Chapter 5

Discussion, summary and future perspectives

The aim of the present thesis was to use an iron sensitive MRI imaging technique (SWI), in the context of the inflammatory, demyelinating and (neuro)degenerative disease multiple sclerosis (MS). For several decades, MRI studies using techniques sensitive to iron, such as SWI but also T2, T2*, R2*, FDRI and post-mortem studies have been conducted in neurodegenerative disorders such as Alzheimer's disease. However, MS has received much less attention, most likely because many have regarded this disease as a strict white matter (WM) disorder, whereas increased iron levels are usually observed in the gray matter (GM). More recent developments in MS research have led to a shift in the focus of research to also include GM pathology. Throughout the present work, the SWI-filtered phase technique, which is sensitive to (para-) magnetic substances, most notably iron, was used as a surrogate *in vivo* marker of iron deposition. In Chapter 2, we first established a baseline for the behavior of SWI phase in healthy aging. From there on, in Chapter 3, we investigated SWI-derived phase measures in deep GM structures in different stages of MS, starting in early MS cases (pediatric and clinically isolated syndrome) and later stages (relapsing-remitting and secondary progressive MS). In Chapter 4, the hallmark pathological finding in MS, white matter signal abnormalities/lesions, are investigated for appearance, prevalence, and diagnostic value using SWI.

Deep gray matter

Hallgren and Sourander published a hallmark histological study in 1958 where they observed that non-heme iron was elevated in older people; occurring mostly in the putamen, caudate nucleus, globus pallidus, substantia nigra, dentate nucleus, and thalamus, as well as the prefrontal, sensory, cerebellar, and motor cortices.[1] Iron levels of the globus pallidus, thalamus, red nucleus, substantia nigra, and sensory and cerebellar cortices increase rapidly during the first decades of life, but remain relatively constant starting at 30 years, with the exception of the thalamus where iron levels slowly decreases after young adulthood (Chapter 2). In contrast, iron contents of the putamen, caudate nucleus, and motor cortex increase somewhat slower, with maximum levels being reached in older age. Many recent studies have used MRI to investigate the link between healthy aging and brain iron levels. Among the MRI techniques used are SWI, magnetic field correlation, quantitative susceptibility mapping, and T2 and R2 relaxometry.[2-7] Collectively, their main findings imply that MRI changes suggestive of increased iron increase with age. In order to have a reference framework, it is important to also understand SWI phase behavior in healthy individuals. In Chapter 2, we extend previous findings in the normal population to include not only mean phase measures as obtained by SWI, but also mean phase measures of low phase voxels (MP-LPV) as a measure of highly affected tissue, as well as volumetric analysis of deep GM structures. Our findings are in line with the literature with respect to the association of increased age and lower mean phase and MP-LPV measures, indicative of higher iron levels. However, a novel finding was that –unlike in previous studies– the association of SWI mean phase measures and age was not strictly linear but are plateauing or even reversing in middle age. In several prominent brain structures, including the caudate nucleus and thalamus, the association of age with mean phase was better explained by a quadratic fit, as opposed to a linear fit. This suggests that deep GM iron levels peak between 40 and 60 years of age, after which iron levels tend to slowly decrease again. However, when considering only MP-LPV, i.e. highly affected voxels, the associations with age were strictly linear. This finding sheds light on brain phase behavior

in healthy individuals; the total iron concentration peaks in middle-age, after which it rebounds, whereas the iron content of tissues with already high levels of iron increases steadily with age. In a recent SWI study by Haacke et al.,[8] it was shown that not only did measures of such high iron content areas increase with time, they appeared to even accelerate with age. This increase of high iron tissues can potentially have deleterious effects in the form of atrophy. Indeed, we observed that mean phase and MP-LPV measures from deep GM structures, especially the thalamus, were strongly associated with neocortical and lateral ventricle volumes. One proposed hypothesis postulates that the excessive iron levels cause free radical damage to cells through chemical (Fenton and Haber-Weiss) reactions.

After establishing that SWI-filtered phase measures are altered in older age, and that mean phase and MP-LPV behave differently (quadratic vs. linear), it was important to highlight differences of these measures among different MS disease types and stages. First, we scanned adolescent MS patients (Chapter 3.1). Twenty patients with a mean age of 15 were recruited and phase measures of deep GM structures were compared to 21 age- and sex-matched healthy individuals and eight adolescent patients with other neurological disorders. In this study, multiple measures of abnormally high iron content were utilized: (1) as considered previously, MP-LPV which takes into account the mean phase values only of voxels 2 standard deviations below the mean of a reference healthy control group, (2) LPV volume which quantifies the volumetric size (in milliliters) of these low phase voxels, and (3) the inverse; normal phase tissue volume (NPTV) which is defined as the volume of tissue that is not severely affected (voxels within the >2 standard deviations normal range). Consistently the pulvinar nucleus of the thalamus had the lowest MP-LPV, increase in LPV volume, and biggest decreases in normalized volume and NPTV, all of which are suggestive of increased atrophy and iron content. In addition, the putamen and caudate nucleus had prominent healthy tissue (NPTV) reductions. Although not always significantly so, in other structures among adolescent MS patients (caudate, putamen, globus pallidus, thalamus, amygdala, nucleus accumbens, substantia nigra), the MP-LPV and NPTV was consistently the lowest, whereas the LPV volume was consistently the

highest. Showing both measures of mean phase and volumes serves to create a link between SWI phase measurements suggestive of iron increases and volume reduction. Clearly, longitudinal studies can shed more light on the causality of this relationship. However, an interaction between iron and cell destruction appears evident. This study showed that excessive iron is present at the earliest stages of MS, a finding also observed in the caudate nucleus by Ceccarelli et al.[9] using T2 hypointensity, lending credence to the notion that its detrimental effects could potentially not only cause localized damage, but may also lead to eventual disease progression.

Investigating clinically isolated syndrome (CIS), as done in Chapter 3.2, is another crucial step in understanding the phase behavior of early MS disease course. Similar to adolescent MS patients, patients diagnosed with CIS have significantly lower MP-LPV values and increased LPV volumes in especially the pulvinar nucleus of the thalamus. In addition, they also show signs of iron deposition in the caudate and putamen compared to age- and sex-matched healthy individuals. Interestingly, no global or regional volumetric differences were found between the study groups. It stands to reason that pathology measured by SWI-filtered phase images is not reflected in atrophy yet. This study supports the concept that iron deposition is present in early MS disease stages, even in patients with a single clinical attack, which may contribute to disease development and brain damage. In these early stages, volume loss is minimal yet pathology is visible on SWI phase. It would be expected that in later stages of MS the atrophy of deep GM structures is concomitantly present along with increased iron content, a finding which has been observed previously.[10]

To test whether SWI-filtered phase metrics have any clinical relevance, we recruited an adult sample of relapsing-remitting and secondary-progressive MS patients in order to assess the association of SWI mean phase and Kurtzke Expanded Disability Status Scale (EDSS)[11] and disease duration. In Chapter 3.3 it is shown that deep GM MP-LPV is independently related to increases in EDSS, even when the statistical model is corrected for age and gender as well as conventional MRI measures (T2 and T1 lesion volume, and normalized cortical and WM volume). Specifically, caudate and red nucleus

MP-LPV were associated with EDSS increases. Interestingly, in stepwise models, the deep GM MP-LPV measure was retained, whereas conventional MRI metrics commonly found to be associated with MS were not. These results suggest that decreased mean phase, indicative of increased iron content, in the deep GM is clinically relevant.[12] In addition, loss of thalamus volume was associated with longer disease duration.

In chapters 2, 3.1 and 3.2 the thalamus, especially the pulvinar nuclei of the thalamus are consistently shown to have lower phase values and thereby elevated iron content, in addition to volume loss. This begs the question, are the thalamic nuclei, specifically the pulvinar, more heavily involved than previously thought in MS? Several recent studies have found that extensive volume loss (1) occurs in the thalamus[13] (2) is related to cognitive decline[14] and (3) is associated with conversion from CIS to clinically definite MS.[15] The pulvinar nucleus of the thalamus has not been researched as extensively, although researchers have found atrophy of this structure among relapsing-remitting MS patients.[14] Because of the extensive cortical connections of the thalamus and pulvinar nucleus,[16] research efforts will have to be made to investigate their involvement in MS. In recent years, the focus of research has already shifted somewhat away from WM, toward GM.[17, 18] Several studies,[19-21] including the present work, have shown that elevated levels of iron, as assessed using different MRI techniques, are associated with GM atrophy. Therefore, increased levels of iron could potentially be a piece of the puzzle of, or biomarker for, GM pathology and the associated clinical signs.

White matter phase signal abnormalities (lesions) in multiple sclerosis

White matter signal abnormalities (WM-SAs) are a hallmark feature of MS, yet the clinical importance of the occurrence of such lesions as observed on T2-weighted imaging are disappointing.[22-25] T2- and T1-weighted WM-SAs are thought to represent focal pathology, and to be caused by inflammation, edema, demyelination and/or gliosis.[23] Even though T2 WM-SAs are present at the first demyelinating episode, the poor specificity of conventional

MRI[22,26] limits their predictive value. Previously, the differential diagnosis of MS vs. other central nervous system disorders was considered using brain and spinal cord MRI, and incorporating number, localization and morphology of T2 WM-SAs in the diagnostic criteria of MS,[27] or by using non-conventional MRI techniques.[26,28-30] We sought to add SWI-filtered phase to the mix of non-conventional techniques in order to examine focal brain pathology in CIS and MS, and presence, prevalence, localization, and clinical relevance of observed white matter phase lesions.

A substantial subset of WM-SAs have negative phase shifts,[27] and have morphological differences.[10,27-30] Recent studies have confirmed histologically, that WM-SAs visible on MRI phase and $R2^*$ correspond to focal iron deposits, although other factors are likely to influence MRI phase as well (e.g. demyelination, deoxyhemoglobin, tissue microstructure and fiber orientation).[31-33] Phase changes of WM-SAs have been proposed to specifically signal early lesion development.[33, 34] WM-SAs visible on SWI-filtered phase images may appear initially, but signal intensity will be lost when the pathology advances, possibly due to lesion microstructural changes.[33] Even though phase WM-SAs may disappear over time, it has to be noted that they are not synonymous to active lesions (ie. they are not contrast enhancing). In fact, in a recent pilot study by Bian et al.[35] some phase visible WM-SAs persisted over an extended period (>2 years), far longer than one would expect a contrast-enhancing lesion to be active. Also, in one case, the phase WM-SAs even preceded hyperintensity on magnitude images.[35] In a combined MRI and histopathological study of WM lesions, Bagnato et al.[36] found that activated microglia (CD68) co-localize with ferritin and iron (Perl's/Turnbull Blue). Since oligodendrocytes possess the highest concentrations of iron in the healthy brain, the breakdown of myelin in the context of WM lesions would cause the extracellular release of iron, followed by macrophages ingesting copious amounts of iron as a means of detoxification (by transforming the more toxic $Fe^{2}+$ to $Fe^{3}+$, and binding it to ferritin).[36] Furthermore, leakage of the blood brain barrier may lead to an increase in iron concentration by allowing iron to seep into perivascular regions.[37] This mechanism may have further importance especially in ring-

like phase WM-SAs, which tend to be situated around central penetrating veins and where macrophages may remain for an extended period of time in an anti-inflammatory, protective state. The previously mentioned distinct pathologies, including increased iron levels, are most likely strongly associated with each other, have the potential to influence phase shift in WM-SAs, and are observed in MS and related disorders.[10, 38-42] Because of this, investigating both the occurrence and relevance of phase WM-SAs remains important.

First, in Chapter 4.1, we investigated the number, volume, and mean phase of SWI-filtered phase visible WM-SAs in a sample of 135 MS patients. As expected, MS patients had more, and higher volumes of phase WM-SAs compared with healthy individuals. However, phase WM-SAs were much less prevalent than T2 lesions. Of T2- and T1-weighted imaging WM-SAs, only 23.6% and 37.3% respectively overlapped with phase WM-SAs, indicating that the majority of T2 and T1 lesions are independent of phase lesions. This suggests that some of the phase lesions are unique to SWI-filtered phase images. Phase WM-SAs were also observed to consist of several morphological subtypes: the most prevalent of which were nodular and scattered, while ring lesions were more rarely observed. The presence of multiple (>5) phase WM-SAs could readily distinguish MS patients from healthy control subjects with a sensitivity of 75.6% and specificity of 89.9%. Indeed, presence of phase WM-SAs appears to be a good differentiator of patients from healthy individuals, and may be a valuable tool in differential diagnosis, in addition to the classic, hallmark, T2 WM-SAs. However, the lack of significant correlations between phase WM-SA volume, number, or mean phase with clinical outcomes mirror the limited clinical relevance that T2 WM-SAs have.[24]

Even though the presence of WM-SAs lacks strong clinical and phenotypical associations, they do seem to constitute genuine brain pathology. In order to be of value as a diagnostic measure, disease stages before relapsing remitting MS, such as CIS, will have to be assessed for phase WM-SAs. As is described in Chapter 4.2, phase WM-SAs possess diagnostic value in patients with a single demyelinating episode. In addition to corroborating findings from the

previous study that phase WM-SAs are more prevalent in patients, it was also determined that the mere presence of phase WM-SAs could not only distinguish between CIS and healthy subjects, but also between CIS vs. patients with other neurological disorders, and CIS vs. neurological autoimmune disorders, with a high sensitivity and specificity. An interesting finding relates to the commonness of T2 WM-SAs. Because it is not unusual to have at least one T2 WM-SA, the presence of such abnormality is not highly specific in classifying CIS patients. In order to still have valuable diagnostic properties, additional lesions and localization data are necessary, such as with the McDonald 2005[43] and 2010[27] criteria. However, differentiating CIS patients from patients with other neurological disorders using 1 or more phase WM-SA could be done with an accuracy of 73.1%. Compared to an accuracy of 64.1% for the presence of any T2 WM-SA, 62.8% for satisfying the McDonald 2005, and 67.9% for satisfying the McDonald 2010 (based on the dissemination in space of T2 WM-SAs; longitudinal analyses were not conducted). The mere presence of phase WM-SAs could classify the CIS patients. Furthermore, presence of multiple phase WM-SAs was associated with progression to clinically definite MS from CIS and approximately half of phase WM-SAs were not detected by T2 and may represent unique pathology. Phase lesions may be less prevalent, but when they occur they can classify CIS patients with relatively high specificity, on par with, or even exceeding, McDonald criteria for dissemination in space, rendering it a potential tool for differential diagnosis.

Brain iron as a biomarker; genetics and underlying biology

Throughout this work it has been argued that the detection of brain iron using MRI techniques provides valuable information in healthy brain aging and disease states. However, as opposed to for example demyelination and inflammation, iron deposition is not regarded a classic hallmark pathology of MS. It is therefore imperative to describe what biological mechanisms may underlie these changes seen on MRI, and what subsequent pathologies those may cause, in order to fully understand the implications of the presented MRI findings.

It has long been known that iron is present in the brain, a finding first published by Zaleski in 1886 using the Perl's stain on a single human brain.[44] Guizetti and Spatz first described that staining for iron was most evident in deep GM structures of the extrapyramidal system,[44] and Hallgren and Sourander published a hallmark study in 1958 where they observed that deposition of non-heme iron in the brain was correlated with age. Basal ganglia structures tend to have the highest level of iron, with oligodendrocytes being the most prominent cell-type to stain for iron,[45] while ferritin is the most common iron-storage protein. In the substantia nigra, neuromelanin is the location of the most prominent iron storage, with levels also increasing with age.[46]

Mutations of several iron metabolism genes can have severe implications in age related diseases, such as cardiovascular disorders, but also more importantly for the present work, in disorders of the central nervous system. Homozygous carriers of the relatively common Cys282Tyr mutation of the hemochromatosis (HFE) gene, which causes systemic iron overload, have a significantly increased risk for acute myocardial infarction.[47, 48] Male carriers with hereditary hemochromatosis seem to be especially vulnerable to develop further iron overload related problems, such as cirrhosis and hepatocellular carcinoma.[49] It has also been observed that healthy male carriers of the Cys282Asp and/or transferrin C2 mutations have significantly higher brain ferritin levels than non-carriers, which could possibly be a contributing factor for gender differences observed in neurodegenerative disorders.[50] Furthermore, these iron metabolism genes have been located within the brain, indicating that they not only affect systemic iron levels. Studies have linked increased incidence of several neurodegenerative disorders such as amyotrophic lateral sclerosis and Alzheimer's disease, to the presence of HFE gene mutations such as Cys282Tyr and His63Asp. However, the role of these genetic mutations remains uncertain in other disorders such as MS, Parkinson's disease, and ischemic stroke,[51-53] and would thus require further research.

Even though brain iron deposits have been found in healthy individuals, it may still be related to functional impairment. For example, Penke et al.[54]

showed that elevated levels of brain iron are inversely related to cognitive ability and successful cognitive aging in a cohort of healthy elderly individuals. Furthermore, in a group of 10 healthy elderly individuals, caudate and putamen iron estimates corresponded to lower scores on the Dementia Rating Scale.[55]

On the molecular level, excessive levels of iron, as well as other redox active metals, have the ability to promote the generation of reactive oxygen species. These subsequently overwhelm antioxidant protection mechanisms and cause harm to membranes and DNA, and can promote or exacerbate protein misfolding and aggregation.[56] Specifically, free (labile) iron, has the ability to catalyze the generation of highly reactive hydroxyl radicals ($^{\cdot}OH$). In the Fenton reaction, ferrous iron (Fe^{2+}) and hydrogen peroxide (H_2O_2) react to generate ferric iron (Fe^{3+}), hydroxyl anion (OH-), and the reactive hydroxyl radical ($^{\cdot}OH$), potentially resulting in molecular damage. The Haber-Weiss reaction, wherein superoxide ($^{\cdot}O_2^-$) participates in the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}), is followed by the Fenton reaction to also produce hydroxyl radicals (see Chapter 1).[57, 58] Hydroxyl radicals can cause DNA strand breakage and chemical alterations of the deoxyribose and purine and pyrimidine bases, as well as damage to membranes through lipid peroxidation, causing mitochondrial dysfunction.[57] Oxidative damage has been known for some time to occur in and around MS lesions.[59, 60] For example, lesions contain significant amounts of mRNA encoding for human inducible nitric oxide synthase.[61] Other mechanisms leading to nitric oxide radical increases in WM lesions include the widespread expression of NADPH oxidase, which induces oxidative damage and may act in targeting oligodendrocytes for microglia destruction.[60] Other factors may exacerbate or amplify oxidative damage. For example, anterograde or retrograde axonal degeneration can lead to microglia activation and lesion formation in areas which are connected to previously injured sites.[62] Clearly, excessive iron is not the sole cause for oxidative injury in the MS brain. However, iron may hold an important key in that iron in the healthy brain is mostly stored in oligodendrocytes and myelin sheets.[63] Therefore, widespread destruction of specifically these cells in MS could mean that there is a particular

susceptibility to a vicious cycle of iron-induced oxidative injury.[64] This would suggest that demyelination and neurodegeneration in MS is at the least partly driven by free radical induced damage. At this stage it is impossible to implicate excessive iron levels as a causative factor in MS disease initiation. However, it seems likely that in early disease stages both inflammation and oxidative stress together with excess iron, play an important role. Even though increased iron levels may play a role in the early stages by causing oxidative injury, it stands to reason that the more severe the demyelination, the more labile iron is released, resulting in a cascade of oxidative damage and microglia activation, leading to even more severe pathology. Because excessive iron has the potential to cause harm in the brain, *in vivo* monitoring of patients with CNS disorders may prove valuable in MS.

Conclusions and future perspectives

Even though several factors can influence MRI phase (inflammation, microstructure, myelin, fiber orientation), it remains likely that a substantial contribution of the observed signal comes from differences in iron concentration, as it is the most abundant paramagnetic substance. Some effort has already been put into histopathological studies;[65] however, future experimental studies should extensively expand on the investigation of excessive iron levels using a combination of histopathology and MRI, to further validate what the observed MRI signals represents in both human studies and animal models of MS. In either case, regardless of the source of signal change, if it can distinguish between disease and health states, as well as disease type, and provide clinically relevant information, it should be considered a promising measure. Combined, the results from the described studies demonstrate that imaging MS patients using SWI or other iron sensitive imaging techniques may enhance the understanding we have about some of the underlying pathologies, and can potentially aid in the detection and diagnosis in early disease development.

The described studies are all cross-sectional in nature. We attempted to overcome the issue of causality by investigating patients at different disease stages and different ages, and concluded that in patients with a single

demyelinating event there are MRI phase changes present in the deep GM structures, though no structural volume reductions are observed. However, what the exact temporal relationship of phase pathology with hallmark MRI abnormalities (such as atrophy and lesion formation) observed in MS is, can only be addressed by longitudinal follow-up studies, preferably among patients recruited at or before first onset. Several studies[66] have proposed that excessive iron levels can cause tissue destruction through its free radical properties, whereas others[67] have argued that excessive iron levels may be an after-the-fact epiphenomenon. There is currently no definitive proof which of these hypotheses, and to what extent, is true.

Further research would also need to address whether phase measures and WM-SAs are associated with clinical and cognitive measures. Results from Chapter 3.3 showed correlations with EDSS. However, it will be crucial to utilize extensive neuropsychological and memory test batteries to investigate whether phase WM-SA presence or deep GM phase measures can predict alterations in cognition. Among MS patients, such studies would have to be carefully designed, due to confounding factors such as fatigue and fluctuating disease severity in the form of relapses.

In the future, SWI and other iron sensitive MRI methods could potentially be used in clinical trial designs to monitor the brain iron levels of MS patients. Although mostly hypothetical at this stage, longitudinally following patients with MS using such MRI measures could hold potential in studies of iron-chelating agents and anti-oxidant therapies targeting reactive oxygen species catalyzed by excessive iron. For example, a recently (2013) FDA approved therapy (BG-12/Tecfidera, Biogen-Idec) has been shown to have beneficial effects by reducing oxidative stress mostly by mediating the nuclear 1 factor (erythroid-derived 2)–like 2 (Nrf2) antioxidant response pathway.[68, 69] Although studies on iron chelators are in their infancy, and are mostly conducted in animal models, selective chelators have the potential to remove excessive levels of brain iron, and studies are currently being carried out in several other neurodegenerative disorders.[70-72]

In conclusion, the main findings of this thesis are:

- SWI-filtered phase allows indirect imaging of deep GM iron content, although other physical properties are also likely to influence phase changes.
- In the deep GM, mean phase, an overall measure of phase visible pathology where more negative values represent more severe pathology, decreases with age until middle-age, after which mean phase increases again (a quadratic effect).
- The mean phase of low phase voxels of the deep GM, a measure of the severity of only highly affected tissues, likely due to excessive iron content, continuously decreases (ie. more putative iron) with age.
- Both adolescent MS patients and CIS patients have lower deep GM SWI phase measures indicative of higher iron levels, even though structural atrophy is minimal or not present yet.
- In MS patients, disability (EDSS) can be better explained by the deep GM MP-LPV of some structures than with conventional MRI measures.
- Phase WM-SAs partially overlap with T2 and T1 WM-SAs. However, the remaining (approximately 50% among CIS patients) may represent unique pathology.
- Phase WM-SAs are much more common in CIS patients than in healthy individuals and individuals with other neurological disorders, and may be useful as part of diagnostic criteria because of high sensitivity and specificity.
- Overall, these (indirect) MRI measures suggest that increased iron levels play a role in MS disease pathology, although no conclusions about causality can be drawn. Reduction of both paramagnetic substances and oxidative stress may prove to be viable therapeutic targets.

References

1. Hallgren B, Sourander P. The effect of age on the non-haemin iron in the human brain. J Neurochem 1958;3:41-51.
2. Martin WR, Ye FQ, Allen PS. Increasing striatal iron content associated with normal aging. Mov Disord 1998;13:281-286.
3. Schenker C, Meier D, Wichmann W, Boesiger P, Valavanis A. Age distribution and iron dependency of the T2 relaxation time in the globus pallidus and putamen. Neuroradiology 1993;35:119-124.
4. Hardy PA, Gash D, Yokel R, Andersen A, Ai Y, Zhang Z. Correlation of R2 with total iron concentration in the brains of rhesus monkeys. J Magn Reson Imaging 2005;21:118-127.
5. Xu X, Wang Q, Zhang M. Age, gender, and hemispheric differences in iron deposition in the human brain: an in vivo MRI study. Neuroimage 2008;40:35-42.
6. Adisetiyo V, Jensen JH, Ramani A, et al. In vivo assessment of age-related brain iron differences by magnetic field correlation imaging. J Magn Reson Imaging 2012;36:322-331.
7. Cherubini A, Peran P, Caltagirone C, Sabatini U, Spalletta G. Aging of subcortical nuclei: microstructural, mineralization and atrophy modifications measured in vivo using MRI. Neuroimage 2009;48:29-36.
8. Haacke EM, Miao Y, Liu M, et al. Correlation of putative iron content as represented by changes in R2* and phase with age in deep gray matter of healthy adults. J Magn Reson Imaging 2010;32:561-576.
9. Ceccarelli A, Rocca MA, Perego E, et al. Deep grey matter T2 hypo-intensity in patients with paediatric multiple sclerosis. Mult Scler 2011;17:702-707.
10. Zivadinov R, Heininen-Brown M, Schirda CV, et al. Abnormal subcortical deep-gray matter susceptibility-weighted imaging filtered phase measurements in patients with multiple sclerosis: a case-control study. Neuroimage 2012;59:331-339.
11. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 1983;33:1444-1452.
12. Zhang Y, Metz LM, Yong VW, Mitchell JR. 3T deep gray matter T2 hypointensity correlates with disability over time in stable relapsing-remitting multiple sclerosis: a 3-year pilot study. J Neurol Sci 2010;297:76-81.
13. Cifelli A, Arridge M, Jezzard P, Esiri MM, Palace J, Matthews PM. Thalamic neurodegeneration in multiple sclerosis. Ann Neurol 2002;52:650-653.
14. Houtchens MK, Benedict RH, Killiany R, et al. Thalamic atrophy and cognition in multiple sclerosis. Neurology 2007;69:1213-1223.
15. Zivadinov R, Havrdova E, Bergsland N, et al. Thalamic Atrophy is Associated with Development of Clinically Definite Multiple Sclerosis. Radiology 2013;268:831-841.

16. Zhang D, Snyder AZ, Fox MD, Sansbury MW, Shimony JS, Raichle ME. Intrinsic functional relations between human cerebral cortex and thalamus. J Neurophysiol 2008;100:1740-1748.
17. Fisniku LK, Chard DT, Jackson JS, et al. Gray matter atrophy is related to long-term disability in multiple sclerosis. Ann Neurol 2008;64:247-254.
18. Geurts JJ, Calabrese M, Fisher E, Rudick RA. Measurement and clinical effect of grey matter pathology in multiple sclerosis. Lancet Neurol 2012;11:1082-1092.
19. Khalil M, Enzinger C, Langkammer C, et al. Quantitative assessment of brain iron by R(2)* relaxometry in patients with clinically isolated syndrome and relapsing-remitting multiple sclerosis. Mult Scler 2009;15:1048-1054.
20. Bakshi R, Dmochowski J, Shaikh ZA, Jacobs L. Gray matter T2 hypointensity is related to plaques and atrophy in the brains of multiple sclerosis patients. J Neurol Sci 2001;185:19-26.
21. Khalil M, Teunissen C, Langkammer C. Iron and neurodegeneration in multiple sclerosis. Mult Scler Int 2011;2011:606807.
22. Poloni G, Minagar A, Haacke EM, Zivadinov R. Recent developments in imaging of multiple sclerosis. Neurologist 2011;17:185-204.
23. Filippi M, Rocca MA, De Stefano N, et al. Magnetic resonance techniques in multiple sclerosis: the present and the future. Arch Neurol 2011;68:1514-1520.
24. Barkhof F. The clinico-radiological paradox in multiple sclerosis revisited. Curr Opin Neurol 2002;15:239-245.
25. Barkhof F. MRI in multiple sclerosis: correlation with expanded disability status scale (EDSS). Mult Scler 1999;5:283-286.
26. Rovaris M, Holtmannspotter M, Rocca MA, et al. Contribution of cervical cord MRI and brain magnetization transfer imaging to the assessment of individual patients with multiple sclerosis: a preliminary study. Mult Scler 2002;8:52-58.
27. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol 2011;69:292-302.
28. Govoni M, Castellino G, Padovan M, Borrelli M, Trotta F. Recent advances and future perspective in neuroimaging in neuropsychiatric systemic lupus erythematosus. Lupus 2004;13:149-158.
29. Triulzi F, Scotti G. Differential diagnosis of multiple sclerosis: contribution of magnetic resonance techniques. J Neurol Neurosurg Psychiatry 1998;64 Suppl 1:S6-14.
30. Zivadinov R, Bergsland N, Stosic M, et al. Use of perfusion- and diffusion-weighted imaging in differential diagnosis of acute and chronic ischemic stroke and multiple sclerosis. Neurol Res 2008;30:816-826.
31. Yao B, Bagnato F, Matsuura E, et al. Chronic multiple sclerosis lesions: characterization with high-field-strength MR imaging. Radiology 2012;262:206-215.

32. Schweser F, Deistung A, Lehr BW, Reichenbach JR. Quantitative imaging of intrinsic magnetic tissue properties using MRI signal phase: an approach to in vivo brain iron metabolism? Neuroimage 2011;54:2789-2807.
33. Yablonskiy DA, Luo J, Sukstanskii AL, Iyer A, Cross AH. Biophysical mechanisms of MRI signal frequency contrast in multiple sclerosis. Proc Natl Acad Sci U S A 2012;109:14212-14217.
34. Wiggermann V, Hernandez Torres E, Vavasour IM, et al. Magnetic resonance frequency shifts during acute MS lesion formation. Neurology 2013;81:211-218.
35. Bian W, Harter K, Hammond-Rosenbluth KE, et al. A serial in vivo 7T magnetic resonance phase imaging study of white matter lesions in multiple sclerosis. Multiple sclerosis 2013;19:69-75.
36. Bagnato F, Hametner S, Yao B, et al. Tracking iron in multiple sclerosis: a combined imaging and histopathological study at 7 Tesla. Brain : a journal of neurology 2011;134:3602-3615.
37. Craelius W, Migdal MW, Luessenhop CP, Sugar A, Mihalakis I. Iron deposits surrounding multiple sclerosis plaques. Archives of pathology & laboratory medicine 1982;106:397-399.
38. Haacke EM, Makki M, Ge Y, et al. Characterizing iron deposition in multiple sclerosis lesions using susceptibility weighted imaging. J Magn Reson Imaging 2009;29:537-544.
39. Hagemeier J, Heininen-Brown M, Poloni GU, et al. Iron deposition in multiple sclerosis lesions measured by susceptibility-weighted imaging filtered phase: a case control study. J Magn Reson Imaging 2012;36:73-83.
40. Craelius W, Migdal MW, Luessenhop CP, Sugar A, Mihalakis I. Iron deposits surrounding multiple sclerosis plaques. Arch Pathol Lab Med 1982;106:397-399.
41. Ferguson B, Matyszak MK, Esiri MM, Perry VH. Axonal damage in acute multiple sclerosis lesions. Brain 1997;120 (Pt 3):393-399.
42. Trapp BD, Ransohoff R, Rudick R. Axonal pathology in multiple sclerosis: relationship to neurologic disability. Curr Opin Neurol 1999;12:295-302.
43. Polman CH, Reingold SC, Edan G, et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". Ann Neurol 2005;58:840-846.
44. Koeppen AH. The history of iron in the brain. J Neurol Sci 1995;134 Suppl:1-9.
45. Connor JR, Menzies SL, St Martin SM, Mufson EJ. Cellular distribution of transferrin, ferritin, and iron in normal and aged human brains. J Neurosci Res 1990;27:595-611.
46. Zecca L, Gallorini M, Schunemann V, et al. Iron, neuromelanin and ferritin content in the substantia nigra of normal subjects at different ages: consequences for iron storage and neurodegenerative processes. J Neurochem 2001;76:1766-1773.
47. Tuomainen TP, Kontula K, Nyyssonen K, Lakka TA, Helio T, Salonen JT. Increased risk of acute myocardial infarction in carriers of the hemochromatosis gene

Cys282Tyr mutation : a prospective cohort study in men in eastern Finland. Circulation 1999;100:1274-1279.
48. Roest M, van der Schouw YT, de Valk B, et al. Heterozygosity for a hereditary hemochromatosis gene is associated with cardiovascular death in women. Circulation 1999;100:1268-1273.
49. Allen KJ, Gurrin LC, Constantine CC, et al. Iron-overload-related disease in HFE hereditary hemochromatosis. N Engl J Med 2008;358:221-230.
50. Bartzokis G, Lu PH, Tishler TA, et al. Prevalent iron metabolism gene variants associated with increased brain ferritin iron in healthy older men. J Alzheimers Dis 2010;20:333-341.
51. Nandar W, Connor JR. HFE gene variants affect iron in the brain. J Nutr 2011;141:729-739.
52. Ristic S, Lovrecic L, Brajenovic-Milic B, et al. Mutations in the hemochromatosis gene (HFE) and multiple sclerosis. Neurosci Lett 2005;383:301-304.
53. Rubio JP, Bahlo M, Tubridy N, et al. Extended haplotype analysis in the HLA complex reveals an increased frequency of the HFE-C282Y mutation in individuals with multiple sclerosis. Hum Genet 2004;114:573-580.
54. Penke L, Valdes Hernandez MC, Maniega SM, et al. Brain iron deposits are associated with general cognitive ability and cognitive aging. Neurobiol Aging 2010;33:510-517.
55. Sullivan EV, Adalsteinsson E, Rohlfing T, Pfefferbaum A. Relevance of Iron Deposition in Deep Gray Matter Brain Structures to Cognitive and Motor Performance in Healthy Elderly Men and Women: Exploratory Findings. Brain Imaging Behav 2009;3:167-175.
56. Andersen JK. Oxidative stress in neurodegeneration: cause or consequence? Nat Med 2004;10 Suppl:S18-25.
57. Halliwell B. Reactive oxygen species and the central nervous system. J Neurochem 1992;59:1609-1623.
58. Jomova K, Valko M. Advances in metal-induced oxidative stress and human disease. Toxicology 2011;283:65-87.
59. Oleszak EL, Zaczynska E, Bhattacharjee M, Butunoi C, Legido A, Katsetos CD. Inducible nitric oxide synthase and nitrotyrosine are found in monocytes/macrophages and/or astrocytes in acute, but not in chronic, multiple sclerosis. Clin Diagn Lab Immunol 1998;5:438-445.
60. Trapp BD, Bo L, Mork S, Chang A. Pathogenesis of tissue injury in MS lesions. J Neuroimmunol 1999;98:49-56.
61. Bo L, Dawson TM, Wesselingh S, et al. Induction of nitric oxide synthase in demyelinating regions of multiple sclerosis brains. Annals of neurology 1994;36:778-786.

62. Kolasinski J, Stagg CJ, Chance SA, et al. A combined post-mortem magnetic resonance imaging and quantitative histological study of multiple sclerosis pathology. Brain : a journal of neurology 2012;135:2938-2951.
63. Lassmann H. Multiple sclerosis: Lessons from molecular neuropathology. Exp Neurol 2013:262;2-7.
64. Hametner S, Wimmer I, Haider L, Pfeifenbring S, Bruck W, Lassmann H. Iron and neurodegeneration in the multiple sclerosis brain. Annals of neurology 2013:74(6);848-861.
65. Bagnato F, Hametner S, Yao B, et al. Tracking iron in multiple sclerosis: a combined imaging and histopathological study at 7 Tesla. Brain 2011;134:3602-3615.
66. Smith MA, Harris PL, Sayre LM, Perry G. Iron accumulation in Alzheimer disease is a source of redox-generated free radicals. Proc Natl Acad Sci U S A 1997;94:9866-9868.
67. Stankiewicz J, Panter SS, Neema M, Arora A, Batt CE, Bakshi R. Iron in chronic brain disorders: imaging and neurotherapeutic implications. Neurotherapeutics 2007;4:371-386.
68. Linker RA, Lee DH, Ryan S, et al. Fumaric acid esters exert neuroprotective effects in neuroinflammation via activation of the Nrf2 antioxidant pathway. Brain : a journal of neurology 2011;134:678-692.
69. Gold R, Kappos L, Arnold DL, et al. Placebo-controlled phase 3 study of oral BG-12 for relapsing multiple sclerosis. The New England journal of medicine 2012;367:1098-1107.
70. Weinreb O, Mandel S, Youdim MB, Amit T. Targeting dysregulation of brain iron homeostasis in Parkinson's disease by iron chelators. Free Radic Biol Med 2013;62:52-64.
71. Guo C, Wang T, Zheng W, Shan ZY, Teng WP, Wang ZY. Intranasal deferoxamine reverses iron-induced memory deficits and inhibits amyloidogenic APP processing in a transgenic mouse model of Alzheimer's disease. Neurobiol Aging 2013;34:562-575.
72. Ritchie CW, Bush AI, Mackinnon A, et al. Metal-protein attenuation with iodochlorhydroxyquin (clioquinol) targeting Abeta amyloid deposition and toxicity in Alzheimer disease: a pilot phase 2 clinical trial. Arch Neurol 2003;60:1685-1691.

Nederlandse samenvatting

De ironie van ijzer

Multipele sclerose (MS) is met een prevalentie van ongeveer 1 op 1000 een relatief veel voorkomende chronische aandoening van het centrale zenuwstelsel. De ziekte wordt meestal gekenmerkt door terugvallen ("relapses"), waarbij een patiënt neurologische klachten heeft zoals visuele, motorische (bijvoorbeeld balans), sensorische, en cognitive (bijvoorbeeld aandacht) problemen. Dit ziektebeeld wordt in de loop van tijd in de meeste gevallen steeds sterker tot het punt bereikt is waarbij neurologische klachten aanhouden. Met een gemiddelde diagnose-leeftijd van rond het dertigste levensjaar zijn het vaak jong volwassenen en voornamelijk vrouwen die getroffen worden door deze ziekte. Bij mannen lijkt daarentegen de ziekte agressiever te zijn en zij hebben dan ook een slechtere prognose. Er is veel onduidelijkheid over de oorzaak van MS, al is de incidentie beduidend hoger in landen ver verwijderd van de evenaar (bijvoorbeeld in Noord-Europa, Noord-Amerika en Oceanië) dan in landen dicht bij de evenaar. Factoren die het risico op MS lijken te beïnvloeden zijn onder andere zonlicht en vitamine D deficiëntie, virale infecties zoals het Epstein-Barr virus, hygiënische factoren, en genetische aanleg.

MS wordt gekenmerkt door verschillende ziektebeelden (fenotypen). Wanneer een persoon voor de eerste keer MS-symptomen vertoont (een MS-aanval) wordt dit klinisch geïsoleerd syndroom (CIS) genoemd. Een veelvoorkomend eerste symptoom is het tijdelijk verlies van zicht door demyelinatie van de gezichtszenuw. Het merendeel van deze patiënten zal binnen enkele jaren opnieuw een aanval krijgen, waarna een MS-diagnose waarschijnlijk is. De meest voorkomende vorm van MS is "relapsing remitting" MS (RRMS), waarbij terugvallen ("relapses") gepaard gaan met tussenliggende periodes van remissie. In deze periodes trekken de symptomen zich gedeeltelijk of geheel terug. Op den duur, wanneer de schade in het brein accumuleert, trekken de symptomen zich minder terug en zijn ze blijvend. Deze fase wordt "secundair progressieve" MS (SPMS) genoemd en wordt gekenmerkt door geleidelijk verlies van functie. Een kleine subgroep van MS-patiënten heeft "primair progressieve" MS (PPMS), waarbij

er geen sprake is van terugvallen, maar symptomen van begin af aan geleidelijk verergeren.

MS werd voor het eerst in 1887 door de Franse neuroloog Jean-Martin Charcot omschreven als een op zichzelf staande aandoening. Historisch gezien werd MS beschouwd als een ziekte van de witte stof (myeline) in de hersenen, al is het in de laatste decennia duidelijk geworden dat de ziekte niet alleen de witte stof maar ook grijze stof (zenuwcellen) in de hersenen aantast. MS is een demyeliniserende ziekte gekenmerkt door een immuunreactie tegen de isolatielaag van de axonen (lange uitlopers van zenuwcellen die zorgen voor de connecties tussen neuronen). Myeline is essentieel voor de snelle overgang van informatie tussen neuronen. Wanneer de myeline is aangetast wordt in feite het hele communicatienetwerk van het brein aangetast. De aanvallen van het immuunsysteem zorgen ervoor dat er "laesies" (gelokaliseerde schade aan de myeline) ontstaan. Deze littekens worden ook wel "plaques" of "scleroses" genoemd. Deze laesies zijn post-mortem te herkennen, maar dankzij MRI is het mogelijk om een groot deel hiervan te visualiseren *in vivo*. Er is sprake van een contradictie tussen de symptomen die patiënten ervaren, en de laesies die we tot dusver hebben kunnen visualiseren: het aantal laesies hoeft namelijk niet in relatie te staan met de ernst van de MS-symptomen. Door deze paradox zijn wetenschappers op zoek naar andere, mogelijk belangrijkere, kenmerken ("biomarkers"). Zo is er naast de schade in de witte stof door de jaren heen dankzij nieuwe post-mortem en MRI-technieken duidelijk geworden dat ook de grijze stof veel schade ondergaat. Waar myeline zorgt voor snelle communicatie tussen zenuwcellen zijn de miljarden neuronen verantwoordelijk signalen te initiëren en vooruit te sturen. Bij MS-patiënten is er in de grijze stof tevens sprake van verlies van zenuwcellen en ontstaan er, net zoals in de witte stof, focale laesies. Het feit dat uitgebreide schade zich voordoet in zowel de myeline als in de neuronen, uit zich in de diversiteit van symptomen en biedt een verklaring voor de klinisch-radiologische paradox. Hierdoor is het van groot belang om onderzoek te doen naar onderliggende factoren die mogelijk laesies en atrofie veroorzaken en wellicht een betere voorspeller zijn van het klinische beeld. In dit onderzoek hebben wij MRI-technieken gebruikt om het

niveau van ijzer in de hersenen, een mogelijke oorzaak van de alom geobserveerde hersenschade, te onderzoeken.

Ijzer (Fe) is een belangrijk element dat in het menselijk lichaam voorkomt, zo ook in het brein. Er zijn twee vormen: ijzer dat gebonden is aan rode bloedcellen (hemoglobine) en ongebonden ijzer. Dit ongebonden ijzer heeft de mogelijkheid om schade te veroorzaken door te reageren met waterstofperoxide waarbij vrije radicalen ontstaan. Deze vrije radicalen (voornamelijk hydroxylradicaal) kunnen leiden tot celschade aan bijvoorbeeld het DNA, celmembranen en de mitonchondria. Onderzoek heeft aangetoond dat er meer ijzer aanwezig is in de grijze stof van oudere mensen. Tevens zijn er hogere ijzer niveaus te vinden bij patiënten met neurodegeneratieve ziektes zoals de ziekte van Alzheimer en Parkinson. Beide ziektes zijn ook gekenmerkt door atrofie van bepaalde hersenstructuren. Als ijzer in hogere mate aanwezig is in het brein van MS-patiënten, zou dit mogelijk een belangrijk onderdeel van het proces kunnen zijn dat leidt tot laesies, atrofie en MS-symptomen. Hoewel een overschot aan ijzer schadelijk kan zijn, is een deficiëntie hiervan -vooral in de jeugd- ook nadelig. Dit kan zich uiten in bijvoorbeeld verminderde productie van myeline. De homeostasis van ijzer is klaarblijkelijk belangrijk, te veel of te weinig is schadelijk. Dit contrast is de ironie van ijzer.

Dankzij nieuwe MRI-technieken die gevoelig zijn voor de magnetische eigenschappen van ijzer kan er onderzoek worden gedaan naar het niveau van ijzer in hersenstructuren. Derhalve hebben wij in dit onderzoek gekeken naar ijzer accumulatie bij zowel een gezonde populatie als bij verschillende groepen MS patiënten. Hierbij trachten wij een breed beeld te scheppen over de ontwikkeling van zowel een gezonde populatie, alsmede een duidelijk inzicht te verkrijgen van vroege en latere fases van MS.

Na een algemene introductie in **hoofdstuk 1**, bespreken we in **hoofdstuk 2** een groep gezonde individuen. In de studie die is omschreven in **hoofdstuk 2.1** hebben we gekeken naar mensen die variëren in leeftijd van adolescenten tot senioren. We gebruikten de MRI-techniek “susceptiblity weighted imaging” (SWI) die een schatting kan geven van de hoeveelheid ijzer in het

brein. Tevens keken we naar het krimpen van de hersenen (atrofie). Uit het onderzoek kwam naar voren, dat naarmate men ouder wordt, het ijzergehalte van verschillende grijze stof structuren ook hoger wordt. Dit was vooral te zien in bepaalde “diepe” grijze stof structuren, zoals de nucleus caudatus en thalamus en dit ondersteunt onderzoeksresultaten van post-mortem studies. Het ijzergehalte vertoonde ook een sterke relatie met atrofie van het brein. Een andere interessante bevinding van deze studie is dat de totale ijzer concentratie niet lineair lijkt te stijgen met leeftijd. In plaats daarvan is er een piek rond het vijftigste levensjaar, waarna –bij deze gezonde populatie- de stijging stagneert of zelfs enigszins vermindert. Echter, wanneer we exclusief kijken naar de zwaarst aangetaste hersengebieden, dan is de relatie met leeftijd lineair.

Na het onderzoek bij gezonde individuen ligt de focus in **hoofdstuk 3** op verschillende stadia van MS. In **hoofdstuk 3.1** onderzochten we adolescente MS patiënten. Om deze groep te kunnen vergelijken gebruikten we een controle groep bestaande uit gezonde individuen van dezelfde leeftijd en hetzelfde geslacht. Onze bevindingen laten zien dat er bij jonge patiënten grootschalige ijzer opstapelingen voorkomen, alsmede atrofie van dezelfde structuren. De meest aangetaste structuur is een onderdeel van de thalamus: de pulvinar nuclei. Deze structuur is een belangrijk station voor connecties van-en-naar de cortex, waardoor schade aan deze regio een breed scala aan gevolgen kan hebben.

In **hoofdstuk 3.2** onderzochten we patiënten met CIS, patiënten die tot dan slechts één MS aanval hebben gehad. Dit wordt gezien als een vroeg stadium van MS. Pas wanneer er zich een tweede aanval voordoet kan er sprake zijn van een MS-diagnose. Evenals bij adolescente MS patiënten, is hier sprake van een verhoogde mate van ijzer in de diepe grijze stof, vooral in de pulvinar nucleus maar ook in de nucleus caudatus en putamen. Er lijkt bij deze patiënten daarentegen geen sprake te zijn van grootschalige atrofie. Dit zou kunnen aantonen dat er al wel sprake is van ijzer acummulatie aan de hand van de SWI fase scan, maar dat de ziekte zich nog in een te vroeg stadium bevindt voor de manifestatie van atrofie van de grijze stof. Ook al is er geen

sprake van causaal bewijs, toch is dit een interessante bevinding omdat het aantoont dat ijzer opstapelingen mogelijk aan de atrofie voorafgaan.

In **hoofdstuk 3.3** onderzochten we RRMS en SPMS patiënten. Deze twee ziektebeelden zijn gekenmerkt door verergering van symptomen en pathologische veranderingen die zichtbaar zijn met MRI. Naast het onderzoeken of RRMS en SPMS patiënten verschillen van de controle groep, trachten we hier ook te bezien of ijzer opstapelingen gerelateerd zijn aan MS-symptomen. Zoals verwacht was het ijzer-peil hoger bij RRMS en vooral bij SPMS patiënten in vergelijking tot gezonde individuen. Zowel op medisch- als wetenschappelijk gebied wordt de Kurtzke Expanded Disability Scale (EDSS) maat gebruikt als gouden standaard om de hevigheid van symptomen te kwantificeren. Een hoge mate van ijzer in de diepe grijze stof is gerelateerd aan een verhoogde EDSS score, zelfs wanneer er in de statistische modellen rekening wordt gehouden met leeftijd en geslacht. Ook in deze studie laten we zien dat ijzer gemeten met SWI sterker gerelateerd is aan EDSS dan klassieke MRI maten (zoals de hoeveelheid laesies en atrofie).

MS laesies worden meestal onderzocht met de T1 en T2 MRI methoden. Deze methoden kunnen laesies visualiseren. T1 toont “black holes”, langdurige littekens, terwijl met T2 nieuwere laesies worden gevisualiseerd. In **hoofdstuk 4.1** en **4.2** onderzochten wij laesies in de witte stof die niet worden gevisualiseerd met behulp van T1 of T2, maar met SWI fase. Hoogstwaarschijnlijk hebben deze laesies een andere etiologie, zijn ze zichtbaar in een andere fase van MS en zijn ze hoog in ijzer concentratie. Voorgaand onderzoek heeft aangetoond dat SWI fase laesies voor langere periodes zichtbaar kunnen zijn (tot meer dan twee jaar) en al zichtbaar zijn voordat ze op conventionele MRI te zien zijn. Een mogelijke oorzaak van de verhoogde ijzerwaarden in deze MS laesies komt voort uit microglia die de myeline afbreken waardoor er ijzer van de cellen vrijkomt en een cluster vormt rond de laesie. In **hoofdstuk 4.1** onderzochten we bij MS patiënten T1-, T2- en SWI fase laesies. We vonden dat er meer SWI fase laesies bij MS patiënten aanwezig waren dan bij gezonde individuen. Ook toonden we aan dat SWI fase laesies slechts gedeeltelijk overlappen met T1 en T2 zichtbare laesies. Dit toont aan dat er unieke laesies zichtbaar zijn met deze techniek,

en dat een gedeelte onafhankelijk is van de T1 en T2 laesies. De aanwezigheid van meerdere (>5) SWI fase laesies kon met hoge zekerheid -zonder enige andere informatie- MS patiënten onderscheiden van gezonde individuen. De klinisch-radiologische paradox die zo prominent aanwezig is bij T1 en T2 laesies is ook van toepassing op laesies met hoge ijzer concentraties: er waren geen sterke correlaties met klinische maten zoals EDSS.

In **hoofdstuk 4.2** onderzochten we of deze SWI fase laesies enige diagnostische waarde hebben in een vroeg stadium van MS. Om dit te onderzoeken werden patiënten met CIS onderzocht. In deze studie werd aangetoond dat de aanwezigheid van laesies met hoge ijzerwaarden CIS patiënten kon onderscheiden van zowel gezonde individuen, als van patiënten met andere neurologische aandoeningen. De aanwezigheid van meerdere T2 laesies in meerdere locaties is noodzakelijk voor een MS diagnose. De reden hiervoor is dat T2 laesies veel voorkomend zijn, zelfs bij gezonde mensen kan dit worden aangetroffen. De aanwezigheid van één of meerdere SWI fase laesies is daarentegen al een goede indicatie van pathologie; de kans dat een gezond persoon een fase laesie heeft is erg laag.

Samenvatting

In deze onderzoeken schetsen wij een beeld van de verschillende stadia van MS door middel van het gebruik van een MRI techniek die indirect ijzer concentraties meet. Alhoewel meerdere factoren dit MRI signaal kunnen beïnvloeden blijft het waarschijnlijk dat het merendeel veroorzaakt wordt door ijzer opstapelingen. Histopathologische studies hebben bevestigd dat er een sterke associatie is tussen het signaal dat zichtbaar is met SWI fase en ijzer concentraties. We hebben aangetoond dat met deze MRI techniek vroege MS patiënten kunnen worden onderscheiden van de gezonde populatie, en dat de vermeende ijzer accumulatie in de diepe grijze massa gerelateerd is aan klinisch relevante maten. Ook schijnt er een sterke samenhang te zijn tussen ijzer concentratie en atrofie. MRI technieken die gevoelig zijn voor ijzer concentraties kunnen mogelijk in de toekomst gebruikt worden bij de diagnose van MS. Onderzoek naar medicatie die ijzer concentraties kunnen verlagen zijn momenteel in gang gezet en zouden kunnen helpen bij het verminderen van schadelijke vrije radicalen.

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List of publications

Kavak KS, Teter BE, **Hagemeier J**, Zakalik K & Weinstock-Guttman B. (2014). Higher weight in adolescence and young adulthood is associated with an earlier age at MS onset. *Multiple Sclerosis Journal* 2014, epub ahead of print, pii: 1352458514555787.

Uher T, Blahova-Dusankova J, Horakova D, Bergsland N, Tyblova M, Benedict RH, Kalincik T,Ramasamy DP, Seidl Z, **Hagermeier J**, Vaneckova M, Krasensky J, Havrdova E, Zivadinov R. (2014). Longitudinal MRI and neuropsychological assessment of patients with clinically isolated syndrome. *Journal of Neurology* 2014, 261(9), 1735-1744.

Zivadinov R, Chin J, Horakova D, Bergsland N, Weinstock-Guttman B, Tamaño-Blanco M, Badgett D, **Hagemeier J**, Tyblova M, Carl E, Krasensky J, Vaneckova M, Seidl Z, Dwyer MG, Havrdova E, Ramanathan M. (2014). Humoral responses to herpesviruses are associated with neurodegeneration after a demyelinating event: results from the multi-center set study. *Journal of Neuroimmunoly* 2014, 15;273(1-2), 58-64.

Hagemeier J, Heininen-Brown M, Gabelic T, Guttuso T, Silvestri N, Lichter D, Fugoso LE, Bergsland N, Carl E, Geurts JJG, Weinstock-Guttman B, Zivadinov R (2014). Phase White Matter Signal Abnormalities in Patients with Clinically Isolated Syndrome and Other Neurological Disorders. *American Journal of Neuroradiology* 2014, epub ahead of print doi:10.3174/ajnr.A3969.

Browne RW, Weinstock-Guttman B, Zivadinov R, Horakova D, Bodziak ML, Tamaño Blanco M, Badgett D, Tyblova N, Vaneckova M, Seidl Z, Krasensky J, Bergsland N, Ramasamy DP, **Hagemeier J**, Qu J, Havrdova E, Ramanathan M. Serum lipoprotein composition and vitamin D metabolite levels in clinically isolated syndromes: Results from a multi-center study. *The Journal of Steroid Biochemistry and Molecular Biology* 2014, 143, 424-433.

Zivadinov R, Bergsland N, Cappellani R, **Hagemeier J,** Melia R, Carl E, Dwyer M, Lincoff N, Weinstock Guttman,B, Ramanathan M. Retinal Nerve Fiber Layer Thickness and Thalamus Pathology in Multiple Sclerosis Patients. *European Journal of Neurology* 2014, 21(8), 1137-e61.

Jacobsen C, **Hagemeier J,** Myhr, K-M, Nyland H, Lode K, Bergsland N, Ramasamy D, Dalaker TO, Larsen JP, Farbu E, Zivadinov R. Brain atrophy and disability progression in multiple sclerosis patients: A 10-year follow-up study. *Journal of Neurology, Neurosurgery, and Psychiatry 2014,* 85(10),1109-1115.

Browne RW, Weinstock-Guttman B, Horakova D, Zivadinov R, Bodziak ML, Tamaño Blanco M, Badgett D, Tyblova M, Vaneckova M, Seidl Z, Krasensky J, Bergsland N, Ramasamy DP, **Hagemeier J**, Havrdova E, Ramanathan M. Apolipoproteins are associated with new MRI lesions and deep grey matter atrophy in clinically isolated syndromes. *Journal of Neurology, Neurosurgery, and Psychiatry* 2014, 85(8), 859-864.

Gabelic T, Ramasamy DP, Weinstock-Guttman B, **Hagemeier J**, Kennedy C, Melia R, Hojnacki D, Ramanathan M, Zivadinov R. Prevalence of Radiologically Isolated Syndrome and White Matter Signal Abnormalities in Healthy Relatives of Patients with Multiple Sclerosis. *American Journal of Neuroradiology* 2014, 35, 106-112.

Cappellani R, Bergsland N, Weinstock-Guttman B, Kennedy C, Carl E, Ramasamy DP, **Hagemeier J,** Dwyer MG, Patti F, Zivadinov R. Subcortical Deep Gray Matter Pathology in Patients with Multiple Sclerosis Is Associated with White Matter Lesion Burden and Atrophy but Not with Cortical Atrophy: A Diffusion Tensor MRI Study. *American Journal of Neuroradiology* 2014, 35, 912-919.

Hagemeier J, Dwyer MG, Bergsland N, Schweser F, Magnano R, Heininen-Brown M, Ramasamy DP, Carl E, Kennedy C, Melia R, Polak P, Deistung A, Geurts JJG, Reichenbach JR, Zivadinov R. Effect of Age on MRI Phase Behavior in the Subcortical Deep Gray Matter of Healthy Individuals. *American Journal of Neuroradiology* 2013, 34(11), 2144-2151.

Weinstock-Guttman B, Horakova D, Zivadinov R, Tamaño-Blanco M, Badgett D, Tyblova M, Vaneckova M, Seidl Z, Krasensky J, Bergsland N, Ramasamy DP, **Hagemeier J**, Havrdova E, Ramanathan M. Interactions of serum cholesterol with anti-herpesvirus responses affect disease progression in clinically isolated syndromes. *Journal of Neuroimmunology* 2013, 263(1-2), 121-127.

Zivadinov R, Havrdová E, Bergsland N, Tyblova M, **Hagemeier J**, Seidl Z, Dwyer MG, Vaneckova M, Krasensky J, Carl E, Kalincik T, Horáková D. Thalamic Atrophy is Associated with Development of Clinically Definite Multiple Sclerosis. *Radiology* 2013, 268(3), 831-841.

Cappellani R, Bergsland N, Weinstock-Guttman B, Kennedy C, Carl E, Ramasamy DP, **Hagemeier J**, Dwyer MG, Patti F, Zivadinov R. Diffusion Tensor MRI Alterations of Subcortical Deep Gray Matter in Clinically Isolated Syndrome. *Journal of Neurological Sciences* 2014, 338(1-2), 128-134.

Hagemeier J, Yeh EA, Brown M.H., Bergsland N, Dwyer MG, Carl E, Weinstock-Guttman B, Zivadinov R. Iron content of the pulvinar nucleus of the thalamus is increased in adolescent multiple sclerosis. *Multiple Sclerosis* 2013, 19 (5), 567-576.

Zivadinov R, Karmon Y, Dolic K, **Hagemeier J**, Marr K, Valnarov V, Kennedy C, Hojnacki D, Carl E, Hopkins LN, Levy EI, Weinstock-Guttman B, Siddiqui AH. Multimodal noninvasive and invasive imaging of extracranial venous abnormalities indicative of CCSVI: results of the PREMiSe pilot study. *BMC neurology* 2013, 13:151.

Zivadinov R, Magnano C, Galeotti t, Schirda C, Menegatti E, Weinstock-Guttman, B, Marr K, Bartolomei I, **Hagemeier J**, Malagoni AM, Hojnacki D, Kennedy C, Carl E, Beggs C, Salvi F, Zamboni P. Changes of Cine Cerebrospinal Fluid Dynamics in Patients with Multiple Sclerosis Treated with Percutaneous Transluminal Angioplasty: Case-control Study. *Journal of Vascular and Interventional Radiology* 2013, 24(6), 829-838.

Karmon Y, Zivadinov R, Weinstock-Guttman B, Marr K, Valnarov V, Dolic K, Kennedy C, Hojnacki D, Carl E, **Hagemeier J**, Hopkins LN, Levy EI, Siddiqui AH. Comparison of intravascular ultrasound with conventional venography for detection of extracranial venous abnormalities indicative of chronic cerebrospinal venous insufficiency. *Journal of Vascular and Interventional Radiology* 2013, 24(10), 1487-1489.

Zivadinov R, Magnano CM, Galeotti R, Schirda C, Menegatti E, Weinstock-Guttman B, Marr K, Bartolomei I, **Hagemeier J**, Malagoni AM, Hojnacki D, Kennedy C, Carl E, Beggs C, Salvi F, Zamboni P. Changes of Cine Cerebrospinal Fluid Dynamics in Patients with Multiple Sclerosis Treated with Percutaneous Transluminal Angioplasty: Case-control Study. *Journal of Vascular and Interventional Radiology* 2013, 24(6), 829-838.

Hagemeier J, Weinstock-Guttman B, Heininen-Brown M, Poloni GU, Bergsland N, Schirda C, Magnano CR, Kennedy C, Carl E, Dwyer MG, Minagar A, Zivadinov R. Gray matter SWI-filtered phase and atrophy are linked to disability in MS. *Frontiers in Bioscience Elite Edition* 2012, 5, 525-532.

Hagemeier J, Geurts JJG, Zivadinov R. Brain iron accumulation in aging and neurodegenerative disorders. *Expert Reviews of Neurotherapeutics* 2012, 12(12), *1467-1480*.

Tipirneni A, Weinstock-Guttman B, Ramanathan M, Abdelrahman N, Hussein S, **Hagemeier J**, Durfee J, Teter BE, Hojnacki D, Dwyer MG, Zivadinov R. MRI characteristics of familial and sporadic multiple sclerosis patients. *Multiple Sclerosis* 2012, 19(9), 1145-1152.

Dolic K, Weinstock-Guttman B, Marr K, Valnarov V, Carl E, **Hagemeier J**, Kennedy C, Kilanowski C, Hojnacki D, Ramanathan M, Zivadinov R. Heart disease, overweight, and cigarette smoking are associated with increased prevalence of extra-cranial venous abnormalities. *Neurological research* 2012, 34(8), 819-827.

Hagemeier J, Heininen-Brown M, Poloni GU, Bergsland N, Magnano CR, Durfee J, Kennedy C, Carl E, Weinstock-Guttman B, Dwyer MG, Zivadinov R. Iron deposition in multiple sclerosis lesions measured by susceptibility-weighted imaging filtered phase: a case control study. *Journal of Magnetic Resonance Imaging* 2012, 36(1), 73-83.

Hagemeier J, Weinstock-Guttman B, Bergsland N, Heininen-Brown M, Carl E, Kennedy C, Magnano C, Hojnacki D, Dwyer MG, Zivadinov R. Iron deposition on SWI filtered phase in the subcortical deep gray matter of clinically isolated syndrome patients may precede structure-specific atrophy. *American Journal of Neuroradiology* 2012, 33(8), 1596-1601.

Zivadinov R, Heininen-Brown M, Schirda CV, Poloni GU, Bergsland N, Magnano CR, Durfee J, Kennedy C, Carl E, **Hagemeier J**, Benedict RH, Weinstock-Guttman B, Dwyer MG. Abnormal subcortical deep-gray matter susceptibility-weighted imaging filtered phase measurements in patients with multiple sclerosis: a case-control study. *Neuroimage* 2012, 59(1),331-339.

Dolic K, Weinstock-Guttman B, Marr K, Valnarov V, Carl E, **Hagemeier J**, Brooks C, Kilanowski C, Hojnacki D, Ramanathan M, Zivadinov R. Risk factors for chronic cerebrospinal venous insufficiency (CCSVI) in a large cohort of volunteers. *PLoS One* 2011, 6(11), doi:10.1371/journal.pone.0028062.

Dolic K, Marr K, Valnarov V, Dwyer MG, Carl E, **Hagemeier J**, Kennedy C, Brooks C, Kilanowski C, Hunt K, Hojnacki D, Weinstock-Guttman B, Zivadinov R. Sensitivity and specificity for screening of chronic cerebrospinal venous insufficiency using a multimodal non-invasive imaging approach in patients with multiple sclerosis. *Functional Neurology* 2011, 26(4), 205-214.