further studies are required to investigate the relation of CSF NCAM levels with modern MRI techniques, including magnetization transfer imaging for structural analyses.

In conclusion the results of this thesis led to several new insights into the clinical significance of MRI and body fluid biomarkers for disease progression in MS, focusing on the earliest stage of the disease, i.e. in CIS.

The use of non-conventional MRI techniques, such as R2* relaxometry for brain iron quantitation and MT imaging to quantitatively measure structural brain changes, enabled new possibilities to establish markers associated with MS disease progression. The relation of R2* relaxometry with tissue destruction suggests that this iron mapping technique may potentially be used as a marker for neurodegeneration in MS. Cortical MT imaging may be an early sign of structural tissue alterations related to impaired mental processing speed in CIS patients.

Novel identified body fluid candidate biomarkers, including serum secretogranin III and NCAM, to indicate disease progression in a subset of MS patients are currently being validated in large MS cohorts. We demonstrated that serum antibodies against MOG peptides and native refolded MOG may indicate disease activity and progression in MS. However, recent studies indicate that antibodies directed against the native, membrane bound and glycosylated form of MOG are more relevant in children with acute disseminated encephalomyelitis. CSF neurofilaments are reliable markers to indicate the extent of axonal damage in CIS and MS and may thus be helpful to monitor clinical status and treatment efficacy in future studies.

Ultimately, the results of the present thesis support the notion that combined approaches of biochemical and quantitative MRI measures together with improved clinical outcome measures in clinical and MRI follow-up settings might be fundamental to establish assessment tools for axonal damage and/or neuronal repair in the earliest phase of MS.