Brain morphometry in Multiple Sclerosis

Short title: Brain morphometry in MS

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Abbreviations

BMS: benign multiple sclerosis
CNS: central nervous system
CSF: cerebrospinal fluid
DMT: disease-modifying treatment
DTI: diffusion tensor imaging
EDSS: expanded disability status scale
GM: gray matter
MRI: magnetic resonance imaging
MS: multiple sclerosis
MSFC: multiple sclerosis functional composite
RRMS: relapsing-remitting multiple sclerosis
PPMS: primary progressive multiple sclerosis
SPMS: secondary progressive multiple sclerosis
VBM: voxel-based morphometry
WM: white matter

Introduction

Multiple sclerosis (MS) is an autoimmune condition of the central nervous system (CNS) characterized by recurrent episodes of inflammation with demyelination and neurodegeneration[1]. These episodes manifest themselves through a variety of symptoms, most commonly affecting motor, sensory, visual and cognitive functions[2]. MS usually onsets in young adulthood (aged 20-40 years) and is more common in women. In the majority of cases, after a clinically isolated syndrome, MS evolves in a relapsing-remitting form, followed by secondary progression (SPMS); a progressive worsening of clinical disability over time, with an insidious onset, characterizes the primary progressive form of the disease (PPMS)[3].

MS is characterized by an immune attack against the myelin sheaths surrounding the axons of the neurons[4]. This inflammation can lead to axonal damage[5]. Evidence of neurodegeneration that extends beyond the areas of inflammation suggested that there is a primary neurodegenerative component in MS, followed by secondary inflammation[6–8].

Inflammatory damage in MS can be detected with magnetic resonance imaging (MRI)[9], which has been used both as a diagnostic and a prognostic tool. MS lesions in the white matter (WM) can be seen as hyperintense on T2-weighted acquisitions. Active lesions are detected as hyperintense areas on T1-weighted scans acquired after the injection of the contrast agent Gadolinium-DTPA and indicate a breakdown in the blood-brain barrier. Hypointense lesions visible on post-contrast T1-weighted scans reflect severe tissue damage and are called “chronic black holes”[10,11]. Lesions also occur in the gray matter (GM)[12] and are characterized by transection of axons and dendrites as well as cell death[13]. GM lesions histopathologically differ from WM lesions, as they do not involve breakdown of the blood brain barrier, and show significantly less inflammation[14–16].

MS lesions have traditionally been the main focus of disease diagnosis[17], prognosis[18] and response to treatment[19,20]. More recently, MS research has turned its focus to GM abnormalities and brain volume loss not only as prognostic factors[21], but also as outcome measures of clinical trials assessing the efficacy of disease modifying treatments (DMTs)[22]. In this chapter, we discuss the available methods for measuring MS pathology in the brain GM using MRI. We then discuss potential causes for GM changes and the relationship with damage in WM. Last, we outline the

relationship between GM damage and disability, and its potential applicability to monitor and predict disease evolution. This chapter will not cover global or tract-specific WM atrophy, or atrophy of the spinal cord and optic nerve, despite their undisputed contribution to symptom and disease severity[23–25]. The term *atrophy* will be used to describe a decrease in GM volume over time. In contrast, we will use the term *volume loss* to describe a mean difference between patients with MS and healthy controls.

Measuring atrophy in MS: common methods and challenges

Brain volume is commonly measured using high-resolution T1-weighted MRI scans. Isotropic resolutions of, or around, 1 mm can be acquired within clinically feasible times (<10 minutes) and have sufficient contrast for distinguishing separate tissue classes (e.g. GM, WM, cerebrospinal fluid [CSF]). Delineation of specific structures is occasionally carried out using manual tracing, but the vast majority of studies use automated analytic techniques for estimating volume. These automated techniques benefit from less user bias, are highly reproducible and demonstrate comparable results to manually-defined region-of-interest approaches[26] .

Segmentation algorithms first separate the brain images into different tissue classes. Tissue fractions can be generated by dividing each tissue volume by the summed GM, WM and CSF volumes, called the intracranial volume. Regional brain volumes can also be estimated by voxel-based morphometry (VBM) or shape-based parcellation algorithms. The former relies on registration of each voxel within the image to a common template, with the amount of warping needed to move voxels from their original, native space into the common space serving as an index of regional volume. Common analysis routines used for VBM are implemented in the Statistical Parametric Mapping (SPM; Wellcome Trust Centre for Neuroimaging, UCL Institute of Neurology, http://www.fil.ion.ucl.ac.uk)[27] software and in FSL (the FMRIB Software Library, where FMRIB is the Oxford Centre for Functional MRI of the Brain[28]). Shape-based parcellation of sub-cortical structures (such as the hippocampus, thalamus and caudate nucleus) can be carried out using Freesurfer[29,30] or FSL's FIRST (FMRIB's Integrated Registration and Segmentation Tool[31]), which exploits the probabilistic relationships between the intensity and shape of these structures. Use of shape-based parcellation routines is particularly advantageous for determining volumes of striated structures (i.e. those containing both GM and WM), like the thalamus and globus pallidus, which can be challenging to segment using standard techniques. Alternatively, surface-based analysis methods can be used to measure the cortical surface area or cortical thickness, using the Freesurfer software[32]. The variety of software tools available provides a number of choices for measuring brain tissue volumes in people with MS. These methods are not free from confounds, as the algorithms used by each software package can affect the final results. The results of VBM analysis can differ depending on whether it is carried out in SPM or FSL[33,34]. This can be relatively small differences introduced by segmentation algorithms, through to large differences introduced by registration algorithms and statistical approaches. The lack of a gold-standard for measuring volume precludes calibration of algorithms, or even an assessment of which software offers the most accurate results.

A number of potential confounds are known to affect findings in studies of brain volume and cortical thickness. VBM results may be influenced by the field strength, with regional differences seen when comparing images acquired on 1.5 T and 3 T MRI scanners[35]. Many clinical studies are conducted on hospital scanners with 1.5 or 1 T field strengths. The choice of smoothing kernel can also influence findings[36], as well as the use of modulation, a scaling method, which maintains local volume size during registration to standard space[37]. Additional factors specific to MS should also be considered. In MS, the presence of WM lesions can affect both estimates of GM volumes[38] and cortical thickness[39]. This bias can occur due to an inappropriate assignment of lesion signal

intensities into WM signal intensity distributions, affecting the estimation of the boundary between different tissue classes, and subsequent tissue volumes. The bias caused by WM lesions can be minimised by marking and "filling" lesions using mean WM intensities from the whole brain[38] or from the surrounding neighbouring voxels of lesions[39–42].

Regional distribution of GM volume changes

Most studies on GM volume in MS have examined global whole brain or GM volume. Most commonly used measures have been brain parenchymal fraction (BPF; e.g. Bermel et al.[43]) or the width of the third ventricle (a surrogate marker of brain atrophy). Both suggest decreased total brain volume in MS patients relative to healthy controls[44–48], and ventricle size is highly correlated with BPF[49]. Enlarged ventricles are commonly observed in MS brains with long disease duration (FIGURE 1).

include FIGURE 1

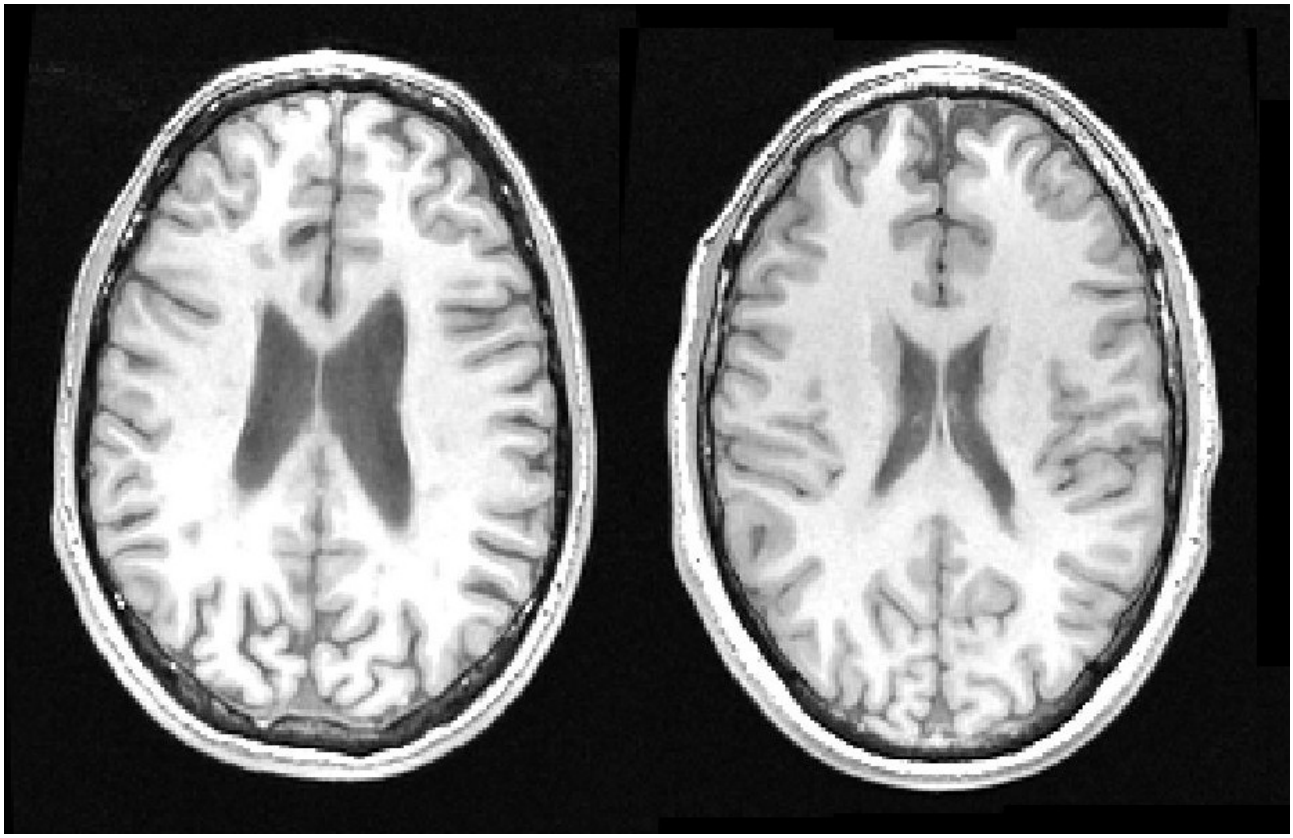

Figure 1: left: T1 weighted axial brain slice of a 36 year old male with RRMS and a disease duration of 12 years. Right: scan acquired with the same parameters in similar location of a male of the same age.

MS related damage is not randomly distributed over the brain[1]. In the WM, lesions are particularly common within periventricular regions, such as those neighbouring the lateral ventricle and in the superior and posterior corona radiata. This observation has been quantitatively confirmed by demonstrating highly consistent lesion probability maps across studies[50–53]. Similarly, within GM, demyelinated lesions, which are more frequently sub-pial, are seen along the entire cortical ribbon, but their incidence and size are significantly larger in cortical sulci, and in deep invaginations of the brain surface[1]. GM and WM damage do not happen completely independently and histochemistry shows that GM neurodegenerative processes are mostly pronounced in the cortex overlaying sub-

cortical demyelinated lesions[1]. Histopathological[16,54,55] and neuroimaging studies[56] show the greatest number of cortical lesions in the frontal and temporal lobes. However, comparing patients with healthy controls, GM volume loss has been reported throughout cortical and sub-cortical areas. Individual studies have reported significant loss within the cingulate cortex[57,58], temporal lobe[57,59], insula[59,60] and cerebellum[57,60,61]. A meta-analysis of 19 studies[62] has concluded that GM loss in RRMS and CIS is most pronounced in the thalamus and basal ganglia, within the cingulate cortex and around the central gyrus. Cortical thinning in MS patients compared to healthy controls has been found in frontal, parietal, occipital, temporal and insular lobes[63–66]. The volume loss in MS patients compared to healthy controls is likely a result of higher atrophy rates. In a longitudinal study conducted with patients with PPMS, Eshaghi et al.[67] showed regional differences in GM atrophy, with the greatest atrophy observed in the cingulate gyri. Regional differences in GM atrophy studies may be influenced by methodological factors, as discussed above. Despite this caution, it is clear that atrophy in MS occurs in both cortical and sub-cortical GM.

The basal ganglia form complex circuits with cortical and sub-cortical structures and are involved in motor and cognitive functions[68]. In RRMS, focal atrophy within basal ganglia structures has been reported, with up to 20% volume loss within the caudate nuclei[49,69]. Many basal ganglia circuits also involve the thalamus, which coordinates several sensory-motor pathways and has been viewed as a hub between cortical and sub-cortical regions. Thalamic volume changes are frequently reported in MS[70], with neuronal volume loss at levels up to 30%[45,71]. Thalamic atrophy impacts significantly upon cortical functional networks in MS[72]. Indeed given the role of the thalamus as a hub region in the brain, it has become clear that GM volume within this region may be one of the most important predictors of clinical and cognitive dysfunction in MS[73–75].

Interplay between GM and WM pathology

The processes that characterise MS occur primarily through loss of myelin, followed by axonal degeneration as a result of demyelination and subsequent metabolic changes[76]. MRI research in MS was initially dominated by a focus on WM changes. Despite increasingly common investigations of GM changes, at a theoretical level these effects are persistently attributed mainly to prior WM changes[66]. This attribution is partly supported by evidence that the distribution of GM damage throughout the brain is in part related to damage to connected WM. This relationship has been shown using WM lesions and also more subtle changes in "normal appearing" WM. For example, the ventricular enlargement seen in the lateral and third ventricles is associated with the total volume of WM lesions[77]. Also, regional GM atrophy in MS can be statistically predicted by diffusion tensor imaging (DTI) metrics (probing microstructural alterations) in connected WM tracts[66]. This relationship between GM and WM damage was observed in both deep and cortical GM in those with RRMS, and with deep GM in those with SPMS. These findings provide a clear link between WM and GM changes, but also suggest that pathology in different subtypes of MS may be driven by separate pathophysiological mechanisms[1]. We do not yet know the order in which different forms of pathology occur, or what drives each form of pathology, but well-designed longitudinal imaging studies in MS can help to address these questions.

The thalamus has also been well studied in MS, with evidence suggesting that it may be both directly affected by GM pathology and indirectly affected by WM pathology in connected tracts. A correlation between total lesion volume and GM volume in the thalamus has been reported repeatedly[78–81]. In people with clinically isolated syndrome (CIS), thalamocortical tracts show a tenfold higher lesion density than other tracts[82]. In the same study, lesion volume specifically within these thalamo-cortical tracts was found to significantly predict thalamic volume. Similarly in MS, thalamic volume has been found to be associated with WM lesion volume and diffusion MRI

metrics (fractional anisotropy) in its connected tracts[66]. At histopathology, substantial deep GM pathology is seen in MS, with focal demyelinating lesions, inflammation and diffuse neurodegeneration[83]. However, a recent MRI study has identified substantial GM abnormalities on magnetisation transfer imaging in GM regions neighbouring ventricles, suggesting the role of CSF factors in driving pathology[84]. This interesting hypothesis is supported by the general finding that deep GM lesions tend to follow ventricles and CSF, providing an alternative pathophysiological mechanism for atrophy in deep GM structures[16].

Atrophy and the influence of disease stage

Throughout adulthood the normal brain shrinks and the rate of shrinkage accelerates throughout the lifespan. In MS this shrinking happens faster, at a rate of about 0.5% per year compared to 0.3% per year in healthy controls[85–87]. Some studies report even higher atrophy rates of up to 2%[88,89], and atrophy rates may be higher still in those with faster disease evolution[90]. This increase in atrophy rate can explain the, often substantial, brain volume differences between MS patients and healthy controls[90,91]. Volume loss compared to controls has been reported in RRMS[62,92], as well as in progressive forms[67,93,94].

It has been argued that MS is a neurodegenerative disease and it enters the progressive stage when the brain's capacity to compensate for damage has exceeded its limit[6]. With this in mind, one would expect GM damage to be more extensive and accelerated in progressive stages, which is supported by histological studies[83,95], but which is not always found in MRI studies[46,96,97]. Some studies suggest that the spatial distribution of atrophy differs between disease subtypes and could be responsible for the different clinical phenotypes[1,77].

GM volume loss has been observed at the earliest stages of the disease, with changes apparent in patients with CIS, i.e. those who have experienced a single inflammatory event and in whom MS has not yet been diagnosed. Cross-sectional studies report GM volume loss in whole brain GM[98], the thalamus, basal ganglia and brain stem in CIS[99] and within both deep GM and cortical areas[60]. However, these findings are not consistently seen, with many failing to show GM alterations[98,100,101]. Differences in the nature of samples could help to explain these inconsistencies. For example, CIS patients with higher lesion load have been found to have lower sub-cortical GM volumes[102]. Additionally, there are likely to be differences between those with CIS who eventually convert to MS and those who do not. “Converters” have been found to show lower GM volume before conversion compared to “non-converters”[61], as well as higher GM atrophy rates within the first few years after the clinical event[103,104].

Ceccarelli et al.[100] found significantly different rates of GM volume loss among clinical phenotypes (CIS, RRMS, SPMS, and PPMS), with it being highest in SPMS patients. Similarly, lower GM volume has been reported for RRMS when compared to patients with progressive MS[98,105,106]. Such comparisons may sometimes be confounded by the typically longer disease duration and older age of SPMS compared to RRMS patients, as disease duration influences brain volumes in both RRMS and SPMS[89]. However, other factors are likely to have a greater influence, as a longitudinal study has demonstrated that RRMS patients who later convert to progressive course show lower baseline BPF and GM and greater decreases in these metrics than those who do not convert within the subsequent four years[87]. Therefore, it is likely that pathophysiological mechanisms relating to the progressive pathology of MS accelerate volume changes over and above the effects of age or disease duration. Additionally, the regional distribution of volume loss seems to differ between early MS and later stages of progressive MS[107].

One approach to study the influence of disease severity on GM volume is by comparing patients with benign MS (BMS)[108] to MS patients with a similar disease duration but more severe disease. Patients with BMS show lower GM volumes than healthy controls[79], but also lower cortical lesion volume[109] and less severe GM volume changes than patients with RRMS[98,110] or SPMS[79]. Some evidence indicates that it may be the spatial distribution of GM changes, rather than the extent, that differs between patients with BMS and other patients[53]. Additionally, patients with clinically BMS, but cognitive deficits do have GM volume loss similar to more severe forms of MS[111].

Only a few longitudinal studies have directly compared atrophy rates between disease subtypes. Ge et al.[89] compared rates between RRMS and SPMS, reporting a 2% annual decrease in brain volume in SPMS and a 1.5% decrease in RRMS, but this difference was not statistically significant. Similarly, Kalkers et al.[112] and De Stefano et al.[85] showed no differences in annualized atrophy rate between MS subtypes. Korteweg et al.[113] suggest that patients with higher baseline lesion volume have a greater atrophy rate over the subsequent two years. Looking at cortical thickness, Calabrese et al.[107] demonstrated a faster change over time in SPMS and late RRMS than in early RRMS. Studies examining BMS suggest that it may be characterized by slower rates of atrophy than patients with more active early MS[110,114]. Possible confounds for studies comparing atrophy rates across different disease subtypes are the baseline characteristics of the cohorts[115]. Larger scale studies are needed to identify predictors of brain atrophy rates.

Mechanisms leading to GM atrophy

As GM volume loss seems to be a hallmark in MS and relates to both disease severity and disease prognosis[116], identifying the mechanisms underlying GM atrophy in MS could offer useful tools for both monitoring the disease and measuring the efficacy of therapeutic interventions[117]. In order to identify and understand the molecular and cellular processes underlying the GM changes visible on MRI, a combination of histopathological evidence and advanced brain imaging tools is crucial.

Initial views on disease mechanisms in MS focused on axonal loss as the cause for degeneration of connected GM tissue (i.e. Wallerian degeneration). A recent line of thought is that MS is not initially triggered by the immune system malfunctioning (outside-in hypothesis[118]) but that demyelination may be the underlying disease mechanism, with myelin debris then triggering the immune system to react, amplifying the tissue damage (inside-out hypothesis[6]). This view was bolstered by Stys et al.[8], amongst others, based on evidence that demyelination also occurs in areas with low levels of inflammation (such as the GM[13]), which argues against a purely inflammation-driven disease process; note that cortical inflammation has been reported in early MS[119,120] and meningeal inflammation might be involved in cortical neuronal loss[121]. In either case, the demyelination that co-occurs with inflammation triggers several mechanisms that can affect the GM.

Recently, attention has been drawn to the involvement of mitochondria and energy metabolism following demyelinating events[122]. These biochemical processes can also trigger neuronal loss in MS tissue. Active microglia and macrophages, which are present at inflammatory sites, release radical oxygen species and nitric oxide, which can oxidize macromolecules in the neurons, inhibit their function, and promote their degeneration[123]. In particular, radical oxygen and nitric oxygen species can impact upon mitochondrial function as they directly inhibit parts of the respiratory chain[124]. Additionally, due to demyelination, ion channels are rearranged along the axons of the neurons, and more energy is needed for ion transport. This can lead to an increase in non-energy demanding ion exchange mechanisms and to a Ca2+ overload in the cell, further triggering mechanisms of neurodegeneration[124]. Iron is stored as ferritin in myelin and if myelin is destructed,

iron starts to move freely and contributes to oxidative stress[76]. An increased demand in energy coupled with energy deficiency can lead to cell death and axonal transection. These findings suggest that axonal loss and demyelination can affect GM damage in both direct and indirect ways.

In a recent paper, Haider et al.[1] distinguish two main patterns of neurodegeneration. The first is characterized by retrograde degeneration and the pattern of damage that follows WM lesions, as discussed above. The second is related to oxidative stress and happens across the entire cortex. Combining ex vivo MRI scanning with histology in post-mortem studies can help to understand which of these molecular processes underlie MRI measures of GM volume. For example, Popescu et al.[125] found that neuronal density, neuronal size and axonal density all contribute to GM volume measures. Therefore, axonal degeneration, as well as demyelination, could contribute to in vivo measures of GM atrophy. In deep GM, there is reduced neuronal density in MS patients compared to healthy controls, with lower neuronal density in demyelinated MS brain tissue than in non-demyelinated tissue, and lower neuronal density in non-demyelinated MS brain tissue compared to healthy control tissue[16,45]. These findings indirectly suggest that the GM volume loss detected with MRI might be at least influenced by neuronal loss triggered by demyelination.

As with most studies of MRI changes in MS, imaging studies investigating GM changes are challenging to carry out. For example, it is estimated that only a small subset of existing cortical lesions can be detected with conventional MRI methods[126,127]. Therefore, abnormalities and changes in GM volume measures may reflect the presence of undetected GM lesions[14]. In support of this view, non-lesional MS cortical tissue shows similar cell density (neuronal, glial and synaptic density) to tissue in healthy volunteers[128]. A recent MRI study investigated the spatial co-localisation of GM lesions and atrophy and found only weak correspondance[129]. In a longitudinal study[107] the appearance of new cortical lesion correlated with cortical atrophy early in the disease course, but only when averaged across the whole GM and not on a region by region basis. Even if undetected GM lesions contribute to findings of GM atrophy in MS, it still needs to be resolved whether this is a methodological effect (e.g. different relaxation time in lesioned GM) or whether the cellular changes within lesions (e.g. cell death) compromise GM volume. Another challenge concerns the registration between MRI data and histological slices. Newer methods such as CLARITY, which permits histological assessment in intact tissue, will allow more direct links and comparisons between MRI images and histology[130].

Application and predictive value of GM volume measures

Whilst the mechanisms underlying GM atrophy are not fully understood, GM volume measures might be useful as markers for disease evolution and prognosis[22]. WM lesion count and volume remain the most commonly used clinical outcome measures for pharmaceutical studies, as DMTs aim to reduce risk of developing new lesions and new symptoms. However, there are substantial drawbacks to an exclusive focus on WM pathology. The correlation between WM lesion load and disability scores is inconsistent, a phenomenon often referred to as the "clinico-radiological paradox"[131]. It is unclear whether this paradox is caused by methodological limitations, such as missed lesional tissue[132,133], or whether parameters other than lesions are better predictors of MS damage. Increasingly, researchers have focussed on the relationship between atrophy and disability. It has been proposed that GM volume can explain some of the remaining variance, and may even be a better indicator of disease progression and clinical outcome than WM damage[134,135]. Only recently studies have started looking at the effects of drugs on GM atrophy, with DMTs having been shown to slow atrophy rates[22].

Clinical disability correlates with whole brain GM volume and regional volume loss

The clinical relevance of whole brain and regional GM volumes can be established by examining their associations with disability scores. The heterogeneity of disability in MS creates obvious challenges when assessing this relationship. The symptoms cover a wide range of sensory, motor and cognitive dysfunctions, which creates difficulties when summarising overall disability levels. The most commonly used measure of disability is the expanded disability status scale (EDSS[136]), which is based on the neurological examination of various functional systems, but is primarily affected by the mobility of a patient. The second most frequently used measure of disability is the multiple sclerosis functional composite (MSFC[137]), which consists of tests of mobility, fine motor skills and cognition. The range of symptoms covered by the EDSS and MSFC makes pinpointing of regional associations between disability and GM atrophy challenging. Additionally, the EDSS scale is not an interval scale, which makes it statistically more challenging to detect clinical-MRI associations[138]. Despite these limitations, a number of studies have reported negative correlations between disability scores and GM volume measures in the form of either whole brain volume, GM volume, ventricular volume or cortical thickness[46,49,87,98,106,139–142].

More regionally specific correlations have been demonstrated for cortical, as well as deep GM. Correlations between EDSS and GM volume and thickness were shown in the precentral, medial and superior frontal cortices, cingulate, insula and other cortical regions[61,65,143], thalamus, putamen and cerebellum[61,102]. In a more symptom specific analysis approach, Calabrese et al. (2007)[63] showed that the severity of motor symptoms was correlated with cortical thickness in the precentral gyrus, while visual symptoms were correlated with thickness in the occipital cortex. Similarly, Henry et al. (2008)[99] related cerebellar system symptoms with atrophy in the cerebellum, and disability measured with the MSFC with atrophy in cerebellum, caudate and putamen.

Due to the variety of methods used, results across studies are far from consistent with some failing to find an association between disability and GM volumes or cortical thickness[64,144]. In an approach to combine existing evidence, Lansley et al (2013)[62] conducted a meta-analysis of 19 VBM studies that had looked at the relationship between EDSS and GM volume. While widespread brain volume loss in patients compared to healthy controls was confirmed, the analysis revealed only a single cluster, in which GM volume was related to EDSS, encompassing the left pre- and post-central cortex.

The relationship between GM damage and cognition

A burgeoning area of MS research is the one exploring links between GM changes and cognitive impairment[145]. This work has become of increasing importance as the drugs used to treat MS have proven effective in slowing disability worsening, but as yet have had limited impact on slowing cognitive decline[145]. GM lesions and volume are significant predictors of cognitive impairment, both in cross-sectional[146] and longitudinal studies[147]. GM lesions and atrophy are independent predictors of cognitive function[129], emphasising the need to consider each separately. In addition, other MRI metrics have proved useful for understanding cognitive changes, such as alterations in the complexity of diffusion in GM[148,149], or abnormally low levels of glutamate in relevant GM regions[150]. These changes offered similar or greater predictive value than measures of regional brain volume alone, so complement the use of conventional imaging techniques when trying to understand the role of subtle structural GM damage on cognitive impairment.

Atrophy rate correlates with disability change

Even stronger evidence that GM volume and MS disability influence each other comes from studies

that show how both of these variables change in synchrony. The change of GM volume per year has been shown to correlate with change of disability as measured by EDSS and MSFC[67,77,87,139,151–155]. This is the case even when correlating the short term change of GM volume with long term change in disability[139], suggesting that brain atrophy may not spontaneously slow down. Even GM volume at baseline can predict disease progression. Most of this evidence comes from studies comparing CIS patients who convert to MS with patients who do not convert. In particular, lower baseline GM volume and more pronounced GM atrophy are reported in "converters"[61,87,103].
Recent studies by Filippi et al.[156] and Popescu et al.[157] showed that baseline GM atrophy can predict EDSS up to 13 years later even when controlling for baseline EDSS[157].

The size of these reported effects are all small but statistically significant at a group level. This makes it difficult to predict an individual patient's prognosis based on their MRI scans. Additionally, the effects seem to be wide-spread across the brain with regional differences between studies, making it challenging to judge which GM measures are the most useful brain imaging markers and predictors of disability progression. In order to identify markers suitable for assessing an individual's prognosis we need further information about "normal" and "pathological" rates of atrophy. De Stefano et al.[85] attempted to find a cut-off in annualised percent brain volume change that differentiates patients from controls, as well as patients with strong disability incline from patients with weak disability incline. As the effects of GM atrophy seem to be regionally dependent, region specific markers might allow even higher specificity and sensitivity. This needs large, multi-centre longitudinal studies with clear validation samples.

Atrophy as an outcome measure in clinical trials

Due to the increasing evidence of the importance of GM pathology in MS, GM volume measures are starting to be used as outcome measures in clinical trials. DMTs such as laquinimod[158], fingolimod[159,160], interferon beta[161–164] and glatiramer acitate[162,164] have been shown to slow down GM atrophy[161–163] (for a review see De Stefano et al.[22]), but no effect[165,166] or the opposite effect has also been reported[167]. A recent trial based meta-analysis has indicated that DMTs may impact upon disability through independent effects on both lesions and atrophy[168].
Clinical trials using GM atrophy as an outcome measure have also had to contend with treatment-related decreases in volume. This "pseudoatrophy" can be seen as a paradoxical acceleration in brain volume loss following the initiation of therapy. It is thought to be caused by the resolution of inflammation, and may reflect a decrease in oedema[169] or changes in the volume of inflammatory cells, such as glial cells[170]. Studies of regional brain volume indicate that pseudoatrophy may be confined to WM regions[171], suggesting that VBM studies of GM volume may be less affected.

Summary & Conclusions

Substantial evidence supports the existence and clinical relevance of both cortical and sub-cortical GM volume loss and atrophy in all MS subtypes. Neuropathology[1] and neuroimaging[172] are progressively clarifying the nature of GM pathology in MS and its relationship with WM damage[173]. Improving further our understanding of the mechanisms underlying GM damage and the relationship between GM and WM damage remains a goal of future research. The clinical relevance of GM damage is supported by the association between clinical characteristics of MS and GM pathology. There is relationship between GM damage and the development of clinical disability, especially cognitive dysfunction[145]. GM plays a role in predicting the evolution of the disease, i.e., the conversion from CIS to MS and from RRMS to progressive MS[61,87]. GM damage is becoming a relevant outcome measure for immunomodulatory[22] and neuroprotective strategies[174]. Despite this emerging role of GM pathology in MS, more work remains to be done in order to translate the

application of GM atrophy measurements in individual patients[175].

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