Chapter 15

English Summary
In patients with multiple sclerosis (MS), inflammatory processes in the central nervous system (CNS) cause demyelination, which ultimately results into neurodegeneration. The disease onset is generally at an age between 20 and 40 years, and therefore MS is the most common neurodegenerative disease among young adults.\textsuperscript{2,3} Several therapies exist for inhibition of the inflammation, but the damage induced by demyelination or neurodegeneration is not repaired. Therefore new treatment strategies for MS aim to restore myelination, which should enhance axonal survival and restore axonal function. However, an accurate biomarker to evaluate treatment efficacy to facilitate the development of these new treatment strategies is currently missing. Imaging techniques like MRI and PET might be suitable for the assessment of remyelination treatment efficacy.

To explore the potential of myelin imaging, we first assessed (\textit{Chapter 2}) how current myelin imaging methods work on a biophysical level, describing how the methods differ from each other with respect to their biological target, how scans can be acquired and what the validation status of these imaging methods is. In general, 5 main types of myelin imaging can be distinguished: imaging of myelin water (e.g. myelin water imaging, MWI), imaging of macromolecules (e.g. ultrashort echo time, UTE; positron emission tomography, PET), indirect imaging of the macromolecules (e.g. magnetization transfer, MT; inhomogeneous MT, ihMT), mapping the magnetizability (e.g. quantitative susceptibility mapping, QSM), and mapping the effects of myelin on water diffusion (e.g. diffusion tensor imaging, DTI). From a biophysical perspective, MWI, PET, ihMT, and QSM seem to be the most promising, as they target aspects of myelin that are unique for myelin. However, MWI already exists since the 1980s and has not yet shown to be able to accurately capture de- and remyelination. In contrast, PET has been shown to be able to visualize and quantify de- and remyelination in animal studies,\textsuperscript{62,63} which has been replicated in a pilot study in humans.\textsuperscript{226} As both ihMT and QSM are relatively new techniques, no studies have yet assessed their efficacy in quantification of de- and remyelination. While PET seems to be a promising myelin imaging method, its invasive nature (e.g. due to arterial blood sampling) hampers routine clinical application. As such, MRI is so far the main technique used for myelin imaging, due to its availability and non-invasive nature.
15.1. Magnetic resonance imaging

15.1.1. Diffusion MRI
With diffusion MRI, several indices regarding axonal integrity and various other diffusion derived parameters can be estimated. As myelin is the protective sheath covering axons, assessing axonal integrity is especially of interest for determining which kind of treatment strategy should be followed: remyelination or both neuronal regeneration and remyelination. The relevance of diffusion parameters for multiple sclerosis or lesion characterization is still not completely clear. Therefore, we investigated the value of various axonal integrity metrics and other diffusion derived parameters for MS lesion characterization (Chapter 3). We found that in addition to the conventional diffusion MRI parameters, especially the fibre density (FD), fibre bundle cross section (FC), and fibre density and bundle cross section (FDC) enhance the insight in MS lesion and peri-lesion characteristics. For instance, in lesions FD, FC, and FDC were affected compared to normal appearing white matter (NAWM), whereas the FD of peri-lesions was not significantly different from NAWM, but FC and FDC were. This reduction in axonal integrity in peri-lesions, despite unaffected fibre density estimates, suggests an effect of Wallerian degeneration, which describes the phenomenon that local axonal injury causes distal degeneration.

15.1.2. Lesion filling
Due to the non-invasiveness of MRI and the high anatomical structural information that can be obtained with this technique, MRI is applied for routine diagnostic purposes in MS. Typically, T₁w, T₂w, and T₂w-FLAIR MRI sequences are acquired, yielding high anatomical information on MS lesions. Aside from the high anatomical information, indices of cortical quantity (e.g. volume, thickness, fraction) can be extracted from T₁w images. As the cortex entails the majority of brain regions important for human functioning, information regarding cortical density per brain region could be used to determine which brain regions are connected with each other, and this is called network analysis. However, accurate assessment of the cortical quantity indices can be affected by the presence of MS lesions, which can be compensated for by application of lesion filling. With lesion filling the voxels of a lesion are replaced by voxels with the intensities of neighbouring voxels. This is supposed to enable more reliable estimates of the cortical quantity indices, and therefore enhance the performance of network analysis. In our study (Chapter 4), however, we found that lesion filling can introduce artifacts, which may affect tissue segmentation, resulting in erroneous allocation of GM and WM. While we found limited alterations due to MS pathogenesis using network analysis without application of lesion filling, the application of lesion filling...
significantly enhanced the number of findings. We observed that lesion filling reduced the heterogeneity among subjects, which would most likely explain the greater number of significant findings. Yet, we recommend cautious implementation of lesion filling and a thorough assessment of potential artefacts, as lesion filling can have a significant impact on both tissue segmentation and the calculation of network parameters, which could lead to erroneous findings and incorrect interpretations of the results.

15.1.3. Advanced MRI analysis and MS subtypes
While MRI is used routinely for supporting MS diagnosis, in some cases the clinician might have difficulties determining the MS type based on radiological and clinical symptoms. However, MR images might contain more “hidden” information that could aid in disease characterization than is currently used. A method that could extract such information is scaled subprofile modelling using principal component analysis (SSM/PCA), which results in the identification of typical disease patterns. Therefore, we investigated whether SSM/PCA could be employed as a tool for aiding in the differential diagnosis between MS subtypes (Chapter 5). Hence, SSM/PCA was performed on \( T_1 \), \( T_2 \), \( T_2^* \), FLAIR, MT ratio (MTR), quantitative MT (qMT), ihMT ratio (ihMTR), and quantitative ihMT (qihMT) for assessing whether SSM/PCA could segregate relapse remitting MS (RRMS) from progressive MS (PMS). We found that SSM/PCA on \( T_1 \) images resulted in the highest sensitivity (93%) and NPV (88%) for segregating RRMS and PMS, but a lower PPV (71%) and specificity (53%), whereas SSM/PCA on qihMT images resulted in the highest specificity (87%) and PPV (88%), with a relatively low NPV (75%) and sensitivity (67%). As such, SSM/PCA might aid in the differential diagnosis between MS subtypes, as the high specificity of qihMT and high sensitivity of \( T_1 \) could complement each other.

15.1.4. Validation statuses of current myelin MRI techniques
As already indicated in Chapter 2, several methods exists for myelin imaging with MRI. Therefore, we assessed the validation statuses of these methods (Chapter 6). We observed that the existing methods overall have only a moderate correspondence with myelin histopathology \( R^2=0.54 \). In animals, both QSM \( R^2=0.85 \) and ihMT \( R^2=0.94 \) perform very well. In contrast, QSM performs poorly on human brain \( R^2=0.07 \), whereas the correspondence of ihMT with histopathology has not yet been assessed in humans. The performance of QSM suggests that there is a need for further standardization and optimization of the employed post-processing algorithms, as the good results obtained in animal studies seem to be difficult to reproduce in humans. In human tissue, the highest correspondence between histopathology and myelin imaging was observed for MWF \( R^2=0.68 \). Using human samples, even the best MRI method only corresponds
moderately with myelin histopathology. This illustrates the need for the development of a more accurate method for assessing de- and remyelination processes in MS.

15.2. Positron emission tomography

15.2.1. Current applications of PET imaging in MS

We searched the literature to assess the utility of another imaging modality, PET, for MS disease characterization (Chapter 7). We found that the primary PET imaging methods employed in MS patients are the assessments of glucose metabolism and neuro-inflammation. Almost all tracers employed for PET imaging of neuro-inflammation bind to the 18-kD translocator protein (TSPO). As TSPO is upregulated in activated innate inflammatory cells, PET imaging of TSPO expression cannot distinguish between cell types and between pro- and anti-inflammatory activation. Nonetheless, increased uptake of the TSPO targeting tracers was found within lesions and also within NAWM of MS patients, as compared to healthy controls, suggesting that neuro-inflammation is more widespread in MS brains and not restricted to active lesions. Glucose metabolism as measured with $^{[18F]}$FDG PET was used in studies in MS patients to depict neuronal integrity, as neurons are assumed to be the primary consumers of glucose within the brain. However, inflammatory cells are also large consumers of glucose and might confound the assumptions regarding the use of $^{[18F]}$FDG as a marker for neuronal integrity. Nonetheless, alterations in glucose metabolism in MS have been related to mobility, fatigue, and cognition. Aside from glucose metabolism and neuro-inflammation, some studies with PET for assessing myelin density have also been performed. These myelin PET studies used repurposed amyloid-beta tracers, due to the high affinity of amyloid-beta tracers to WM. These studies found in general a decrease in myelin density within MS lesions, and thus indicate the potential of myelin PET.

15.2.2. First in human $^{[11C]}$MeDAS PET: myelin imaging in brain and spinal cord

Animal studies have shown that $^{[11C]}$MeDAS PET may be a more accurate and reliable method for myelin imaging than PET with the repurposed amyloid tracers. Therefore, we aimed to evaluate $^{[11C]}$MeDAS PET in a first-in-human study. First the quantification of myelin in brain with $^{[11C]}$MeDAS PET was assessed (Chapter 8), followed by the evaluation of the imaging properties of the tracer in the spinal cord (Chapter 9). The optimal quantification model for analysis of the brain data was the 2T3k model, with which we found a higher uptake in the WM as compared to the GM. Subsequently, the cerebral lesions were categorized according to a radiological classification system based
on the presumed myelin density. With the 2T3k model we also determined the lowest tracer uptake in the lesion types with the lowest myelin density and the highest tracer uptake in lesion types with highest myelin density. This suggests that with $^{11}$CMeDAS PET we indeed can measure myelin density within the brain. From the literature, it is known that the caudal spinal cord has a lower amount of myelin than the rostral spinal cord. Such a gradient in tracer uptake was also observed with $^{11}$CMeDAS PET. $^{11}$CMeDAS PET had a high negative predictive value and specificity, but a moderate positive predictive value and sensitivity for detection of MS lesions in the spinal cord, observed with MRI. This indicates that $^{11}$CMeDAS PET is better in depicting absence than the presence of pathology within the spinal cord. Taken together, these studies show that $^{11}$CMeDAS PET could be a promising tool for myelin imaging of both the brain and the spinal cord.

15.2.3. Non-invasive PET quantification methods
To improve the applicability of $^{11}$CMeDAS PET, we investigated the possibility of applying a non-invasive PET quantification method (Chapter 10). Because the distribution of MS lesions is a very heterogeneous across subjects and myelin density in normal appearing white matter may be affected in MS patients, it is not possible to denominate a suitable reference region for $^{11}$CMeDAS. Therefore, alternatives for the arterial input function (AIF) for pharmacokinetic modelling, other than reference tissue methods, have to be employed in order to make $^{11}$CMeDAS PET less invasive. A literature survey showed that some studies on the use an image derived input function (IDIF) or simultaneous estimation of the input function (SIME) as less invasive alternatives for kinetic modelling with an AIF have shown positive results. However, the correspondence of these alternative methods with kinetic modelling with an AIF have shown positive results. However, the correspondence of these alternative methods with kinetic modelling with an AIF have shown positive results. However, the correspondence of these alternative methods with kinetic modelling with an AIF have shown positive results. However, the correspondence of these alternative methods with kinetic modelling with an AIF have shown positive results. However, the correspondence of these alternative methods with kinetic modelling with an AIF have shown positive results. However, the correspondence of these alternative methods with kinetic modelling with an AIF have shown positive results. However, the correspondence of these alternative methods with kinetic modelling with an AIF have shown positive results. However, the correspondence of these alternative methods with kinetic modelling with an AIF have shown positive results. However, the correspondence of these alternative methods with kinetic modelling with an AIF have shown positive results. However, the correspondence of these alternative methods with kinetic modelling with an AIF have shown positive results.
15.2.4. Imaging myelin changes over time

\[^{11}\text{C}]\text{MeDAS}\) uptake seems to correspond with the expected distribution of myelin density in the brain and spinal cords. However, it is especially important that \[^{11}\text{C}]\text{MeDAS}\) PET can also detect the subtle temporal changes in myelin density over time to enable the assessment of new treatments that promote remyelination. Therefore, we performed a pilot study to assess whether \[^{11}\text{C}]\text{MeDAS}\) PET could detect subtle alterations in myelin density over time (Chapter 12). While no decrease of tracer uptake in whole brain WM was observed, we found decreases of tracer uptake in cerebral MS lesions after an interval of approximately 1.5 year. Our findings from static \[^{11}\text{C}]\text{MeDAS}\) PET scans of the spinal cord indicate that more accurate PET quantification (such as compartment modelling using an AIF) might be needed for capturing changes in myelin density in this region over time.

15.3. Conclusion

In conclusion, the novel MRI methodologies in development for MS (e.g. lesion filling, SSM/PCA) require some more optimization prior to their clinical applicability. Yet, techniques like diffusion MRI may, in a research setting, already help to enhance our understanding of microstructural changes in MS. For myelin imaging, PET is a promising technique, but still requires further evaluation and optimization. Especially, a reduction of the invasiveness of PET, shorter scan duration, and reduction of labour intensity, could further enhance the clinical applicability of myelin PET.