The work presented in this thesis substantiates the relevance of brain volume measurement in MS by: addressing technical and sample size issues relevant to automated brain volume measurement, studying the relationship of brain atrophy with clinical disability and a potential CSF biomarker for axonal damage, and by attempting to find determinants of brain atrophy rate. The following chapter will discuss these findings further in view of methodological aspects of brain volume measurement, biomarkers, lesions, NABT, clinical disability and clinical trials.

METHODOLOGICAL ASPECTS OF BRAIN VOLUME MEASUREMENT

The desired properties of a brain volume measurement technique are: sensitivity to brain atrophy, reproducibility, accuracy, precision and robustness to image quality. To meet these standards, several brain volume measurement techniques have been developed over the years that use automated tissue type segmentation for brain volume estimation. T1 weighted pre-contrast images are frequently used for these brain volume measurement techniques, as they have a clear contrast between brain parenchyma and CSF facilitating reliable tissue type segmentation. When putting these techniques into practice, several methodological issues may arise; including (un)availability of T1 weighted pre-contrast images and errors that may occur within the automated protocol. These issues will be discussed in the following paragraphs.

In our experience, many valuable datasets exist that do not include the validated T1 weighted pre-contrast images. To exploit the full potential of such datasets with regard to brain atrophy, other image types will have to be used. Chapter 1.1 compares SIENAX and SIENA results obtained using ‘alternative’ T1 post-contrast images, T2 weighted images, and composite, ‘pseudo T1’ images, with results obtained using the validated T1 weighted pre-contrast images. The findings suggest that T1 post-contrast images are the best alternative option to T1 pre-contrast images, followed by pseudoT1 and T2 images respectively. This study shows that the use of alternative image types is feasible for SIENA(X) analyses, and lays the foundation for a more flexible use of SIENA(X).

It is important to realise that we used relative agreement to compare brain volume measurement results between image types, because absolute NBV and PBVC values can be expected to differ between image types. Therefore, the image types should be used consistently across the dataset.

SIENAX and SIENA analyses using T1 weighted pre-contrast images are known to be robust, accurate and reasonably insensitive to slice
thickness. The same properties should, to a large extent, apply to brain volume analysis using T1 post-contrast images, because they are acquired using the same pulse sequences and have a similar distinction between brain parenchyma and CSF. In view of this and the results of our study, T1 post-contrast images should be the first image of choice as alternative to T1 pre-contrast.

T2 and PD images have a fundamentally different contrast between CSF and brain parenchyma and their image characteristics may vary between scanners and different scan parameter-settings. Although our study results suggest that T2 and ‘pseudo T1’ image types are possible alternatives for brain volume analyses using SIENA(X), caution should be taken until further validation data is available for these image types.

Ideally, brain volume measurement using SIENAX and SIENA is fully automated. However, errors may arise when using the automated protocol, such as the inclusion of non-brain tissue. These errors can be expected to introduce unwanted variability (noise) to brain volume measurement results, and could thereby diminish sensitivity to detect group differences. The usual cause of these errors is inadequate automated brain extraction, which is the first step in the SIENA(X) method. In principle, the settings of the automated Brain Extraction Tool (BET) used by SIENA(X) can be altered to perform more conservative or lenient brain extraction. However, finding the optimal BET settings for each individual patient or subsets of patients is time-consuming and may introduce bias between subject groups. Instead, a frequently used strategy to correct for errors in brain extraction is to perform an initial brain extraction using BET, followed by the manual removal of remaining non-brain tissue. Furthermore, in view of the increasing need for large datasets that can only be acquired from multi-centre studies and the labour intensive nature of manual editing, it would be advantageous to divide the workload associated with this manual editing between centres. With this background, we studied to what extent the manual editing scheme reduces unwanted variability and introduces inter-centre (and inter-observer) variability to SIENAX and SIENA results.

Chapter 2.2 shows that agreement of SIENAX and SIENA with manual editing is substantial between centres (and thus observers) and confirms the assumption that manual editing reduces subject variability, thereby increasing statistical power, of the percentage brain volume change measured by SIENA. For SIENAX, manual editing reduced overestimation of normalised brain volume, but did not reduce variability in brain volume results. These findings formally support the view that manual editing is a sensible strategy to correct for errors in automated brain
extraction using SIENA(X) and that analysis results from multiple centres can be pooled. Despite the advantage of a decrease in unwanted variability, manual editing does introduce some inter-observer variability and time consuming manual labour, the latter being especially relevant to high resolution 3D MR images with large numbers of slices. To solve these issues, ongoing developments hopefully will provide an optimised brain extraction tool that will be able to automatically process images from different scanners and patients in a uniform and robust way.

**BRAIN VOLUME RELATED TO BIOLOGICAL MARKERS**

Different pathophysiological mechanisms, including inflammation, demyelination, axonal damage and repair mechanisms, are involved in the MS disease process\(^3\). A better understanding of these mechanisms could aid in prognosis, treatment development and monitoring of treatment effects. Post-mortem histopathological studies provide important clues to these mechanisms, but do not give insight into their in vivo dynamics. Substances in the body fluids of MS patients, referred to as biomarkers, could be useful tools for in vivo monitoring of ongoing processes in the central nervous system of MS patients\(^4\). Substances associated with the axon cytoskeleton (neurofilaments, actin, tubulin and tau proteins), axonal membranes (24S-hydroxycholesterol, apolipoprotein E), axonal cytoplasm (NAA, neurospecific enolase, 14-3-3 protein) and axonal injury (APP protein) have been proposed and studied as potential biomarkers for axonal degeneration\(^5\). Besides being potential monitoring tools, these ‘axonal’ biomarkers could provide further support for the hypothesis that brain atrophy reflects neurodegenerative changes. Chapter 3.1 describes an exploratory study relating NAA in cerebrospinal fluid, as a potential CSF marker for neuro-degeneration, to clinical and MRI markers of disease progression. The results of this cross-sectional study show that a lower NAA concentration in CSF is related to a later stage of relapse onset MS, a higher degree of clinical disability, higher lesion load and lower brain volume. The relationship between lower brain volume and lower NAA in CSF suggest that brain atrophy is at least partially reflective neuro-axonal degeneration. In further support of this, a previous study has found a relationship between brain atrophy and higher levels of antibodies directed against neurofilament light chains (anti-NFL) in the CSF, suggesting that brain atrophy is related to the presence of cytoskeletal proteins in CSF\(^6\). Another study found that reduced CSF levels of growth associated protein 43 (GAP43), a protein produced by neurons to promote axonal regeneration and synaptic plas-
ticity, is related to lower brain volume. This relationship could simply be explained by a reduction of the number of axons in the brain. Alternatively, reduced GAP43 expression in the CNS could be reflective of inadequate neuronal repair mechanisms which, in turn, could lead to a higher propensity to decline in brain volume in some patients.

Although these results look promising, further insight into the biological dynamics of axonal biomarkers should be gained and larger, longitudinally followed patient groups should be studied to confirm these findings. Additionally, these studies should also include biomarkers not related to axonal degeneration to assess the specificity and robustness of the relationship of ‘axonal’ biomarkers with brain atrophy.

BRAIN VOLUME RELATED TO LESIONS AND NORMAL APPEARING BRAIN TISSUE

In chapter 4.1 we have shown that lower brain volume and, to a lesser extent, higher T2 lesion volume at baseline partly explain the subsequent rate of brain atrophy in patients with recently diagnosed MS in a regular hospital setting. These findings suggest that lesions have a partial relationship with subsequent development of brain atrophy. Destructive effects associated with inflammatory lesions include demyelination, gliosis and neurodegeneration. These lesion-related changes may all contribute to the development of brain atrophy through local and remote volume effects due to, for instance, wallerian degeneration. In support of this, post-mortem studies have shown that regional lesion load is related to atrophy in the corresponding projection area in the corpus callosum. Furthermore, other MRI based studies have found similar relationships between brain atrophy and T2 hyper intense, T1 hypo intense lesion and gadolinium enhancing lesion volume. Hence, destructive lesion effects can lead to measurable brain tissue loss.

However, in our study, baseline brain and lesion volume could only explain a small fraction of variance of subsequent brain atrophy rates. In addition, not all studies are able to detect a relationship between lesion load and atrophy development. These findings suggest only a weak relationship between lesions and brain atrophy and imply that other factors that go undetected by conventional MRI measures, may play a more prominent role in brain atrophy development.

Histopathological and quantitative MRI studies provide evidence of compromised tissue integrity and axonal degeneration in NABT outside of lesions. These abnormalities probably result from destructive processes ongoing in the NABT and are likely to contribute to brain tissue loss. Further studies are needed to elucidate the relationship between
tissue integrity in NABT measured by quantitative MR and brain atrophy development.

BRAIN VOLUME RELATED TO CLINICAL DISABILITY

The usefulness of a monitoring tool for MS is determined by its relationship to clinical disability. Conventional lesion quantification measures show only poor correlations with clinical disability, which sparked interest in brain volume measurement as a possible monitoring tool. Neuro-axonal degeneration is recognised as an important feature of MS pathology and probably represents the destructive end-stage of the MS disease process. It is considered to be the underlying cause of persisting disability and is likely to result in measurable brain volume loss. Brain volume measurement can therefore be expected to have a better relationship to persisting disability than focal disease activity measured by lesion quantification.

In chapter 4.2 we studied the added value of MR measures, including brain lesions, spinal cord abnormalities and brain volume measurements, over clinical parameters in predicting short term clinical disease progression in a group of MS patients with a relatively short disease course. A higher rate of brain atrophy proved to be the strongest predictor of clinical disease progression. This study shows that MRI parameters have added value over clinical parameters in predicting clinical disease progression and that brain atrophy rate is more closely related to clinical disease progression than the conventional MR markers of focal disease activity in an early stage of MS.

Other studies have shown that significantly greater atrophy can be observed in patients with disease progression over patients without disease progression in RR, SP and PPMS\textsuperscript{14,23–25} and that brain atrophy is the most important predictor of clinical disability status at long-term follow-up in cohort of patients with a considerable disease duration before study inclusion\textsuperscript{13}. Our results add to these findings by showing that the relationship between brain atrophy and subsequent disability progression can already be observed at an early stage of the disease in a relatively unselected cohort of patients with a mild degree of disability.

Thus, brain atrophy has a relationship with clinical disability development at short- and long-term follow-up. However, available studies do not tell us how long-term clinical disability can be predicted from the earliest stages of the disease. Because of the variable rate of disability progression between patients and the overall view that immuno-modulatory treatment should be initiated as early as possible, this would provide important information on whether or not treatment should be
initiated. With this in mind, identification of factors that predispose to the
development of brain atrophy early in the disease course, may give im-
portant clues to possible clinical prognosticators at long-term follow-
up.
In chapter 4.1 we have shown that lower brain volume and, to a lesser ex-
tent, higher lesion volume at baseline partially explain the subsequent
rate of brain atrophy in patients with recently diagnosed MS in a regu-
lar hospital setting. In the future, these baseline variables could hope-
fully be used as prognostic tool for individual patients early in the disease
course. Further long-term follow-up studies on other patient groups, star-
ting in the earliest stages of the disease, using clinical disability as out-
come measures are needed to confirm the prognostic value of these
variables.
The relationship between lower brain volume at baseline and the subse-
quent higher rate of brain atrophy, could mean that some patients have
a higher propensity to decline in brain volume. A greater understanding
of the pathological mechanisms underlying brain atrophy, might help to
identify patients that are more likely to develop brain atrophy, for exam-
ple using genetic screening.
The question of whether the neurodegenerative changes quantified by
brain atrophy measurement are directly responsible for specific clinical
deterioration cannot be answered by studying the relationship between
overall brain atrophy and overall clinical disability. We hypothesized that,
if neurodegenerative changes are directly responsible for clinical disa-
bility, decline in specific aspects of neurological function should be re-
lated to atrophy of the associated brain regions. In the study described in
chapter 4.3, we used a novel technique to perform voxel-wise statistics
on brain edge displacement maps provided by SIENA to study the rela-
tionship between regional brain atrophy development and specific as-
psects of clinical disability. The results show that decline in ambulatory
function is related to atrophy development of the ventricles and brain-
stem, whereas decline in neurologically more complex tasks for coordi-
nated hand function are related to atrophy development of both central
and peripherally located brain structures. These findings suggest that
worsening on specific aspects of clinical disability is directly related to
the atrophy development in the associated brain regions, implying that
brain volume measurement gives a direct reflection of clinically rele-
vant pathology.
This study only focused on regional brain atrophy development and cli-
nical disability, whereas both disability and brain atrophy can be affec-
ted by lesion activity. Future studies, using methodologies specifically
designed to analyse regional lesion load and regional brain atrophy are
needed to elucidate these relationships.
The analyses presented in this chapter could be improved in several ways. The tasks used to measure clinical disability in this study rely on the integrity of a range of neurological systems, which gives only a rough perspective on the relationship between clinical dysfunction and atrophy of associated brain regions. In addition, spatial resolution was reduced due to dilation and smoothing effects which are necessary to correct for brain geometry differences between patients. Future studies might aim to identify more specific relationships between local brain atrophy and clinical dysfunction by including tasks testing specific neurological systems and by optimising spatial resolution in order to provide more anatomical detail.

BRAIN ATROPHY MEASUREMENT AS OUTCOME MEASURE IN CLINICAL TRIALS

Brain volume measurement is becoming increasingly important in clinical trials, especially as treatment development is increasingly focused on prevention of neuro-axonal degeneration and neuro-axonal repair mechanisms. Treatment effects on brain atrophy development have been observed in clinical trials on interferons, glatiramer acetate and natalizumab. Although these findings need to be interpreted with caution, they suggest that immunomodulatory treatments appear to slow, but not stop, progression of brain atrophy and thereby neuro-axonal degeneration. As discussed previously, brain atrophy is clinically meaningful, appears to be reflective of neuro-axonal degeneration and can be accurately and robustly quantified by SIENA(X).

It is important for clinical trials that use brain atrophy as a marker of disease progression to have sufficient power to detect treatment effects. Sample size calculations are therefore needed to ensure that an adequate number of patients is included and to identify the best method to use in future studies. Previous sample size estimates of four different methods for longitudinal brain volume measurement (Brain volume difference, Brain boundary shift Integral (BBSI), SIENA and Ventricular Enlargement) suggest that these methods are feasible for detection of treatment effects in clinical trials on RRMS. Relative sample sizes were lowest for SIENA and only slightly higher for BBSI. This study was based on serial MRI scans obtained from a single scanner and including only RRMS patients.

Chapter 2.3 reports sample size calculations for two different longitudinal brain volume measurement methods using serial MRI scans obtained from a large group of placebo treated SPMS patients from different...
The results show that SIENA and Central Cerebral Volume (CCV) are both feasible longitudinal brain volume measures for detection of treatment effects in clinical trials. Required sample sizes were smaller for SIENA compared to CCV, suggesting that the former measure is the best method to use for detection of treatment effects using longitudinal brain volume analysis.

This study adds to the previous study by providing sample size estimates for patients in a different stage of MS, possibly having different dynamics with regard to brain atrophy rates, using MR scans obtained in a multi-centre setting. Compared to current clinical trials, sample sizes to detect treatment effects would be greatly reduced if longitudinal brain volume measurement was used as sole outcome measure. However, brain volume measurement is currently regarded to be a secondary outcome measure to be used in combination with other clinical and radiological outcome measures. In light of this, both sample size studies suggest that SIENA should provide sufficient power to detect treatment effects with the usual inclusion numbers and follow-up durations needed to detect clinical and lesion effects in clinical trials.

The relationship we found between baseline brain and lesion volume with subsequent atrophy rates in chapter 4.1 may have implications for the interpretation of studies using brain atrophy rate as outcome measure. For example, the ability to detect treatment effects could be reduced when a large number of placebo treated patients have relatively high brain volume at baseline compared to the treated patient group. These placebo treated patients are expected to have a low rate of brain atrophy development, thereby reducing the ability to detect treatment induced differences in brain atrophy rate between trial arms. Analysis of brain atrophy rate in clinical trials could take our findings into account by, for instance, controlling for baseline brain volume in the eventual statistical analyses or at treatment randomisation.

As discussed previously, destructive lesion effects lead to measurable brain tissue loss. Another way in which lesions can lead to brain volume changes is by inflammation related oedema. Resolution of oedema is proposed to be the cause of increased atrophy rates seen at the initiation of interferon and natalizumab treatment and after corticosteroid treatment. This pseudo-atrophy effect is an important nuisance when attempting to quantify actual tissue loss. Future studies will hopefully provide adequate measures to correct for this effect.
FUTURE PERSPECTIVES

The results presented in this thesis provide further support that brain volume measurement is a valuable addition to the arsenal of MRI monitoring tools in MS, by showing that it has clinical relevance, is likely to represent neuro-axonal degeneration, represents clinically relevant pathology not quantified by conventional lesion measures, and is able to detect significant treatment effects within a reasonable sample size. In addition, our results suggest that brain atrophy development is mainly driven by pathology ongoing in the NABT outside of lesions. Lastly, the results from this thesis provide solutions to a number of technical problems that could be encountered when using SIENA(X) as brain volume measurement tool. The results from this thesis and other studies pave the way for future research, which will be discussed in the following paragraphs.

In this thesis, several technical issues on brain volume analyses using SIENA(X) were addressed. Firstly, although manual editing is a sensible strategy to correct for errors in brain volume analysis, this approach does introduce inter-observer variability and time consuming manual labour, the latter being especially relevant for high resolution 3D MR images with a large number of slices. To solve this, a new automated BET version has been developed that is very likely to make manual editing inappropriate in the very near future. Secondly, several image types seem to be feasible alternatives to T1-weighted pre-contrast images in performing brain volume analysis. Before these alternative image types can be used confidently, additional validation studies are necessary.

Although brain atrophy measurements are being used in clinical trials and although a number of trial related topics have been discussed in this thesis, there are still a number of issues that need to be clarified regarding sample size and confounding effects. Further sample size calculations are needed for PPMS and multi-scanner studies in RRMS. Most importantly, as disease modifying treatment is currently available for RRMS, future clinical trials will probably move towards study designs that test the benefit of new treatments against existing treatments. This is likely to increase required sample sizes and therefore additional sample size calculations should be obtained for patients using existing disease modifying treatment.

Pseudo-atrophy effects due to inflammation related changes in brain water content are an important nuisance for reliable measurement of tissue loss. Longitudinal studies using frequent MRI and MR measures of brain water content are needed to further elucidate this pseudo-atrophy phenomenon. One possible way to correct for this effect in clinical
trials is to include an additional MRI scan after treatment initiation. Frequent MRI studies could provide an adequate timing scheme for post-treatment-initiation scans.

Brain atrophy is thought to be at least partly representative of neuro-axonal degeneration. However, other destructive changes, including effects of de- and remyelination and gliotic effects, are also likely to contribute to brain volume changes and should be taken into account when interpreting brain atrophy data. To what extent each of these destructive changes contribute to brain volume changes is unknown and should be an important goal for future studies using advanced MR techniques and biological markers.

What mechanisms underlie brain tissue destruction in MS is not well understood. Recent pathology data suggest that inflammation and neurodegeneration occur in parallel and are partly interrelated, involving both immune and non-immune pathological processes\(^\text{36,37}\). This is obviously an important field for current and future research in close collaboration between neuro-pathologists, neuro-immunologists, molecular biologists, geneticists, neuro-radiologists and neurologists. Better understanding of these mechanisms will hopefully aid in the identification of patients that are more sensitive to destructive consequences of MS and thereby more likely to develop progressive brain atrophy and progressive clinical disability.

Processes in focal white matter lesions were initially thought of as the main contributor to destructive pathology seen in MS. Combined evidence from MRI and histopathological studies from the last decade, however, suggest that processes in the NABT contribute considerably, if not even more, to destructive brain tissue changes than focal inflammation. More studies on NABT abnormalities are needed, elucidating their underlying pathological mechanisms, destructive consequences and relative contribution to brain atrophy. The latter goal will hopefully be achieved with the use of advanced MRI and post-processing techniques, like MTR, T1 mapping, diffusion weighted imaging and MR spectroscopy, in relation to brain atrophy measures. As brain atrophy is likely to be a resultant effect of destructive processes, these studies should, apart from concurrent relationships, also focus on how these quantitative MRI measures predict the subsequent rate of brain atrophy.

The studies presented in this thesis focussed mainly on whole brain volume, representing the net resultant effect of ongoing MS pathology. In recent years, gray matter pathology has been recognised as an important feature of MS pathology\(^\text{38}\), and could prove to be very relevant to disability progression and cognitive decline. Recent technical advances have provided methods to reliably study atrophy of gray matter and white matter separately. Using these techniques, a number of studies
have shown that cortical thickness is decreased in MS patients compared to controls and is related to progression of disability.\textsuperscript{39-41} Future studies and technical developments will hopefully provide more insight into the balance between GM and WM atrophy and their relative contributions to clinical disability.

Besides the distinction between GM and WM, specific brain structures should also be investigated in more detail. Different regions of the brain could be differentially affected and may therefore prove to be more sensitive markers of ongoing MS pathology than overall brain volume measures. For instance, because of its highly reciprocal connections with cortical regions throughout the brain and its well-defined anatomical boundaries, atrophy of the thalamus could be a sensitive and reproducible marker of pathology in widespread areas of the brain. Neuronal loss and decreased volume of the thalamus have been found in post-mortem and MRI based studies\textsuperscript{42,43}. 3D high resolution MRI images and recently developed post-processing techniques\textsuperscript{44,49} may shed further light on atrophy of specific brain structures.

This thesis focused mainly on RRMS patients, whereas brain atrophy measurement could be especially relevant to progressive forms of MS. Diffuse tissue abnormalities in the NABT and diffuse spinal cord abnormalities are common features of PPMS, whereas focal inflammatory lesions are less evident\textsuperscript{45-47}. For SPMS, a lower frequency of active inflammatory lesions is observed compared to RRMS, while progression of brain atrophy is still evident\textsuperscript{48}. These differences in disease characteristics suggest that diffuse pathology is more relevant to disease progression than focal inflammation. This might explain why conventional immunomodulatory treatments known to specifically reduce overt inflammatory activity, such as interferon beta and glatiramer acetate, are found to be ineffective in clinical trials on PPMS and SPMS. In the future, brain atrophy measurement could play a key role in the detection of treatment effects in clinical trials, and provide valuable clinical prognostic information for progressive forms of MS.

The long-term clinical course of MS varies considerably between patients, and remains highly unpredictable. Early prognosticators for clinical disability at long term follow-up may have great impact on patient care, especially with regard to disease modifying treatment. We proposed that the extent of brain tissue loss early in the disease course may have clinical prognostic value. Future long-term follow-up studies using clinical disability as outcome, are needed to confirm this hypothesis. The next challenge would be to translate these findings from group level to individual level and to simplify and standardise brain volume measurement techniques to enable use in a regular hospital setting.
REFERENCE LIST


