9. General Discussion, Summary, Future Perspectives and Conclusion
This thesis describes studies that examined applications of high field MRI in Multiple Sclerosis (MS). In particular, feasibility and increased sensitivity of MR sequences that have proven to be valuable in MS diagnosis and treatment monitoring at lower field strengths were investigated. Also the role of iron accumulations in MS patients was reviewed and the possible use of a new iron oxide based contrast agent in an animal model of MS has been investigated.

9.1 High field imaging of inflammation in the central nervous system

MRI has shown to be an excellent modality to visualize inflammatory CNS diseases. The benefits of being able to visualize soft tissue like the brain therefore have been of great value for MS research and diagnosis. Vice versa, its value in MS diagnosis boosted developments in MR techniques. It resulted in new sequences that were more sensitive for pathology.

This started with detection of white matter (WM) lesions in deep white matter, followed by the introduction of sophisticated sequences like fluid attenuated inversion recovery (FLAIR) that were able to increase sensitivity for periventricular lesions. Administration of gadolinium contrast agents showed enhancement of active MS lesions and enabled radiologists to observe differentiation in time (1). MS diagnostic criteria could now be complemented with radiological findings that increased their confidence (2–4). However, correlation of MS lesions observed on MR images with clinical symptoms was only moderate. Advances in sequences and field strength resulted in increased lesion detection that also showed more subtle and/or diffuse MS pathology. In addition, measurement of brain atrophy improved correlations, but did not solve the so-called clinico-radiological paradox (5, 6).

While it was known for long that also grey matter (GM) was affected in MS, imaging GM lesions was more difficult as the available contrast mechanisms mostly relied on the oedema effect from inflammatory lesions. Most GM lesions hardly showed inflammation and therefore remained invisible on MRI. An MR sequence (3D double inversion recovery (DIR)) that suppressed both WM and cerebrospinal fluid (CSF) was able to show many GM lesions on standard field strength (7, 8). Still, most of the clinical studies looking into questions regarding correlations of lesion visibility and clinical impact used standard (1.5T) field. With the development of clinical 3T MR scanners boundaries shifted regarding spatial resolution and signal to noise ratios (SNR). It resulted in increased lesion sensitivity on both WM and GM, but did not result in advancing the diagnosis of MS. Although counter intuitive, high field MRI did not directly increase clinical value (9–11).

Chapter 2 reviews developments in high field MRI and its value for MS research and diagnosis. Not only 3T but also ultra-high field (7T) is discussed together with the possible necessity to revise diagnostic criteria. New applications and challenges for high field MRI in MS may have direct clinical value, but high field MRI may play an even more important role in studying disease aetiology. As a research tool it will help to understand results obtained from MRI studies at lower field. It can also be used for treatment monitoring as its high sensitivity may help to detect efficacy of novel treatments in a very early stage. This would help individual patients to benefit from ‘tailor made’ treatment regimes that could lower side effects. While at this moment application of 7T MRI in standard clinical practise may still be premature, it is conceivable that in the future MS patients would benefit from 7T MRI examination to define disease type or get insight on expected disease progression and future prospects of treatment.

Therefore ultra-high field MRI needs optimization of dedicated hardware, MR pulse sequences and protocols, and, especially regarding the radiofrequency transceiver system, to overcome
high field specific problems. At ultra high field, the RF wave lengths approach typical head size resulting in standing waves that reduce transmit homogeneity. This reduces signal uniformity over the field of view, deteriorating image quality. In addition, problems regarding maximum specific absorption rates (SAR) of RF radiation at the moment limit clinical feasibility.

In spite of these technical challenges, substantial progress is made in this field. In chapter 3 a study is described that examined the clinical feasibility of 7T MRI with sequences that are valuable for MS diagnosis at lower field. These sequences need clinically acceptable acquisition times and have to visualize MS pathology with preferably higher sensitivity than 3T MRI. At 7T field strength, T₁ relaxation times are prolonged which necessitates the use of longer repetition times. Also SAR limitations prevent fast imaging. Previous studies (12, 13) dealt with design of FLAIR- and DIR- sequences that overcome these issues, while still providing desired contrasts. By using magnetization preparation (MP) prior to the standard implementation and using sophisticated readout trains, acquisition times and SAR could be kept low, while high spatial resolution in 3D could be obtained. However, using a single transmit coil induced heterogeneity of the B1 field due to standing wave issues. This resulted in a signal intensity losses most noticeably in the temporal lobes. Also the sensitivity of the applied 16 receive-channel head coil limited imaging of the infratentorial part of the brain in subjects with larger head size. Five MS patients and five healthy controls participated in this study and were imaged using 7T 3D T₁- and 2D T₂-weighted sequences as well as the new 3D-MP-FLAIR and 3D-MP-DIR sequences. The entire imaging protocol took 42 minutes. Images were analyzed for artefacts and homogeneity, contrast ratios were measured and lesions were counted and categorized according to their anatomical location: periventricular (PV), deep white matter (DWM), juxtacortical (JC), mixed GM/WM and intracortical lesions (IC).

The appearance of WM and GM was different on 3D-MP-FLAIR and 3D-MP-DIR images compared to lower field strength images. In addition, the high spatial resolution resulted in appearance of many perivascular spaces (PVS) in the WM. While PVS consist mainly of CSF, they were not completely suppressed by inversion recovery on 3D-MP-FLAIR and 3D-MP-DIR images. Areas with high signal intensities could therefore represent both MS lesions and PVS. By following the abnormalities across slices, geometry characteristics revealed clear differences between lesions and PVS. Also, the high signal intensity of the outer layers of the cortex needed attention as it may obscure cortical lesions. And although not artefactual, currently no clear explanation can be given for this phenomenon. Since lesions also appear hyperintense, some subpial lesions may go undetected using the 3D-MP-FLAIR and 3D-MP-DIR sequences. Overall 3D-MP-FLAIR images showed the highest number of WM and GM lesions (592), while T₁- and T2-weighted sequences showed the least (384 and 442, respectively). In between, 3D-MP-DIR showed 558 lesions. A significantly increased sensitivity was found for juxta-cortical lesions on 3D-MP-FLAIR vs. T₂-weighted images and for mixed lesions both 3D-MP-FLAIR and 3D-MP-DIR outperformed T₂-weighted images. These results show that at 7T a clinical MS protocol is feasible and that FLAIR and DIR sequences on a number of locations significantly show more lesions than more conventional T₁- and T₂-weighted sequences.

In chapter 4 a study was performed that compared a clinical multi-contrast protocol on 7T with comparable 3T images for 38 MS patients and 8 healthy controls. A protocol was used that consisted of 3D-T₁- and 2D-T₂-weighted sequences as well as a 3D-FLAIR sequence. At 7T, the previously described 3D-MP-FLAIR sequence was applied. Together, these sequences are commonly used in clinical MS protocols. Subjects underwent MRI examinations on both 3T and 7T MRI scanners within 2 weeks without the use of a contrast agent. Images were analyzed in random
order or lesions in consensus by three observers. Raters were unaware of clinical and paraclinical information. Lesions were counted for the same anatomical locations as for the study described in chapter 3: PV, DWM, JC, Mixed and IC. Again, in this study no deep gray matter or infratentorial lesions were included since the field of view did not include those regions in all subjects. Multilobular abnormalities resulting from possible confluency of lesions were counted as one lesion when no obvious signal change could be observed between them. Special care was given to PVS as not to mistake them for lesions.

This direct comparison showed that the overall number of lesions observed with 3T and 7T does not differ significantly for $T_1$-weighted and FLAIR sequences. However, $T_2$-weighted images showed increased overall detection rates at 7T MRI. Our results would indicate that the clinical impact of 7T MRI in terms of increased sensitivity on earlier diagnosis is low. This trend was already seen for 3T (14, 15) and our results may confirm that sensitivity for WM lesion detection is limited.

However, when results are compared for specific brain regions some remarkable results were found: periventricular lesions were less frequently seen at 7T than at 3T for all sequences. We expect this effect to be partly artificial: image characteristics for 7T show high signal intensity in the centre of the brain. To properly display areas with lower intensity (e.g. temporal lobes), window and level settings may result in saturation of PV and DWM lesions. This then reduces clear separation of lesions resulting in an ‘artificial’ confluence of hyperintense periventricular lesions. These lesions already were more likely to be confluent and this effect may be strengthened by the image characteristics. Regarding our scoring guidelines, multiple lesions were counted as one lesion when no obvious signal changes could be observed. Looking into this possible explanation we compared results from patients with the lowest and highest number of lesions in PV and DWM areas. It was expected that patients with a low number of PV and DWM lesions would show less confluence because of low lesion density. Patients with a high lesion density for PV and DWM areas would then be more prone to ‘artificial’ confluence. It was found that patients with the highest number of lesions showed 22% less lesions on 7T compared to 3T, while no significant difference was observed for the patients with the lowest number of lesions. This might explain lower lesion counts at 7T.

Images from healthy controls also showed a small number of mostly WM lesions that are not considered MS lesions. At 7T MRI more lesions were seen compared to 3T, but in a subject-wise analysis this result did not reach significance. These lesions are presumed to be of vascular origin and are a normal aging phenomenon. In MS patients therefore a number of the observed lesions may also not be caused by MS.

Regarding cortical lesions 7T significantly outperformed 3T MRI for FLAIR and $T_2$-weighted sequences. Cortical lesions are expected to have high clinical relevance since cortical damage differs between disease stage and disease type. They also have substantial influence on clinical symptoms, both physical and cognitive (16, 17). Presence of cortical lesions is currently not included in diagnostic criteria for MS, but it might increase accuracy of the criteria (18–20). This would then largely increase the clinical value of 7T MRI.

Other sequences applied to 7T MRI in MS have also shown that it can be a valuable tool to depict cortical lesions. $T_2^*$-weighted sequences are able to show many cortical lesions that were verified by pathological examination (21, 22). Also other characteristics of MS lesions like their periventricular orientation (23–25) or hypointense rim (26), that would refer to iron depositions can be even
better depicted by ultra-high field MRI (27, 28).

Although in our study several imaging parameters were different for 3T and 7T (e.g. spatial resolution and coil type), we think it would be artificial not to utilize the increased signal strength available from 7T MRI. Also many parameters depend on field strength and matching one would skew others. Therefore in our opinion the applied configuration reflects the potential of ultra-high field MR imaging in a clinically feasible setting.

At lower field DIR sequences showed increased cortical lesion detection compared to FLAIR, $T_1$-weighted and $T_2$-weighted sequences (8, 10, 29). Since the double inversion regime decreases SNR of DIR images, the increased signal strength of 7T MRI was expected to be advantageous. In Chapter 5 we examined this hypothesis. For a cohort of 37 MS patients and 7 healthy controls we compared 7T 3D-MP-DIR, 3D-MP-FLAIR, $T_1$-weighted and $T_2$-weighted images. Lesions were counted into several locations as was performed for the studies described in chapter 3 and 4. In contrast to results obtained at lower field strength, 3D-MP-DIR unexpectedly showed 47% fewer lesions in GM compared to 3D-MP-FLAIR. Also the number of WM lesions was 4.4% lower. However, differences in lesion numbers between patients may confound lesion-wise analysis, together with the non-normal distribution of lesions. A subject-wise analysis that is less sensitive but more reliable in this situation showed that the higher GM lesion detection at 3D-MP-FLAIR was statistically significant. More specifically, this was caused by the considerably inferior detection of mixed GM/WM lesions on 3D-MP-DIR. Compared to the conventional sequences at 7T, the 3D-MP-DIR sequence showed no significant increase in total lesion detection rate, nor in WM neither in GM lesion detection. The lower detection rates observed were unlikely to be caused by lower overall image quality: the sequence was based on the 3D-MP-FLAIR sequence described by Visser et al. (13, 30) that performed well on 7T.

However, a double inversion works as a filter that attenuates tissue with specific relaxation times. In an optimized implementation this attenuation works well for the specified tissues, but it also lowers resulting signal intensity for the remaining tissue types. This might also affect lesions with $T_1$ relaxation times that only slightly differ from normal brain tissue. Furthermore, a relaxation time gradient may be present from the outer boundary of the lesion to the centre. A combination of two in inversion pulses as in DIR may then lower the intensity of the outside of the lesion compared to FLAIR. This would then result in a lower lesion conspicuity on DIR. In addition, the applied scoring guidelines (31) exclude lesions smaller than 3 voxels.

Another issue that might have hampered lesion detection is the abundance of small high contrast vessels on DIR images. These may have distracted raters resulting in lower scores. Finally, the consensus policy of the three raters was quite defensive: focal hyperintensities were only specified as lesions if the raters were beyond any doubt.

In 1.5T and 3T studies (8, 10, 29) a reclassification phenomenon was observed. There, a lower sensitivity for JC lesions was counterbalanced by increased detection of mixed or IC lesions. In our results we could not find this effect. We would presume from our study that the advantages of 7T (increased SNR and spatial resolution) are more beneficial for depiction of GM lesions by conventional sequences such as 3D-MP-FLAIR. The performance of 3D-MP-FLAIR at 7T was shown in chapter 3 and 4 of this thesis. However, developments in hardware and software configurations of 7T scanners may result in even higher signal strengths and increased image homogeneity. Current shortcomings may then be solved and increase the value of DIR for GM lesion sensitivity.
9.2 High field quantitative MRI in MS: T₁ relaxation time of normal appearing white matter

It has been shown that white matter in the brain of MS patients that visually appears normal on qualitative MR images differs from healthy controls when examined using a variety of quantitative MRI techniques (32, 33). Changes in normal appearing white matter (NAWM) have been reported in all MS disease types (Relapsing Remitting, Primary Progressive and Secondary Progressive) and can be found throughout the brain (32). In T₁ relaxation time mapping studies, normalized histograms of NAWM in MS patients typically show lower peak heights (increased dispersion of T₁ values) and increased T₁ peak locations compared to normal WM of controls. This finding also holds for normal appearing grey matter (NAGM) although this difference is more subtle. At higher field strength, e.g. at 7T, the gain in signal to noise ratio (SNR) can be used to enhance spatial resolution and thereby also accuracy. In addition, T₁ relaxation times increase with field strength.

The clinical study in Chapter 6 used a T₁ relaxation time mapping sequence at 7T that acquired high spatial resolution images. The sequence was based on a multi-slice echo planar method by Ordidge et al (34) that was implemented and adapted for 7T. Twenty-nine MS patients and 8 healthy controls participated in this study. Besides a T₁ map also T₂- and T₁-weighted images were acquired. Images were analyzed using three different methods: firstly normalized whole brain histograms were calculated for each subject, showing the general distribution of T₁ relaxation times. The second method looked at regions of interest (ROI) that were drawn in the white matter on a single axial slice just above the lateral ventricles both left and right for all subjects on T₁-weighted images that were registered to the T₁ maps. These ROIs were copied to the T₁ maps to determine mean relaxation times. For the third method however, ROIs were drawn in specific brain areas, i.e. WM, GM, basal ganglia and corpus callosum. For all methods, care was taken not to include lesions. For the third method additional care was taken not to include Virchow Robin spaces (VRS) that were visible on conventional image types at 7T. Results showed that for whole brain histograms in patients, a shift and broadening of the WM peak occurred. This was in accordance with previous studies (33, 35). Also results from the second method using large WM ROIs showed increases in T₁ relaxation times for MS patients compared to healthy controls (33). However, the third method showed no T₁ relaxation time difference for MS patients and healthy controls now that VRS was excluded. Only the head of caudate nucleus and thalamus in MS patients showed a small but statistically significant difference. This would suggest that the influence of (increased) VRS in MS patients is substantial. In our study a mono-exponential fitting algorithm was used to calculate T₁ relaxation times.

Although multiple components are expected in brain tissue, our results suggest that at least two components with different T₁ values with a large influence need to be considered. This effect was then simulated using a two component model that was analyzed using a mono-exponential fit. This showed that an 8.5% increase of VRS in MS patients compared to healthy controls was able to match results obtained in our experiment. Regarding the observed differences for the second analysis method using large ROIs, it was found that the intra-subject coefficient of variation differed statistically significant between MS patients and controls. This would also suggest increased heterogeneity of WM tissue in patients.

Partial volume effects therefore may have affected results obtained at lower field. We then hypothesize that observed differences in relaxation times may therefore reflect altered VRS volumes rather than purely changes in normal appearing white matter itself. In doing so, this study may also show that current T₁ relaxation time mapping is not able to show primary MS induced changes in WM that are reflected by histopathology. On the other hand, the method
may be used as an early indicator of brain atrophy if no differences in $T_1$ relaxation time of pure WM of MS patients can be observed.

### 9.3 Imaging iron concentrations in MS

Iron deposition in brain tissue occurs in the process of normal aging as well as in many neurodegenerative diseases. Elevated iron levels in certain brain regions are also an increasingly recognized finding in MS. The exact mechanism(s) for this phenomenon and its implication in terms of pathophysiology and clinical significance are still largely unknown and debated. Many studies are described that try to reliably visualize and/or measure iron concentrations in the brain. Those methods mostly use $T_2$- and $T_2^*$-weighted sequences. The latter offers more options as it is both sensitive and easier to implement on most systems. It can then be used to obtain $T_2^*$ maps. It also has shown that $R_2^*$ (=1/$T_2^*$) values are linearly related to iron concentrations.

These and other findings are reviewed in chapter 7. Besides relaxation time mapping, also phase maps, susceptibility maps and magnetic field correlation imaging is discussed as well as saturation imaging. After discussing their advantages and disadvantages, existing MRI clinical correlations with brain iron concentration in MS are summarized and future research directions are shown. They address the idea of correlating iron concentrations near lesions and in deep grey matter with disease mediated and disease-modulating effects. Also the expected lower contribution of iron in WM to relaxation times compared to GM should be elucidated. Finally it is expected that quantitative susceptibility mapping at ultra-high field could help to eliminate confounding relaxation effects that may obscure accurate iron quantification.

As reviewed in chapter 7, $T_2^*$ weighted sequences have shown to be sensitive for iron. Besides imaging of endogenous iron concentrations also administrated exogenous iron can be visualized (36–38). Iron oxide based contrast agents have a complementary function to gadolinium contrast agents in MS. In chapter 8 an experiment is described that looked in to the application of a new ultra-small particle of iron-oxide (USPIO) contrast agent. This was performed on Lewis rats that are sensitive for EAE immunization, an established animal model for MS. A previously applied agent, Sinerem, showed contrast enhancement in lesions in both MS and EAE. Due to efficacy reasons this agent is no longer available for application in CNS inflammatory or other pathology. A new agent - P904 - by Guerbet, France with many similar characteristics was examined for comparable performance.

Although animals developed severe EAE symptoms, after administration during disease onset only one animal showed contrast enhancement by P904 on a location that also showed gadolinium enhancement. This contrast was best observed by $T2^*$ weighted imaging and $T2^*$ mapping.

Immunohistochemistry showed (diffuse) inflammation in many locations in the brain, but no iron could be observed. Staining of cervical lymph nodes resulted in clear depiction of iron. Besides clustering also diffuse staining was observed. Although drainage of USPIO from the brain to the cervical lymph nodes may have occurred, this routing is not expected to be exclusive. Therefore only presence of the particles in the bloodstream can be confirmed but not trafficking into the brain parenchyma.

We think effective concentrations may be even higher than applied. Contrast enhancement was found in one animal while using a double dose compared to recommended doses for Sinerem. Creating a high concentration of USPIO in the blood during a prolonged period (using the
increased half time compared to Sinerem) could then result in increased concentrations of USPIO laden macrophages. This effect may be created by a single high dose (e.g. triple or quadruple doses) or repeated administrations in days before imaging. In conclusion, contrast enhancement by P904 in EAE could therefore not be confirmed in this study, and its clinical utility therefore remains an enigma.

9.4 Future Perspectives
The topics described in this thesis have shown that ultra-high field has certain potential for MS. However, not all MS patients may need ultra-high field MRI as studies have shown that high field may not advance MS diagnosis itself, certainly not without adaptation of criteria. And although not without problems that need to be solved, various imaging methods and techniques that are valuable at lower field may increase in value using ultra-high field. Next to its feasibility for clinical application, an increased value can be found for MS research.

Ultra high field MRI may be able to explain findings from lower field as was shown in chapter 6. Increased Virchow Robin spaces in MS patients could reflect focal brain atrophy due to lesion formation or demyelination. The tissue loss effect is normally only observed at lower field MRI when it is visible as global atrophy. On high spatial resolution 3D T1-weighted images the high contrast between WM and perivascular spaces may even result in a qualitative measure to define atrophy.

The increased sensitivity for GM lesions that was observed using a FLAIR sequence may also help to increase correlation between clinical symptoms and radiological findings. As sensitivity for WM lesions may have reached its limit - as might be concluded from chapter 4 - much may be gained by studying GM damage. Not only in the cortex but also visualization of affected deep GM structures may benefit largely from ultra high field MRI. Especially with the development of multi-transmit send and receive coils, sensitivity in this area may increase. 3D T1-weighted sequences that show strong WM/GM contrast could be used for highly accurate atrophy measurements and show disease progression in an early stage.

Ultra high field T2*-weighted images have shown to be able to detect even more lesions in the cortex, but can also be used to characterize lesions and follow development stages. This could help to verify treatment efficacy in an early stage. Finally application of iron based contrast agents have shown complementary aspects of MS pathology in clinical studies. Since these contrast agents are able to induce T2* weighted contrast and this contrast type is also highly feasible on ultra high field, this good be a valuable combination.
9.5 Conclusions

The studies described in this thesis have shown that

- High field MRI will aid in understanding pathogenesis and heterogeneity of MS and its role in individual patient care, regarding diagnosis and treatment following, will increase with new developments in this field.

- 3D-MP-FLAIR and 3D-MP-DIR sequences allow high quality $T_2$-weighted MR imaging in MS at 7T, improving (cortical) lesion detection.

- Using a clinical multi-contrast MR imaging protocol for MS at 7T, significantly increased lesion detection was observed in cortical GM but not in WM compared to 3T MRI.

- While at 1.5T and 3T, the 3D DIR sequence showed increased sensitivity for cortical MS lesions, this effect was not observed at 7T. Here the highest number of lesions was found using a 3D-MP-FLAIR sequence, for WM, GM and total lesion counts.

- The increased $T_1$ relaxation times of normal appearing white matter in MS patients observed at lower field could not be confirmed when Virchow Robin spaces were excluded using 7T MRI. The previously observed differences may therefore be caused by partial volume effects from increased Virchow Robin spaces.

- The expected association of iron accumulations in deep grey matter structures with disease severity and brain atrophy could be assessed by implementation of high field MR sequences that can reliably detect and quantify iron concentrations.
References

1. F. Barkhof et al., Comparison of MRI criteria at first presentation to predict conversion to clinically definite multiple sclerosis., Brain : a journal of neurology 120 ( Pt 1), 2059-69 (1997).


