Chapter 13

General discussion
The objective of this thesis was to assess multiple sclerosis (MS) pathology with imaging, first with advanced magnetic resonance imaging (MRI) techniques and subsequently with positron emission tomography (PET). To this end, several approaches for myelin imaging have been carefully assessed, in particular the use of several diffusion MRI techniques and advanced statistical approaches on MRI. These techniques can help enhancing our understanding of pathogenesis of MS and aid in differential diagnosis. Subsequently, the role of PET imaging in MS is discussed, a proof-of-concept study on myelin imaging in the brain and spinal cord with $^{11}$CMeDAS PET was performed, and the use of invasive and non-invasive PET quantification methods was assessed. Together, this thesis provides a multifocal view on imaging of the biological mechanisms involved in MS pathology, which will be further discussed in the following sections.

### 13.1. Vascular integrity

Perturbations of the blood-brain barrier (BBB) seem to be instrumental in onset of MS pathogenesis. However, it is not yet known what occurs first: BBB perturbations, or inflammatory infiltrations. Insight in the integrity of the microvasculature might enable elucidation of this enigma. A better insight in the cerebral microvasculature not only could enable a better characterization of the MS pathogenesis, but also enhance the understanding of processes that are involved in tracer delivery (see Chapter 1, section 1.4.2.). Microvasculature integrity can be assessed with perfusion MRI, with which the contrast bolus is followed over time. To evaluate whether BBB perturbations occur prior to disease onset, a MRI study on cerebrovascular integrity should be performed in healthy controls (HC) and patients with radiologically isolated syndrome (RIS), clinically isolated syndrome (CIS), and relapse remitting MS (RRMS). In combination with assessment of inflammation markers, like TSPO PET, such a study might elucidate which biological processes are going on during the early pathogenesis. Such a study using perfusion MRI for vascular integrity and TSPO PET for inflammation, should follow HC, RIS, CIS, and RRMS longitudinally for approximately 1 year with monthly follow-up. From a practical point of view, however, such a study would be challenging, since the changes of capturing the early onset of MS are small and therefore a large study population will be required.
13.2. Myelin imaging

Whether BBB perturbations lead to an influx of inflammatory cells, which subsequently cause demyelination, or demyelinating inflammatory cells cause BBB perturbations remains unclear. Yet, both hypotheses have the demyelination of axons in common. So far, an accurate validated method for visualisation and quantification of demyelination processes in patients is not available yet. In this thesis, we evaluated the validation status of various myelin imaging methods (see Chapters 2 and 6). For a proper evaluation of these myelin imaging methods, there is actually a need for a validated histological myelin quantification method, as histological quantification is considered the gold standard and most close to the actual ground truth. However, we observed that there is no consensus yet about the gold standard for histological assessment of myelin density. Therefore, the effects of various fixative agents, variations in post-mortem interval, and tissue handling should be carefully assessed for their effects on histological quantification. Studies suggested that fixation would alter the physical characteristics of macromolecules, which might influence the histological quantification of myelin density, but the effects of fixation on histological myelin quantification have remained largely unexplored. Likewise, the macromolecular alterations caused by fixation could also impact the accuracy of myelin MRI methods for the same reasons, as the physical alterations of macromolecules might influence the hydrogen content and therefore influence the MRI signal. Alterations of the physical characteristics of macromolecules could of course also have a major influence on the correspondence of myelin MRI methods with myelin histopathology. Since no studies have yet been performed that carefully assess the effects of several fixative agents on histopathological quantification or MRI of myelin density, the potential effects of fixation remain speculative. This is also reflecting the difficulties that arose during our study. For instance, the choice for the development of $[^{11}\text{C}]$MeDAS as a PET tracer for clinical use, was based on animal studies showing a high correspondence between $[^{11}\text{C}]$MeDAS PET and myelin histopathology. However, because a thorough evaluation of the various myelin histological methods is missing, this could have resulted in biased results and potentially result in incorrect conclusions. In addition, a thorough histopathological evaluation of myelin density across brain regions in both animals and humans is missing. Currently, we can only discriminate the known differences in myelin density between grey matter (GM), white matter (WM), and corpus callosum. More detailed knowledge about the histopathologically assessed myelin density across brain regions in both animals and humans could aid future studies on the accuracy of myelin imaging methods. A histopathological dataset of myelin densities should preferably contain data from mice, rats, and humans in healthy conditions, and across several
disease states or disease models (e.g. MS, Alzheimer’s disease (AD), Parkinson’s disease (PD), traumatic brain injury (TBI)) to have a thorough understanding of myelin density and distribution in the healthy brains and the effects of various pathological states on myelin density and distribution. Nonetheless, $^{11}$C MeDAS PET was able to visualise remyelination and demyelination processes in animals, and $^{11}$C MeDAS uptake in the human brain has been shown to correspond with physiological expectations, e.g. low uptake in GM, high in WM, and highest in corpus callosum. $^{11}$C MeDAS uptake also corresponded well with the physiological distribution of myelin within the spinal cord. $^{11}$C MeDAS uptake was also reduced beyond MS lesions, which is most likely due to spinal cord atrophy or due to diffuse MS pathology.

13.2.1. Myelin imaging with MRI

The principles of myelin imaging using MRI or PET are explained and discussed in Chapter 2. MRI is a completely non-invasive method and development of new sequences that could be used to visualize or quantify myelin can relatively easy be integrated in clinical practice. One of the best characterized methods for imaging in myelin is assessment of the myelin water fraction (MWF), which already exists since the 1980s. This method has been used for studying brain development, but has also been used to assess myelin damage caused by several neurological diseases. Nonetheless, MWF has not yet been shown to be able to capture de- and remyelination processes in MS lesions. This might be due to the very small contribution of myelin water to the overall MRI signal, making it difficult to disentangle de- and remyelination processes from noise. To overcome this problem, new MRI techniques have recently been developed that have shown promising results, in particular quantitative susceptibility mapping (QSM) and inhomogeneous magnetization transfer (ihMT). These techniques exploit the intrinsic magnetic characteristics of the molecular content of biological tissues and the isolation of the dipolar relaxation time, respectively, and therefore might be more sensitive than MWF in assessing de- and remyelination processes in MS lesions. However, due to the novelty of these techniques, no studies that assessed de- and remyelination processes in MS lesions with either QSM or ihMT have been reported yet.

13.2.2. Validation status of myelin MRI

In Chapter 6, we assessed the validation status of various myelin MRI methods by determining the correspondence of outcome parameters of the MRI methods with myelin histopathology and by assessing the reproducibility of the MRI methods. The average correspondence of the various MRI methods with myelin histopathology was only moderate ($R^2=0.54$), even for the best method (MWF, $R^2=0.68$), whereas the reproducibility was good to excellent for all methods, except for MTR. As a higher
correspondence with myelin is required to quantify de- and remyelination processes, current MRI methods would need further optimization to improve the correspondence with myelin histopathology. Single-exponential $T_1$ and single-exponential $T_2$ are most likely not specific enough, as they are unable to identify the origin of the signal due to partial volume effects, and therefore the signal can originate from various biological sources. MWF suffers from a low signal-to-noise ratio (SNR). Enhancing the SNR by increasing the number of echo's used for signal decomposition would improve MWF estimates, but also increases scan times significantly beyond current clinically feasible times. Therefore, MWF would benefit most from techniques that enable faster acquisition, like compressed sensing. MTR and qMT suffer from the low offset frequency often employed in a clinical setting, which is 1.5 kHz, whereas an optimal saturation is only achieved with an offset frequency of 7 – 10 kHz.\textsuperscript{176} This larger offset frequency requires hardware optimizations, but this is in general not an option for traditional clinical MRI scanners. In contrast, for ihMTR and qihMT these scanners actually employ the optimal offset frequency, but these methods require an exact determination of the myelin $T_{1D}$. Once the myelin $T_{1D}$ is known, the ihMT sensitization can be focussed on myelin by adjusting the power, offset frequency, and timing parameters of the pulse sequence.\textsuperscript{124,178} For QSM already great potential has been shown in preclinical studies, but improving the post-processing algorithms of QSM might enhance the correspondence with myelin histopathology in humans.\textsuperscript{263} Until these MRI methods have been further optimized for myelin imaging, their usage for myelin imaging will be limited, and their applications remain for mono-exponential $T_1$ improving dynamic contrast enhanced (DCE) perfusion MRI analysis, for mono-exponential $T_2$ improving dynamic susceptibility contrast (DSC) perfusion MRI analysis, and for QSM the generation of an iron map ($R_2^*$) or detection of the central vein sign, which is an important characteristic in MS pathology and is depicted as a small hypointense line or dot within an MS lesion.\textsuperscript{618}

\textbf{13.2.3. Myelin PET}

PET imaging studies aiming to capture de- and remyelination processes in MS lesions have shown promising results. However, the radiation exposure due to administration of a radiotracer, the laborious procedures, the long scan durations used so far, and the relatively high costs may hinder the implementation of PET imaging of myelin in clinical practice. It seems plausible that the technique will initially be primarily used in clinical research on disease characterisation and the evaluation of the efficacy of remyelination therapies. Optimizations of current PET imaging strategies might reduce the invasiveness of PET and may open the way to myelin PET being employed in clinical routine. In \textit{Chapter 8}, the feasibility of myelin imaging with $^{11}$CMeDAS PET was
investigated. In preclinical studies, $[^{11}\text{C}]$MeDAS PET has been shown to be more accurate than the repurposed amyloid tracer $[^{11}\text{C}]$PiB in visualisation and quantification of de- and remyelination processes. However, $[^{11}\text{C}]$MeDAS had not yet been investigated in humans. In a proof-of-concept study, we showed that $[^{11}\text{C}]$MeDAS PET can distinguish brain regions with known differences in myelin density, and more importantly can quantify differences in myelin density in different types of MS lesions in the brain. Furthermore, in Chapter 9, we showed that $[^{11}\text{C}]$MeDAS uptake in the spinal cord corresponds with the known physiological WM gradient and that there is a decreased myelin density in the spinal cord of MS patients as compared to HC. Therefore, $[^{11}\text{C}]$MeDAS PET might be a promising tool for efficacy evaluation of remyelination therapies. In Chapter 12, we assessed whether changes in myelin density can be measured over time with $[^{11}\text{C}]$MeDAS PET. Here we found that over a time span of approximately 1.5 years, the whole brain WM $[^{11}\text{C}]$MeDAS uptake remained constant, whereas the $[^{11}\text{C}]$MeDAS uptake in cerebral MS lesions decreased.

With the aim to enhance the clinical utility of $[^{11}\text{C}]$MeDAS PET, the performance of several PET quantification methods that do not require arterial blood sampling were assessed. Current literature suggests that an image-derived input function (IDIF) or simultaneous estimation of the input function (SIME) seem to be the most promising methods to substitute arterial blood sampling to generate an input function for pharmacokinetic modelling (Chapter 10). We therefore investigated the feasibility to use an IDIF for $[^{11}\text{C}]$MeDAS PET quantification (Chapter 11), in order to reduce the invasiveness of $[^{11}\text{C}]$MeDAS PET. The applied IDIF method does not seem to be good enough for absolute quantification of $[^{11}\text{C}]$MeDAS. Especially the lack of adequate accuracy hinders the application of an IDIF method in longitudinal studies determining the efficacy of remyelination therapies.

### 13.3. Axonal integrity

As myelin is wrapped around axons, assessment of axonal integrity could be of interest to determine the severity of the damage caused by inflammation in MS. Using diffusion MRI, several parameters related to axonal integrity can be generated. However, the diffusion MRI parameters have not yet been compared to histopathological data of axonal integrity in MS. As long as these comparisons are lacking, it remains a matter of debate which diffusion MRI measurement is most accurate and thus most representative for axonal density, if any. Assessment of the known differences in axonal density between GM, WM, and corpus callosum is so far the only method that is used to evaluate the accuracy of measurements for axonal integrity. Although diffusion
MRI is supposed to generate values for axonal integrity, these values are only indirect estimates that assume that anisotropic diffusion is related to axonal density. However, biological aspects, such as an influx of inflammatory cells, might affect diffusion and therefore the correlation between diffusion and axonal integrity. The most frequently used PET tracer to assess neuronal integrity is $^{[18F]}$FDG, as neurons are assumed to be the major glucose consumers in the brain. However, this could lead to confounding results, as neuronal $^{[18F]}$FDG uptake is also influenced by brain activity. In addition, inflammatory cells are also major glucose consumers, and it has been proven that there is an increased concentration of inflammatory cells in the brain of MS patients, irrespective of disease activity.\textsuperscript{503–505} Therefore, a more accurate parameter for assessing neuronal or axonal integrity is necessary, which is less sensitive to brain activity, inflammatory activity, and diffusivity artefacts due to influx of inflammatory cells. Such a parameter could possibly be obtained with $^{[11C]}$flumazenil or $^{[11C]}$UCB-J PET, which are used to quantify post- and presynaptic neurons. Another option could be imaging of tau as a measure of neurodegeneration.\textsuperscript{619}

In Chapter 3, we investigated several methods to analyse diffusion MRI for MS lesion characterization. One of the most interesting parameters that can be obtained with diffusion MRI is fractional anisotropy (FA), which is a proxy for the density of fibre bundles. In the brain, these fibre bundles mainly consist of axons. Various methods to obtain information regarding axonal integrity with diffusion MRI are available. Diffusion tensor imaging (DTI) is the most widely applied method to obtain such information. In this thesis, we also applied diffusion kurtosis imaging (DKI) and fixel-based analysis (FBA). We observed that the outcome parameters for axonal integrity extracted from DTI, DKI, and FBA were highly correlated and thus provide similar estimates for axonal integrity. Among the fixel-based parameters extracted from diffusion MRI data, especially the fibre bundle cross-section (FC) and fibre density (FD) provided interesting additive information, as a reduction in FC, but not in FD, in peri-lesions compared to normal appearing white matter (NAWM) was detected. This observation might be suggestive for Wallerian degeneration, as Wallerian degeneration describes a phenomenon, in which focal damage (e.g. MS lesion) leads to antro- and retrograde degeneration.

13.4. Technical aspects and advanced analysis

13.4.1. Current status of PET imaging in MS

The literature regarding the available PET imaging methods that have been employed to investigate MS pathology has been evaluated in Chapter 7. PET imaging methods
can be categorized according to the main biological processes that is visualized: inflammation, myelin integrity, axonal integrity. So far, the main determinant for treatment efficacy was a reduction in the inflammation in MS lesions. Increased inflammation could be observed in NAWM, grey matter (GM), and MS lesions with PET tracers targeting the 18-kD translocator protein (TSPO), which is upregulated in activated microglia, macrophages, and astrocytes. Although the TSPO receptor seem to be a suitable target for imaging of inflammation, the clinical applications remain limited. A drawback of the TSPO receptor is that it is not completely cell type specific. New tracers that are more specific for certain inflammatory cells are currently in development, but are yet far away from clinical application. For assessing neuronal integrity in MS, [18F]FDG is the main tracer used. [18F]FDG is a tracer for imaging glucose metabolism, and as neurons are the primary consumers of glucose within the brain, [18F]FDG might be used as a marker for neuronal integrity. However, [18F]FDG is also upregulated when brain activity is increased and as a result of inflammation, which might be a major confounder, especially in MS. Nonetheless, several studies indicated that reductions in [18F]FDG uptake are related to fatigue, which might be due to increased muscular demands to compensate for a decreased CNS efficiency. With repurposed amyloid tracers for the visualisation of myelin, a decrease of myelin density was observed in MS lesions, while over the results on myelin reductions in NAWM of MS patients as compared to WM of HC were inconsistent. Nonetheless, studies using repurposed amyloid tracers for myelin visualization have also shown that PET can be used to visualise de- and remyelination processes. However, these studies did not use the radiological classification system regarding differences myelin densities across lesions and employed reference tissue methods for PET quantification, which could confound their findings as it seems likely that MS pathology alters the microvascular integrity and thereby tracer delivery. While PET imaging can help to increase our understanding of MS pathology, its main applicability would likely be efficacy evaluation of remyelination therapies.

### 13.4.2. Tracer development

Another option to enhance the clinical utility of myelin PET is the use of a fluorinated MeDAS analogue, such as [18F]TAFDAS, instead of [11C]MeDAS. Due to the relative short half-life time (~20 min) of [11C]MeDAS, the tracer has to be made on site, which requires the need of a cyclotron and a radiochemistry laboratory. Only a limited number of hospitals, mainly academic centers, have these facilities and this limits the clinical use of [11C]MeDAS. [18F]TAFDAS has a half-life of ~2 hours, which enables transport of the tracer to nearby hospitals. For this thesis, we chose to explore the efficacy of [11C]MeDAS for myelin imaging, due to previous preclinical experiences and
the unavailability of $^{[18F]}$TAFDAS at the onset of the studies described in this thesis. The use of a 1-(2-[$^{18F}$fluoroethyl]-1H-1,2,3-triazol-4-yl)methyl analogue instead of the $^{[11C]}$methyl compound could alter the binding affinity and the lipophilicity of MeDAS and thereby its efficacy in myelin visualisation, by altering the amount of non-specific binding, tracer kinetics and tracer distribution. Future research would need to investigate whether $^{[18F]}$TAFDAS shows similar properties as $^{[11C]}$MeDAS as a tracer for myelin imaging. Another advantage of the application of an $^{18F}$-labeled tracer is the lower positron energy, which reduces the travel distance of positrons, and thus enhances the resolution. This could be especially important for high resolution cameras, preclinical studies, or the quantification and detection of small lesions. As amyloid PET tracers have shown high uptake in WM, which is primarily due to the tracers’ affinity to bind to any beta sheet structure (e.g. amyloid, MBP), they have been recently repurposed for myelin imaging. Although the amyloid tracers are already available clinically to support diagnosis of Alzheimer’s disease, animal studies have shown that $^{[11C]}$MeDAS is more accurate than the amyloid tracer $^{[11C]}$PiB for imaging myelin.62

13.4.3. Tracer validation

During the development of a new PET tracer, the PET tracer is first evaluated in exploratory preclinical studies to identify the usefulness of the PET tracer (phase 1). After sufficient studies have confirmed the usefulness of the PET tracer, a first in human trial should be initiated (phase 2). In such a first-in-human trial, often first a dosimetry study is performed, followed by a face validity study to determine whether the PET scan measures the aspects it should measure, which is often confirmed with a blocking study or tracer displacement study. Then the sensitivity of the PET tracer to biological alterations, the reproducibility, and methodological simplifications should be investigated. This should be followed by larger studies with a longitudinal set-up (phase 3) to determine the capacity of the new PET tracer to detect early disease, determine the effects of covariates, compare existing methods that measure the same biological phenomenon (e.g. different PET tracers or MRI techniques). Subsequently, the diagnostic accuracy (phase 4) should be assessed by determining the sensitivity, specificity, NPV, and PPV, as well as the potential clinical benefits for early detection, diagnostics, treatment evaluation, and disease monitoring. Eventually, the cost-effectiveness of the scan should be determined. This illustrates that despite our findings of $^{[11C]}$MeDAS PET as a promising method for myelin quantification, there are still several steps that have to be performed prior completion of the validation of the tracer.
13.4.4. Advanced analysis of PET and MRI data

13.4.4.1. The effect of lesion filling on graph theoretical network analysis in MS

All imaging techniques addressed in this thesis could be used to investigate MS disease course with for example graph theoretical network analysis (Chapter 4), or to improve differential diagnosis between MS subtypes (Chapter 5). In graph theory, brain regions that are somehow related to each other can be identified. In this way, important brain regions involved in MS pathology might be identified that might not be directly affected by the disease, but are essential for functionality as connecting (or central) brain regions. In Chapter 4, we investigated graph theory for T₁w MRI and we explored the effect of lesion filling. Lesion filling is a strategy which is commonly applied in MS to substitute the distorted MRI signal at the lesion with values of voxels surrounding the lesion, which might enhance the accuracy of more advanced network calculations. However, there is no general agreement yet whether lesion filling should be applied or not, as lesion filling removes the effects of pathology from the image. Our study indicated that the application of lesion filling significantly increased the number of findings detected with graph theoretical network analysis, but we also found that lesion filling is more prone to errors with a higher the lesion load, so care should be taken when applying lesion filling on scans with a high lesion load. Furthermore, we observed that the cerebellum is affected across MS types, and the supplementary motor area is primarily affected in progressive MS patients. This is also corresponding with existing literature and clinical symptomology, as cerebellum is responsible for coordination of subtle movements, which is already affected in RRMS, and the supplementary motor area is important for more grotesque movements, which is primarily affected in PMS.²⁴⁷,⁴⁵⁹ Aside from the application of lesion filling, 2 other options could be considered as well: whether undirected graphs (two-way communication between brain regions) or directed graphs (one-way communication between brain regions) should be applied, and whether the analysis should be performed on a group level or a subject level. We performed the analysis using undirected graphs on a group level, as the application of undirected graphs enables information exchange between two brain regions in both directions and network analysis on group level is more often performed, making our findings more translatable to other studies. However, to more thoroughly assess the effects of lesion filling on graph theoretical network analysis, one should also investigate the use of directed graphs with a group level analysis, and investigate graph theoretical network analysis on a subject level. Moreover, we only investigated T₁w MRI, whereas DTI derived metrics like FA are also commonly used for graph theoretical network analysis. DKI derived FA and FBA derived FD could also be used for graph theoretical network analysis, and the discrepancies in the results
obtained from network analyses based on these different estimates for axonal density might be of interest to investigate, as it directly reflects the connections between brain regions. Graph theoretical network analysis could also be applied on other structural imaging methods, like myelin MRI or myelin PET, because myelin density can also be used as a proxy for brain connectivity (high amount of myelin, high connectivity; low amount of myelin, low connectivity). In other words, as we demonstrated a significant effect of lesion filling on graph theoretical network analysis for $T_1$w MRI (Chapter 4), there is still a lot to investigate and we only briefly touched upon the possibilities.

13.4.4.2. SSM/PCA for aiding in differential diagnosis in MS

Whereas graph theoretical network analysis aims to identify important brain regions in MS pathogenesis, SSM/PCA aimed to improve the differential diagnosis between MS types. Sometimes it is difficult for a clinician to exactly determine which type of MS a patient has. This could be especially important for treatment selection, as different treatment strategies are applied to the MS types. We used the conventional MRI methods $T_1$w, $T_2$w, and $T_2$w-FLAIR, and some myelin sensitive methods like magnetization transfer (MT) ratio (MTR), quantitative MT (qMT), inhomogeneous MTR (ihMTR), and quantitative inhomogeneous MT (qihMT) to investigate whether SSM/PCA could aid in the differential diagnosis of MS types. With SSM/PCA, a small set of uncorrelated components is assumed to represent the differences between two conditions. Thus in case of imaging, the components consist of voxels that are highly correlated, but are uncorrelated with voxels of other components, and thus all components together (thus all voxels) explain the image. Therefore PCA is often used as a data reduction method, for which the data variance is described by the principal components. In our study (Chapter 5), we investigated whether with SSM/PCA we could perform differential diagnosis of MS phenotypes. In other words, we investigated whether we could identify principal components that were significantly different between MS phenotypes and therefore might assist in performing differential diagnosis. When SSM/PCA was used to differentiate MS patients from HC, $T_2$w-FLAIR, qMT, and qihMT performed best (AUC of 0.85, 0.86, and 0.81, respectively). For differentiation between MS types using SSM/PCA, $T_1$w and qihMT showed the most promising results (AUC of 0.69 and 0.70, respectively). An AUC value of 1 means a perfect differentiation between groups, however, this is only theoretically possible. In practise, an AUC value higher than 0.8 is considered to be a good discriminative value. Therefore, a clinician might prefer a higher discriminative value, than we obtained for $T_1$w and qihMT (0.69 and 0.70 AUC, respectively). A potential approach to achieve this is differential diagnosis could be to take the interactions between different MRI methods into account. A method like this would combine the features that have been identified as specific to a MS subtype from
one MRI method with another, and therefore the imaging methods might complement each other in differential diagnosis of MS. As such, the interactions could improve the discriminative value between MS types, and thus lead to higher AUC values. Future studies could investigate whether the interaction of imaging modalities might lead to a more robust differential diagnosis.

13.4.4.3. Application of independent component analysis on PET data
Another commonly applied method is the independent component analysis (ICA). Whereas PCA focusses on maximizing the variance of data points, ICA aims to separate mixed signals into independent signals (or components). Thus, the components of PCA could be derived from multiple sources that explain the highest amount of variance, whereas the components of ICA are derived from unique signal sources. For PET imaging, this means that ICA could be used for IDIF generation, as the tissue TACs are very different from blood TACs. We did not apply this method for IDIF generation, but it remains an interesting approach for future studies.

13.4.4.4. Generation of parametric images during reconstruction of PET data
Direct kinetic modelling for obtaining parametric images during the reconstruction is another aspect that could benefit PET imaging. Current approaches use the blood pool of the heart or aorta for determination of the input curve. The difficulties regarding tracer metabolism are circumvented by applying only tracers without plasma metabolites (e.g. \([^{18}\text{F}]\text{FDG}\)). Nonetheless, the application of a priori information of population based metabolites, metabolites assessed with venous blood samples, or metabolites assessed with arterial blood samples might enable direct quantification during reconstruction for metabolizing tracers as well. A clinically more invasive approach would be the integration of blood samples of the individual subject within the reconstruction. For \([^{18}\text{F}]\text{FDG}\), parametric images generated with Patlak graphical analysis can already be performed and studies investigating PET quantification for reversible tracers using spectral analysis are ongoing. Such automated quantification tools could have a major impact on the interpretation of PET images, due to the direct generation of parametric images (\(V_t, K_i, BP_{ND}\)). So far, clinicians use primarily visual inspection or occasionally SUV calculations for the semi-quantitative assessment of PET images, but the SUV is sensitive to blood related aspects, e.g. blood flow, tracer clearance, etc. (see Chapter 1, section 1.4.). Parametric images are devoid of these aspects, which may increase the clinicians’ detection rate and interpretation of subtle abnormalities. Possible disadvantages are that the automatic quantification methods require dynamic scans, which usually take 60 minutes, instead of the 5 – 20 minutes that are required to acquire static scans for SUV measurements. This means increased
costs per scan, due to prolonged occupation of the PET scanner and personnel, and also lower patient throughput, which limits the accessibility of PET. Therefore, a clinician should carefully weigh the benefits of parametric scanning against reduced patient throughput and higher costs. As such, a clinician might request these parametric scans when SUV images may not suffice for diagnostic purposes, for example for treatment monitoring and accurate assessment disease progression during longitudinal follow-up. The use of SUV is less suitable for these purposes due to the effects that the disease or treatment can have on blood hemodynamics, tissue perfusion and clearance rates, which can all have an effect on tracer delivery. While these developments are truly promising, their added clinical value still needs to be established. When proven useful, full clinical implementation will take some time, due to training of clinicians to read these new and improved PET images, upgrade of current hardware that supports the generation of these parametric images during image reconstruction, and availability of the methods for all PET cameras, which would enabling comparisons across sites in multicentre studies.

13.4.4.5. Non-invasive PET quantification

Another approach to increase the clinical utility of PET, would be to develop an accurate alternative for the continuous arterial blood sampling (Chapter 10 and 11), which would be generally applicable. As shown in this thesis (Chapter 10), several methods already exist, but the core limitation is that each method behaves differently per tracer and per pathology. For instance the traditional reference tissue methods are often not suitable for MS patients due to the random diffuse pathology, but might work very well for patients with for instance Alzheimer’s disease. This means that per tracer, per pathology, and per intervention the feasibility of non-invasive methods to substitute the arterial blood sampling has to be determined. Another way would be to develop an artificial intelligence method that could generate a blood curve accurately enough to substitute the arterial blood sampling. A better understanding of the effects of renal function on tracer clearance and liver function on tracer metabolism, might further enhance the accuracy of these non-invasive methods, whether a simple calibration factor or advanced statistical approaches like structural equation modelling would be required remains to be investigated. This would aid the quantitative benefits of PET across sites by easily enabling the generation of parametric images $(V_r, K_r, BP_{ND})$, with limited hardware requirements. In addition, a partial volume correction might further improve the quantification, and therefore enable detection of more subtle changes in myelin density.
13.4.5. Hardware
A comparison of myelin PET tracers with myelin MRI methods using a PET-MRI scanner, enables direct comparison of myelin imaging methods. However, most hospitals with a PET scanner have PET-CT scanners and not PET-MRI scanners and therefore comparing several myelin MRI methods with a myelin PET-CT scan is a very useful, practical, and clinically relevant approach. However, the use of PET-CT instead of PET-MRI also has some drawbacks. For instance, the brain orientation of the patient between the scanners is different, which means that the voxels contain different tissue constituents among the scanners. This could partly be dealt with by re-orientating the scans in the same space, solving the problem for the largest part. The biggest advantage of using a PET-MRI in this context is the co-registration of spinal cord images. The brain is within the skull, in which little movement is possible, whereas the spinal cord is highly flexible and small alterations in the position of the patient can have a large effect on the spinal cord orientation. This could be compensated for using deformable co-registration methods. However, these methods are a research field on their own and primarily decrease the accuracy of anatomical information of the image, because the image is deformed (e.g. MRI) to match another (e.g. CT). A PET-MRI that simultaneously acquires both PET and MRI images of the spinal cord is devoid of these complexities, and enables highly accurate localisation of the spinal cord on the PET images. This is especially advantageous as the spinal cord is not visible on a CT scan. Another advantage of the PET-MRI is the simultaneous acquisition and thereby reducing significantly the patient burden by reducing the total scan time of the PET and MRI acquisition separately. Though, the benefit in terms of partial volume effects for comparing myelin PET with myelin MRI with a PET-MRI scanner compared to a PET-CT and MRI scanner separately, should be minimal regarding assessment of myelin quantification methods. However, because a PET-MRI scanner is devoid a CT, it uses MRI generated data (currently still sub-optimal) for attenuation correction or is simply devoid of an attenuation correction, which suggest that quantification with a PET-CT scanner would be more accurate. Instead of applying the various myelin imaging methods separately, a more advanced approach could be to synergize the most important features of each method and generate a single hybrid myelin image that is more accurate and better quantifiable than the separate images. Artificial intelligence could maybe aid to extract the myelin derived components from both PET and MRI for creating one myelin image.

13.4.5.1. Total body PET-CT
The use of a camera with a larger field-of-view might further enhance the accuracy and robustness of myelin PET. This would for instance enable the imaging of the brain together with the heart and aorta, which subsequently can be used to extract an IDIF.
A population based metabolite curve or elucidation of the effects of renal function on tracer secretion and liver function on tracer metabolism might further enhance the accuracy of the IDIF. Aside from the advantage of improved non-invasive PET quantification, the larger field-of-view provided by the total body PET camera also increases the sensitivity of the camera by about 40-fold for whole body scans and about 2-5 fold for a single organ.624 This increase in sensitivity is primarily due to the detection of more lines-of-response, as only 3-5% isotopically emitted lines-of-response is captured by the detector rings of traditional PET scanners. Nonetheless, the larger FOV also comes with a price, as a larger amount of tissue has to be traversed prior photon detection by the crystals, which increases scatter of the photons and thereby inaccuracies in the localization of annihilation. Aside from that, the larger FOV of the total body PET camera also significantly increases the price of the camera, as it requires substantially larger number of crystals for signal detection. Another disadvantage is the restricted accessibility for arterial sampling, as the wrist will be within the camera. This might have practical implications, reducing the possibilities for trouble shooting when blood clutters the sampling line. Another drawback is the larger dispersion effect of the bolus measured in blood, as a longer sampling line is required for transporting the blood from the wrist to the online detection system to measure blood activity.

13.4.5.2. MRI
MRI is a primary diagnostic tool for which robust quantitative analysis is challenging, as for every scan the settings for signal to noise ratio and contrast to noise ratio need to be optimized to obtain the best diagnostic image. These optimal settings vary with room temperature and per subject. This indicates that the settings are different every time, and may even vary during a single scanning session. Nonetheless, a consistent field-of-view large enough to fit the brain of each subject, consequent use of the same TR, TE, TI, off-resonance frequencies, number of echoes, and voxel sizes might result in more robust quantification with MRI. However, consistent settings also depict a dilemma, as normally MRI scans are generated under optimal settings for diagnostic purposes. Clinicians are reluctant to deviate from such a protocol, as standardized settings may reduce the diagnostic value of MRI. Therefore, this dilemma can be solved if separate protocols are used: a research protocol with fixed settings and a diagnostic protocol with settings optimized for each individual scan. PET imaging was suffering from a similar problem, but with the introduction of the EARL criteria for harmonisation, this was compensated for. Nowadays, clinicians often receive two different reconstructions of the PET scans, one according to the EARL criteria and one with the most optimal settings for the PET-CT scanner. For MRI such an approach is not possible, as changes in TR, TE, TI during acquisition alter the raw data, which cannot be changed afterwards.
However, some of these hurdles might be overcome with techniques like Synthetic MR or MR-STAT, which both aim to generate the most common diagnostic MR images and quantitative MR maps from a single sequence. Using mathematical approximations, the most common diagnostic contrasts are estimated. Nonetheless, this approach is only available for T₁, T₂, T₁', and T₂ imaging due to their static nature, while these methods will not be suitable for more advanced imaging techniques, like myelin MRI, diffusion MRI, or perfusion MRI, as more dynamic processes are involved in these methods requiring fast acquisition protocols.

Nowadays MRI cameras with a high magnetic field strength are available (e.g. 7T). A higher magnetic field strength increases image resolution of MRI, but it also shortens the relaxation times. This actually means that the imaging of UTE and MWF becomes more difficult, as the relaxation times of the macromolecules and respectively the water fractions become more difficult to measure. Moreover, a higher field strength significantly increases the price of the MRI camera and thus the costs per scan.

13.5. Conclusion

The goal of this thesis was to assess imaging methods for MS pathology, using both advanced MRI techniques and PET. As MRI is readily available in the clinic and commonly used to support MS diagnosis, we evaluated the application of sophisticated analysis methods for MRI, like application of advanced diffusion MRI and graph theoretical analysis on T₁w MRI for a better disease characterization. Several diffusion MRI parameters have been investigated, of which especially FBA-FC and FBA-FD might be interesting as they enable detection of Wallerian degeneration. We have also shown the effect of lesion filling on graph theoretical network analysis. This enabled us to show that the cerebellum is affected across all MS types, whereas the supplementary motor area is only affected in progressive MS. Furthermore, we illustrated the feasibility of SSM/PCA to discriminate between MS phenotypes, which could be useful for treatment selection. Aside from that, the feasibility of myelin imaging with [¹¹C]MeDAS PET was evaluated. We have shown that [¹¹C]MeDAS PET could detect the expected differences in myelin density both in the spinal cord in the brain, and in MS lesions. Therefore, [¹¹C] MeDAS PET shows potential as a myelin imaging tool, but needs to be further evaluated before it may be used for efficacy evaluation of remyelination therapies.

In future studies, it could be interesting to investigate the associations between perfusion MRI, diffusion MRI, and myelin imaging. Important questions that could be answered by combining these techniques could be for instance, whether there is an increased MD, decreased FA, and decreased myelin density when there is a decreased
cerebral blood flow? MD provides information regarding microstructural integrity, as a reduced cellular density increases diffusivity, whereas FA provides information regarding axonal integrity. Future research might elucidate these aspects, leading to better lesion characterization, and therefore potentially facilitate personalised therapeutic strategies.

Another aspect that remains to be investigated, is the relation between the various imaging outcome parameters and clinical scores, like cognition, psychological evaluation, fatigue, and physical disability. This might elucidate how well various imaging methods can characterize clinical disability and clinical deterioration. Whereas all the aforementioned imaging techniques are important to better understand the biological phenomena related to MS pathogenesis, clinical associations are important for clinical utility and applicability for e.g. treatment decision making or monitoring. Because what would be the worth of these advanced biomarkers, if nothing associates with clinical disability, clinical improvement, or treatment response? In the end, we do all this research with the aim to help the patient. But if nothing depicts the clinical aspects properly, why spend all the time and effort on improving the data-analysis or clinical utility?

A logical next step after the validation of $[^{11}\text{C}]\text{MeDAS PET}$ is its application in the evaluation of remyelination therapies. This can be done by stimulating oligodendrocyte recruitment, proliferation and migration, and/or optimization of the environmental aspects, like the matrix metalloproteinase (MMP) and extra-cellular matrix (ECM) constituents. So far, multiple potential therapeutical agents have been investigated for their efficacy in promoting remyelination. However, none of these agents has yet been shown efficacy in clinical phase III trials. This could be due to 2 factors: (1) the molecular events involved in remyelination are too complex to allow promotion of remyelination with a single substance and (2) there is no surrogate marker to assess remyelination in clinical trials. As the main aim of this thesis was to establish an accurate, precise, and quantitative myelin imaging marker, we hope to have shown with the work described in this thesis that at least for the latter factor promising progress has been made.

Once remyelination therapy might be possible, neuroregeneration would be the next challenge, and seems to be even more complex. Myelin is composed of multiple proteins, of which some have shown to inhibit neuronal outgrowth, and thus limit neuroregeneration. More specifically, both myelin oligodendrocyte glycoprotein (MOG) and myelin-associated glycoprotein (MAG) have these neuroregeneration inhibitive effects, not only when they are within the myelin layers, but to an even larger extent in soluble state, after they have been released due to myelin damage. Removal of soluble MOG and MAG and/or blocking of MOG and MAG binding might restore neuroregeneration. However, much work is still needed before this may become clinical reality.