**Title:** Surface-in pathology in multiple sclerosis: A new view on pathogenesis?


## Authors:

Matteo Pardini MD PhD*[1,2], J. William L. Brown PhD MRCP*[1,3,4], Roberta Magliozzi PhD[5,6], Richard Reynolds PhD[6,8], Declan T. Chard PhD FRCP[1,7]

*These authors contributed equally to this work

## Affiliations:

1. NMR Research Unit, Queen Square Multiple Sclerosis Centre, University College London (UCL) Institute of Neurology, London, United Kingdom
2. Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genoa, and IRCCS AOU San Martino-IST, Genoa, Italy
3. Department of Clinical Neurosciences, University of Cambridge, Box 165, Cambridge Biomedical Campus, Cambridge, United Kingdom
4. Clinical Outcomes Research Unit (CORe), University of Melbourne, Melbourne, Australia.
5. Department of Neuroscience, Biomedicine and Movement Science, University of Verona, Verona, Italy
6. Division of Brain Sciences, Department of Medicine, Imperial College London, London, United Kingdom
7. National Institute for Health Research (NIHR) University College London Hospitals (UCLH) Biomedical Research Centre, UK.
8. Centre for Molecular Neuropathology, Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore.

## Corresponding author:

Declan Chard

Queen Square Multiple Sclerosis Centre, Department of Neuroinflammation, UCL Institute of Neurology, University College London, Queen Square, London, WC1N 3BG.

Email: d.chard@ucl.ac.uk

## Abstract

While multiple sclerosis can affect any part of the central nervous system, it does not do so evenly. In white matter it has long been recognised that lesions tend to occur around the ventricles, and grey matter lesions mainly accrue in the outermost (subpial) cortex. In cortical grey matter, neuronal loss is greater in the outermost layers. This cortical gradient has been replicated in-vivo with magnetisation transfer ratio and similar gradients in grey and white matter magnetisation transfer ratio are seen around the ventricles, with the most severe abnormalities abutting the ventricular surface. The cause of these gradients remains uncertain, though soluble factors released from meningeal inflammation into the cerebrospinal fluid has the most supporting evidence. In this Update, we review this "surface-in" spatial distribution of multiple sclerosis abnormalities and consider the implications for understanding pathogenic mechanisms and treatments designed to slow or stop them.

## Keywords

Multiple sclerosis; magnetisation transfer ratio; neuropathology, meningeal inflammation; B cell follicle

## Introduction

Since its first description multiple sclerosis (MS) has predominantly been considered an inflammatory demyelinating disease of central nervous system (CNS) white matter (WM), but it is now clear that no part of the CNS is spared. The detailed molecular pathogenesis of MS still eludes us, but the uneven nature of MS pathology may provide important clues. WM lesions, the most visible feature of MS, tend to form adjacent to the ventricles (Dawson, 1916) and GM lesions also usually form close to the brain suface (Kutzelnigg *et al.,* 2005). Extra-lesional abnormalities are also most marked at the inner (periventricular) and outer (subpial) brain surfaces. Recent MRI studies have shown that "surface-in" abnormality gradients are associated with clinical outcomes in MS, independently of WM lesions, and can predict treatment response. The presence of these gradients has potentially important therapeutic implications, as it implicates CSF as being a likely mediator of this, yet many drugs used to treat MS do not readily enter the intrathecal space. In this Update, we review the "surface-in" spatial distribution of MS abnormalities and consider the implications for understanding pathogenic mechanisms and treatments.

## White matter lesions

Classic active lesions, present in greater numbers at earlier stages and in the most rapidly progressive cases, have perivascular and parenchymal infiltrates of lymphocytes and abundant macrophages throughout (Prineas and Wright, 1978). In progressive MS half of all lesions seen still have features suggesting activity (termed chronically active, smouldering or slowly expanding lesions) chartacterised by an inactive core surrounded by a rim of activated microglia and macrophages with on-going tissue injury (Prineas *et al.*, 2001; Frischer *et al.*, 2009; Lassmann, 2011). Virtually all WM lesions form around a vein (for example (Ribbert, 1882)) and using 7T MRI a central vein is visible in 87% of WM lesions (Tallantyre *et al.*, 2009). WM lesions also tend to occur around the ventricles (Dawson, 1916), and on MRI there is a high lesional density around the lateral ventricles (which have a high density of veins draining towards them) but low density surrounding the fourth ventricle (which has few veins draining towards it) (Wang *et al.*, 2010; Pardini *et al.*, 2016). Lesions also occur in the outer cervical cord, again around small veins (Oppenheimer, 1978). These data collectively suggest that most, if not all, WM lesions in MS are venocentric.

Lesion formation around veins may be due to factors originating from the blood (see later) or perivascular space. Perivascular spaces are continuous with the subarachnoid space (Ichimura *et al.*, 1991), though they collapse at post-mortem making them difficult to study. Perivascular spaces are significantly larger in people with MS (independent of atrophy) and increase in size and number at

the time of lesional contrast enhancement, suggesting some inflammatory association (Wuerfel *et al.*, 2008). Perivenular hypoxia may also be a relevant factor (Martinez Sosa and Smith, 2017): veins normally supply oxygen to surrounding brain tissue (Vovenko, 2009), but under conditions of hypoxia, venous blood becomes deoxygenated, draining oxygen from it (Ivanov *et al.*, 1999). In a cross sectional comparison, WM lesions in secondary progressive MS were most prevalent in areas of low cerebral perfusion while in early relapsing-remitting MS, lesions were also seen in well perfused regions (Holland *et al.*, 2012). Longitudinal assessment is needed, but this finding suggests a differential repair capacity within the human brain influenced by cerebral perfusion. Distinguishing this from a CSF-derived factor impairing repair is however difficult as most areas of low perfusion lie close to the ventricles.

It is also worth noting that even amongst lesions their pathology varies by location: endogenous remyelination (Patrikios *et al.*, 2006) and recovery of T1-hypointensity on MRI (a putative marker of remyelination (Papadopoulou *et al.*, 2014)) are markedly limited in lesions close to the lateral ventricles.

## Grey matter lesions

In long-standing MS, lesions account for a quarter of cortical GM compared to 6% of the WM (Bo *et al.*, 2003). GM lesions are seen in the spinal cord (Fog, 1950); deep GM (predominantly in the inner layers closest to the ventricles (Haider *et al.*, 2014)); and cortical GM (most frequently in the outermost (subpial) layer (Kutzelnigg *et al.*, 2005; Magliozzi *et al.*, 2007)). While evident early (Lucchinetti *et al.*, 2011; Bevan *et al.*, 2018) subpial lesions become more prevalent in progressive disease (Kutzelnigg *et al.*, 2005) accounting for two thirds of all cortical lesions (Bo *et al.*, 2003). If active, subpial lesions in progressive disease occur close to focal areas of meningeal inflammation (Howell *et al.*, 2011) which predominantly occur in cortical sulci (Magliozzi *et al.*, 2007; Haider *et al.*, 2016), suggesting that these lesions might arise from soluble factors released by meningeal inflammation, which diffuses across the CSF and damages CSF-bathed tissue (perhaps via microglial activation) (Kutzelnigg *et al.*, 2005; Howell *et al.*, 2011).

## Extralesional WM

Extralesional WM - regions free of lesions seen using conventional (PD/T2-weighted and FLAIR) MRI - are abnormal when assessed with magnetisation transfer ratio (MTR) and diffusion tensor imaging (DTI), both of which correlate with myelin intensity and axonal count (Schmierer *et al.*,

2004; Schmierer *et al.*, 2007). Structural changes at the nodes of Ranvier associated with microglial activation might also contribute to such abnormalities (Howell *et al.*, 2010).

However, extralesional abnormalities are not uniform. First, perilesional tissue is abnormal for up to 4mm from the T2-defined lesion edge (Vrenken *et al.*, 2006). Second, periventricular changes: in healthy controls, WM MTR is highest adjacent to the lateral ventricles and decreases with distance from them; in all types of MS, extralesional WM MTR is lowest (most abnormal) at the ventricular edge, increases over the first ~5 mm (Fig. 1) before mirroring patterns seen in healthy controls (Liu *et al.*, 2015; Brown *et al.*, 2017; Brown *et al.*, 2019a). This periventricular MTR gradient surrounds supratentorial and infratentorial ventricles (Pardini *et al.*, 2016) and has been replicated with DTI where the gradient is most marked in people with oligoclonal bands (Pardini *et al.*, 2019). Another diffusion technique, the restricted signal fraction (FR) from the Composite Hindered and Restricted Model of Diffusion (CHARMED), appears more sensitive to axons (Assaf and Basser, 2005) though to the best of our knowledge this has not been confirmed in pathological studies. While the FR is lowest at the ventricular edge in early MS - and differences between healthy controls and people with early MS also appear greatest at the ventricular edge - no significant interaction was found between disease group and distance from the ventricles (De Santis *et al.*, 2019). It remains unclear whether this disparity reflects differences in statistical approaches, a smaller sample size (n=22 compared to n=71 (Brown *et al.*, 2017)), or that the periventricular gradient is more heavily driven by demyelination than axonal loss.

The extralesional periventricular WM MTR gradient appears independent of lesions: the severity is the same in those with and without T2 or enhancing lesions, and remains unchanged after excluding perilesional tissue; gradients and lesions have differing distributions (gradients surround the fourth ventricle (despite low lesion density)); and gradients occur within lesional tissues (Pardini *et al.*, 2016; Brown *et al.*, 2017; Pardini *et al.*, 2019). Outside-in MTR gradients are also seen in cervical cord WM (Kearney *et al.*, 2014). Taken together this suggests different mechanisms may underlie lesion formation and gradients within extralesional (and lesional) tissue.

The pathologic substrate underlying periventricular WM gradients remains under-explored, but a PET study has shown a periventricular gradient of microglial activation in extralesional WM and GM (Poirion *et al.*, 2017).

## Extralesional GM

Extralesional cortical and thalamic GM show significant axonal loss compared to healthy controls (Cifelli *et al.*, 2002; Choi *et al.*, 2012; Bevan *et al.*, 2018). Neuronal loss appears relatively independent of demyelination (Magliozzi et al., 2010) and no differences in axonal density were seen between demyelinating cortical lesions and extralesional GM (Klaver *et al.*, 2015). Cortical demyelination has typically been quantified in terms of demyelinating lesions, but extralesional reductions in myelin density are seen (Bevan *et al.*, 2018), in part reflecting areas of incomplete remyelination (Albert *et al.*, 2007).

Significantly greater neuronal loss is seen in the outermost cortical layers in some patients with SPMS (Magliozzi *et al.*, 2010), respect to healthy controls. This abnormality is related to an elevated level of meningeal inflammation (Fig. 2) and accompanied by substantial gradient of microglial activation in the most external cortical layers (Magliozzi *et al.*, 2010) in different cortical grey matter regions (Fig 2), including motor cortex and hippocampus. Preliminary neuropathology studies in progressive MS found a thalamic surface-in gradient of neuronal loss and microglial activation, maximal near the ependyma (Magliozzi *et al.*, 2018a) and most marked in cases where meningeal lymphoid aggregates were found in the cortex (Fig. 2). Similar increases in MHCII+cell density associated with significant axonal/dendritic loss and neuronal dystrophy was observed in subependymal lesions along the ventricular surface (Mahajan *et al.*, 2020), further supporting an "ependyma-in" pattern that may mirror the "pial-in" one of cortical injury (Magliozzi *et al.*, 2010). This study also demonstrated the presence of ovoid perivascular lesions contributing to thalamic injury, but with perivascular axons relatively preserved (Mahajan *et al.*, 2020). It may therefore be hypothesized that intrathecal inflammation, compartmentalized in the CSF and possibly in the leptomeninges, may drive and or enhance superficial cortical and subcortical GM damage.

Notwithstanding MRI's limited lesional sensitivity [even at 7T only about a third of GM lesions are seen (Kilsdonk *et al.*, 2016)], mean GM MTR is decreased in all disease stages (Fernando *et al.*, 2005). Surface-in GM MTR gradients are seen in all types of MS, both in the cortex (where the MTR is lowest at the outer cortical surface (Samson *et al.*, 2014; Brown *et al.*, 2019a) and the deep GM (where the MTR is lowest at the inner periventricular surface (Pardini *et al.*, 2016), Fig. 1). A corresponding gradient of thalamic atrophy, maximal at the CSF boundary and lessening with increasing distance from it, is visible in early paediatric MS (Fadda *et al.*, 2019) but not in monophasic acquired demyelinating syndromes (mono-ADS) studied in parallel. T2*, which inversely correlates with myelin and iron content, also shows surface-in cortical gradients from clinical onset (Mainero *et al.*, 2015). Periventricular and cortical MTR gradients are more severe in SPMS compared to

RRMS (Samson *et al.*, 2014; Liu *et al.*, 2015) and worsen with increasing disease duration (Brown *et al.*, 2019a).

## What causes surface-in gradients?

The cause of periventricular and cortical gradients – and if they reflect the same processes – remains unclear. The correlations between the severity of (i) periventricular and cortical MTR gradients; (ii) periventricular DTI gradients and cortical lesion loads; and (iii) periventricular lesion loads and cortical atrophy suggests a common cause (Jehna *et al.*, 2015; Pardini *et al.*, 2016). A spatially consistent feature is proximity to the CSF compartment, which can carry both cellular and soluble factors.

### Meningeal inflammation

While most pronounced in progressive disease, meningeal inflammation is seen histopathologically in all types of MS (Magliozzi *et al.*, 2007; Lucchinetti *et al.*, 2011; Choi *et al.*, 2012; Bevan *et al.*, 2018) and in multiple anatomical locations (Howell *et al.*, 2011; Howell *et al.*, 2015; Reali *et al.*, 2020). Such 'compartmentalised inflammation' may attract and retain further inflammatory cells, that together with immune cells circulating in the CSF may facilitate a microenvironment where inflammation may persist within this relatively sequestered compartment (Krumbholz *et al.*, 2006; Meinl *et al.*, 2008). Although lesions and surface-based gradients may arise from different mechanisms, identical antigen-experienced B-cell clones have been found in meningeal aggregates and WM perivascular spaces (presumably trapped from perivascular infiltrates) (Lovato *et al.*, 2011), suggesting a common origin.

A common molecular pattern of proinflammatory cytokines (IFNγ, TNF, IL2 and IL22) and molecules promoting sustained B-cell activity and lymphoid neogenesis (CXCL13, CXCL10, LTα, IL6 and IL10) is found in the CSF of patients with MS, both at disease onset (where it strongly correlates with cortical lesion number on MRI ($R^2 = 0.88$)) and in patients with SPMS at post-mortem (where it correlates with the percentage of demyelinated cortex; and is also seen in the meninges) (Magliozzi *et al.*, 2018b). This supports the hypothesis that meningeal inflammation may represent the principal source of CSF inflammatory mediators (Gardner *et al.*, 2013). Neither subpial cortical and or thalamic demyelination nor this inflammatory profile are seen in other conditions characterised by meningeal and CSF inflammation, suggesting it is MS-specific (Magliozzi *et al.*, 2018b; Junker *et al.*, 2020). Soluble CSF factors inhibiting remyelination and cell/tissue repair machanisms might be also involved in an MS-specific, surface-in pattern injury, but this has yet to be investigated.

Until recently, MRI detection of meningeal inflammation relied on surrogates (principally cortical lesions), with poor sensitivity and inter-rater reliability (Geurts *et al.*, 2011; Seewann *et al.*, 2012). Postcontrast T2 FLAIR has recently identified changes that might represent leptomeningeal enhancement (LME) in up to half of patients with MS, particularly in those with SPMS, older age, longer disease duration and more disability (Absinta *et al.*, 2015; Eisele *et al.*, 2015; Zivadinov *et al.*, 2017). In two patients proceeding to post-mortem (both with PPMS), areas of LME on T2 FLAIR corresponded to regions of meningeal inflammation overlying cortical demyelination (Absinta *et al.*, 2015). However, LME is not specific for meningeal inflammation - subarachnoid veins, dural venous sinuses and basal meninges all enhance on postcontrast T2 FLAIR (Zurawski *et al.*, 2017). LME correlates with cortical GM atrophy, but not with neocortical CLs (Ighani *et al.*, 2019), suggesting an association with neurodegenerative processes, rather than focal lesion development. It is unclear whether the compartmentalised inflammation that is seen in the leptomeninges, including the build up of lymphoid-like cell aggregates, would be accompanied by LME.

## Lymphoid tissues

The most striking form of meningeal inflammation – lymphoid tissue formation – is seen in about 40% of patients with SPMS at post-mortem (Magliozzi *et al.*, 2007; Howell *et al.*, 2011), and similar proportions of patients that died following their first MS presentation (Bevan *et al.*, 2018). Lymphoid-like tissues are topographically related to subpial cortical demyelinating lesions, but also occur alongside areas of reduced myelin density suggesting partial demyelination or remyelination (Magliozzi *et al.*, 2010; Bevan *et al.*, 2018). Their presence is associated with cortical gradients of damage: greater neuronal and astrocyte loss, accompanied by increased microglial density, occurs close to the CSF/pia surface which lessens through inner cortical layers (Magliozzi *et al.*, 2010).

The absence of lymphoid-like structures in PPMS might reflect the lower numbers of tissue blocks examined from a smaller number of patients or that aggregates are not an absolute requirement for surface-based gradients (simply worsening them when present). Diffuse meningeal inflammation was associated with both cortical lesions and increased clinical progression rates in a subgroup of PPMS patients (Choi *et al.*, 2012). Lymphoid-like structures have also been found in the spinal cord in MS cases with cerebral meningeal lymphoid-like structures (Reali *et al.*, 2020) and the severity of extralesional spinal WM axonal loss clearly correlates with the degree of both extralesional WM microglial/macrophage activation and the density of meningeal T-cells (Androdias *et al.*, 2010).

## Choroid plexus

Inflammatory cells may enter the CSF via the choroid plexus, particularly in early disease, influenced by the expression of a specific acute cytokine/chemokine pattern (Engelhardt *et al.*, 2001; Reboldi *et al.*, 2009). The concentration of the tight-junction protein claudin-3 (responsible for maintaining the blood-CSF barrier) is significantly lower in patients with MS (Kooij *et al.*, 2014).

## CSF cytokines and other soluble factors

CSF from people with MS has been shown to cause axonal damage and oligodendroglial and neuronal death in-vitro (Menard *et al.*, 1998; Alcazar *et al.*, 2000; Vidaurre *et al.*, 2014). The finding of elevated proinflammatory CSF cytokines suggests that neuronal dysfunction and death arise from cytokine-induced synaptic hyperexcitability, glutamate-dependent neurotoxicity or direct cytokine induced death receptor signalling, again pointing to an inflammatory origin (Rossi *et al.*, 2012; Rossi *et al.*, 2014). In support of the latter, an increase in TNF/TNF receptor 1 expression, together with all the downstream signaling pathway genes leading to necroptotic cell death, occurs in MS cortical grey matter in cases with high levels of meningeal infiltrates (Magliozzi *et al.*, 2019). In addition, persistently elevated levels of TNF and IFNγ in the rat subarachnoid space leads to meningeal inflammation, subpial demyelination, microglial activation and neuronal loss, that is similar to that seen in the cortical GM in MS (James *et al.*, 2020). In RRMS, an association found between leptomeningeal enhancement, cortical and thalamic lesion numbers (Zurawski *et al.*, 2020) also supports the hypothesis that CSF-related inflammation links cortical and thalamic injury.

Ceramides are sphingolipids, and the elevated CSF levels seen in patients with MS (Vidaurre *et al.*, 2014; Wentling *et al.*, 2019) particularly C24, probably reflect WM destruction or exosome release from membranes of mature oligodendrocytes. Ceramides may signal via similar pathways to TNF: both impair mitochondrial function, decrease neuronal energy production and increase reactive oxygen species and free radical production, culminating in neuronal damage. In progressive (but not relapsing-remitting) MS, ceramides accentuate this neurotoxicity by decreasing glucose bioavailability, culminating in 'virtual hypoglycosis' in these patients' CSF (Wentling *et al.*, 2019). It could be proposed that, rather than a single CSF factor, a particular combination of inflammatory and cytotoxic mediators may mediate the MS-specific surface-in damage.

## Other contributory factors

Some factors may augment the effects of a CSF-mediated process. In progressive MS the cortical glia limitans is compromised (Magliozzi *et al.*, 2010), allowing greater molecular diffusion into the

underlying cortex. A similar compromise of the ependymal layer has not been investigated, but granular ependymitis is seen in some patients with MS at post-mortem (Adams *et al.*, 1987). The periventricular venous watershed predisposes the region to hypoperfusion and hypoxia (Andeweg, 1996; Dewar *et al.*, 2003; Varga *et al.*, 2009; Beggs, 2013), though a similar watershed has not been demonstrated in the well-perfused cortex.

## Clinical relevance of surface-based gradients in MS

Surface-in gradients occur early in MS (Samson *et al.*, 2016; Brown *et al.*, 2017; Fadda *et al.*, 2019), within 5 months of a clinically-isolated syndrome (even in those without WM lesions), and independently predict a further relapse within 2 years (OR 61.7, p=0.02), Fig. 1, dwarfing the predictive effect of more established metrics like T2 lesions (OR 8.5, p=0.07, (Brown *et al.*, 2017)). Unlike other radiological markers (Wattjes, 2015), the periventricular WM gradient independently predicts on-treatment relapses following alemtuzumab (Brown *et al.*, 2019b), though larger corobboratory studies are required. Surface-based gradients in the cortex (Mainero *et al.*, 2015; Brown *et al.*, 2019a), periventricular WM (Brown *et al.*, 2019a) and spinal cord WM (Kearney *et al.*, 2014) all correlate with disability. In support, post mortem studies noted the most aggressive disease-course during life was associated with more severe gradients of cortical neuronal loss and the most marked meningeal inflammation (Magliozzi *et al.*, 2007; Magliozzi *et al.*, 2018b). Taken together, this suggests that surface-in gradients are related to clinical outcomes independently of WM lesions, and so the process that causes them may represent an important and independent therapeutic target. They also have prognostic value, again independent of WM lesions, however at present the clinical utility of periventricular MTR gradients is limited by the need for offline MRI scan processing.

## Insights from treatment effects on surface-based pathology

There have only been a few studies on treatment effects on putative markers of intrathecal inflammation. In one study, LME persisted for up to 5.5 years despite (unspecified) disease-modifying therapy (Absinta *et al.*, 2015). If LME does reflect meningeal inflammation, this suggests it might not be affected by current MS treatments. However, more recently, periventricular MTR gradients have been ameliorated with two monoclonal antibodies, alemtuzumab (Brown *et al.*, 2019b) and GNbAC1 (Hartung *et al.*, 2017). If the periventricular MTR gradient reflects intrathecal inflammation then its diminuation following alemtuzumab and GNbAC1 suggests that these processes may originate, or at least be sustained, by factors from the periphery (as alemtuzumab does not cross the blood brain barrier (Moreau *et al.*, 1994)). Further, given that the MTR gradient showed continued improvement two years after the last dose of alemtuzumab, aggressive peripheral

immunodepletion may have an enduring effect (Brown *et al.*, 2019b). Importantly, the effect alemtuzumab had on the MTR gradient was related to the risk of further relapses following treatment, demonstrating that an effect on the processes underlying the gradient is associated with an effect on clinical outcomes. However, treatment of an intrathecal process is likely to be slower or diminished unless a treatment is able to penetrate the CSF, and current MS disease modifying treatments (and in particular the more potent monoclonal antibody agents) have not been designed with this in mind. The discrepancy between LME and periventricular MTR findings may indicate that meningeal inflammation is not actually associated with periventricular gradients (although it has been directly linked with gradients in underlying cortical pathology), or that meningeal inflammation and periventricular gradients respond to treatment in different ways or at different rates. However, it should be noted that LME results from increased permeability of meningeal blood vessels and does not indicate a build-up of large immune cell aggregates in the meningeal space. Alternatively, disease duration may significantly modulate treatment effects, and the mean disease duration in Absinta et al. (Absinta *et al.*, 2015) was 11 years, versus 1.6 years in Brown et al. (Brown *et al.*, 2019b).

## Conclusions

Outside-in gradients in lesional and extralesional tissue are seen in all types of MS, can manifest at the earliest stages of the disease, show clear clinical correlations, and have prognostic value. The underlying process(es) represent an attractive treatment target, and appear at least partially distinct from those leading to demyelinating lesion formation. We now need to know the molecular and histopathological substrates underlying MRI-visible periventricular gradients, particularly whether or not they are pathologically equivalent to cortical gradients. A CSF-mediated process, secondary to meningeal intrathecal inflammation, currently represents one of the best explanations for cortical and periventricular gradients.

Potential biomarker candidates - cortical lesions, leptomeningeal enhancement, MTR gradients and CSF molecular profiling - require optimisation, standardisation and a systematic examination of their response to therapies. Subsequent comparisons with traditional metrics may then identify any true additional value, thereby contributing to more personalised prognostication and therapy in MS. MRI surrogates must also be sought in different diseases with meningeal inflammation to ensure, like the intrathecal CSF pattern (Magliozzi *et al.*, 2018b), they are specific to multiple sclerosis.

## Funding

M.P. is partly supported by an University of Genoa Curiosity Grant. J.W.L.B is funded through a Next Generation Fellowship funded by the Grand Charity of the Freemason's. RM is funded by the Italian MS Foundation grant (FISM 16/17/F14). RR is funded by the MS Society of Great Britain and Northern Ireland and the International Progressive MS Alliance. D.C has received research funding from the International Progressive MS Alliance, the MS Society, and the National Institute for Health Research (NIHR) University College London Hospitals (UCLH) Biomedical Research Centre.

## Competing interests

Matteo Pardini has received research support from Novartis and Nutricia. He has received fees from Novartis and Merck.

William Brown has received consulting and travel expenses from Biogen, Sanofi-Genzyme and Novartis.

Richard Reynolds has received speaker honoraria from Roche and Novartis.

In the last 3 years Declan Chard has received honoraria from Excemed for faculty-led education work. He is a consultant for Biogen and Hoffmann-La Roche.

## References


Absinta M, Vuolo L, Rao A, Nair G, Sati P, Cortese IC*, et al.* Gadolinium-based MRI characterization of leptomeningeal inflammation in multiple sclerosis. Neurology 2015; 85(1): 18-28.

Adams CW, Abdulla YH, Torres EM, Poston RN. Periventricular lesions in multiple sclerosis: their perivenous origin and relationship to granular ependymitis. Neuropathol Appl Neurobiol 1987; 13(2): 141-52.

Albert M, Antel J, Bruck W, Stadelmann C. Extensive cortical remyelination in patients with chronic multiple sclerosis. Brain pathology (Zurich, Switzerland) 2007; 17(2): 129-38.

Alcazar A, Regidor I, Masjuan J, Salinas M, Alvarez-Cermeno JC. Axonal damage induced by cerebrospinal fluid from patients with relapsing-remitting multiple sclerosis. Journal of neuroimmunology 2000; 104(1): 58-67.

Andeweg J. The anatomy of collateral venous flow from the brain and its value in aetiological interpretation of intracranial pathology. Neuroradiology 1996; 38(7): 621-8.

Androdias G, Reynolds R, Chanal M, Ritleng C, Confavreux C, Nataf S. Meningeal T cells associate with diffuse axonal loss in multiple sclerosis spinal cords. Annals of neurology 2010; 68(4): 465-76.

Assaf Y, Basser PJ. Composite hindered and restricted model of diffusion (CHARMED) MR imaging of the human brain. NeuroImage 2005; 27(1): 48-58.

Beggs CB. Venous hemodynamics in neurological disorders: an analytical review with hydrodynamic analysis. BMC medicine 2013; 11: 142.

Bevan RJ, Evans R, Griffiths L, Watkins LM, Rees MI, Magliozzi R*, et al.* Meningeal inflammation and cortical demyelination in acute multiple sclerosis. Annals of neurology 2018; 84(6): 829-42.

Bo L, Vedeler CA, Nyland HI, Trapp BD, Mork SJ. Subpial demyelination in the cerebral cortex of multiple sclerosis patients. Journal of neuropathology and experimental neurology 2003; 62(7): 723-32.

Brown JW, Pardini M, Brownlee WJ, Fernando K, Samson RS, Prados Carrasco F*, et al.* An abnormal periventricular magnetization transfer ratio gradient occurs early in multiple sclerosis. Brain : a journal of neurology 2017; 140(Pt 2): 387-98.

Brown JWL, Chowdhury A, Kanber B, Prados Carrasco F, Eshaghi A, Sudre CH*, et al.* Magnetisation transfer ratio abnormalities in primary and secondary progressive multiple sclerosis. Multiple sclerosis (Houndmills, Basingstoke, England) 2019a: 1352458519841810.

Brown JWL, Prados Carrasco F, Eshaghi A, Sudre CH, Button T, Pardini M*, et al.* Periventricular magnetisation transfer ratio abnormalities in multiple sclerosis improve after alemtuzumab. Multiple sclerosis (Houndmills, Basingstoke, England) 2019b: 1352458519852093.

Choi SR, Howell OW, Carassiti D, Magliozzi R, Gveric D, Muraro PA*, et al.* Meningeal inflammation plays a role in the pathology of primary progressive multiple sclerosis. Brain : a journal of neurology 2012; 135(Pt 10): 2925-37.

Cifelli A, Arridge M, Jezzard P, Esiri MM, Palace J, Matthews PM. Thalamic neurodegeneration in multiple sclerosis. Annals of neurology 2002; 52(5): 650-3.

Dawson J. The histology of disseminated sclerosis. Trans R Soc Edinb 1916; 50: 517-40.

De Santis S, Granberg T, Ouellette R, Treaba CA, Herranz E, Fan Q*, et al.* Evidence of early microstructural white matter abnormalities in multiple sclerosis from multi-shell diffusion MRI. NeuroImage Clinical 2019; 22: 101699.

Dewar D, Underhill SM, Goldberg MP. Oligodendrocytes and ischemic brain injury. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 2003; 23(3): 263-74.

Eisele P, Griebe M, Szabo K, Wolf ME, Alonso A, Engelhardt B*, et al.* Investigation of leptomeningeal enhancement in MS: a postcontrast FLAIR MRI study. Neurology 2015; 84(8): 770-5.
Engelhardt B, Wolburg-Buchholz K, Wolburg H. Involvement of the choroid plexus in central nervous system inflammation. Microsc Res Tech 2001; 52(1): 112-29.
Fadda G, Brown RA, Magliozzi R, Aubert-Broche B, O'Mahony J, Shinohara RT*, et al.* A surface-in gradient of thalamic damage evolves in pediatric multiple sclerosis. Annals of neurology 2019; 85(3): 340-51.
Fernando KT, Tozer DJ, Miszkiel KA, Gordon RM, Swanton JK, Dalton CM*, et al.* Magnetization transfer histograms in clinically isolated syndromes suggestive of multiple sclerosis. Brain : a journal of neurology 2005; 128(Pt 12): 2911-25.
Fog T. Topographic distribution of plaques in the spinal cord in multiple sclerosis. Archives of neurology 1950; 63: 382-414.
Frischer JM, Bramow S, Dal-Bianco A, Lucchinetti CF, Rauschka H, Schmidbauer M*, et al.* The relation between inflammation and neurodegeneration in multiple sclerosis brains. Brain : a journal of neurology 2009; 132(Pt 5): 1175-89.
Gardner C, Magliozzi R, Durrenberger PF, Howell OW, Rundle J, Reynolds R. Cortical grey matter demyelination can be induced by elevated pro-inflammatory cytokines in the subarachnoid space of MOG-immunized rats. Brain : a journal of neurology 2013; 136(Pt 12): 3596-608.
Geurts JJ, Roosendaal SD, Calabrese M, Ciccarelli O, Agosta F, Chard DT*, et al.* Consensus recommendations for MS cortical lesion scoring using double inversion recovery MRI. Neurology 2011; 76(5): 418-24.
Haider L, Simeonidou C, Steinberger G, Hametner S, Grigoriadis N, Deretzi G*, et al.* Multiple sclerosis deep grey matter: the relation between demyelination, neurodegeneration, inflammation and iron. Journal of neurology, neurosurgery, and psychiatry 2014; 85(12): 1386-95.
Haider L, Zrzavy T, Hametner S, Hoftberger R, Bagnato F, Grabner G*, et al.* The topograpy of demyelination and neurodegeneration in the multiple sclerosis brain. Brain : a journal of neurology 2016; 139(Pt 3): 807-15.
Hartung H, Curtin F, Schneble H, Pochet H, Glanzman R, Lambert E*, et al.* Week 24 results from a Phase IIb trial of GNbAC1 in patients with relapsing remitting multiple sclerosis (CHANGE-MS; Clinical trial assessing the HERV-W Env antagonist GNbAC1 for Efficacy in MS). Multiple Sclerosis Journal 2017; 23(3_suppl): 980.
Holland CM, Charil A, Csapo I, Liptak Z, Ichise M, Khoury SJ*, et al.* The relationship between normal cerebral perfusion patterns and white matter lesion distribution in 1,249 patients with multiple sclerosis. Journal of neuroimaging : official journal of the American Society of Neuroimaging 2012; 22(2): 129-36.
Howell OW, Reeves CA, Nicholas R, Carassiti D, Radotra B, Gentleman SM*, et al.* Meningeal inflammation is widespread and linked to cortical pathology in multiple sclerosis. Brain : a journal of neurology 2011; 134(Pt 9): 2755-71.
Howell OW, Rundle JL, Garg A, Komada M, Brophy PJ, Reynolds R. Activated microglia mediate axoglial disruption that contributes to axonal injury in multiple sclerosis. Journal of neuropathology and experimental neurology 2010; 69(10): 1017-33.
Howell OW, Schulz-Trieglaff EK, Carassiti D, Gentleman SM, Nicholas R, Roncaroli F*, et al.* Extensive grey matter pathology in the cerebellum in multiple sclerosis is linked to inflammation in the subarachnoid space. Neuropathol Appl Neurobiol 2015; 41(6): 798-813.
Ichimura T, Fraser PA, Cserr HF. Distribution of extracellular tracers in perivascular spaces of the rat brain. Brain research 1991; 545(1-2): 103-13.

Ighani M, Jonas S, Izbudak I, Choi S, Lema-Dopico A, Hua J*, et al.* No association between cortical lesions and leptomeningeal enhancement on 7-Tesla MRI in multiple sclerosis. Multiple sclerosis (Houndmills, Basingstoke, England) 2019: 1352458519876037.
Ivanov KP, Sokolova IB, Vovenko EP. Oxygen transport in the rat brain cortex at normobaric hyperoxia. Eur J Appl Physiol Occup Physiol 1999; 80(6): 582-7.
James RE, Schalks R, Browne E, Eleftheriadou I, Munoz CP, Mazarakis ND*, et al.* Persistent elevation of intrathecal pro-inflammatory cytokines leads to multiple sclerosis-like cortical demyelination and neurodegeneration. Acta neuropathologica communications 2020; 8(1): 66.
Jehna M, Pirpamer L, Khalil M, Fuchs S, Ropele S, Langkammer C*, et al.* Periventricular lesions correlate with cortical thinning in multiple sclerosis. Annals of neurology 2015; 78(4): 530-9.
Junker A, Wozniak J, Voigt D, Scheidt U, Antel J, Wegner C*, et al.* Extensive subpial cortical demyelination is specific to multiple sclerosis. Brain pathology (Zurich, Switzerland) 2020.
Kearney H, Yiannakas MC, Samson RS, Wheeler-Kingshott CA, Ciccarelli O, Miller DH. Investigation of magnetization transfer ratio-derived pial and subpial abnormalities in the multiple sclerosis spinal cord. Brain : a journal of neurology 2014; 137(Pt 9): 2456-68.
Kilsdonk ID, Jonkman LE, Klaver R, van Veluw SJ, Zwanenburg JJ, Kuijer JP*, et al.* Increased cortical grey matter lesion detection in multiple sclerosis with 7 T MRI: a post-mortem verification study. Brain : a journal of neurology 2016; 139(Pt 5): 1472-81.
Klaver R, Popescu V, Voorn P, Galis-de Graaf Y, van der Valk P, de Vries HE*, et al.* Neuronal and axonal loss in normal-appearing gray matter and subpial lesions in multiple sclerosis. Journal of neuropathology and experimental neurology 2015; 74(5): 453-8.
Kooij G, Kopplin K, Blasig R, Stuiver M, Koning N, Goverse G*, et al.* Disturbed function of the blood-cerebrospinal fluid barrier aggravates neuro-inflammation. Acta Neuropathol 2014; 128(2): 267-77.
Krumbholz M, Theil D, Cepok S, Hemmer B, Kivisakk P, Ransohoff RM*, et al.* Chemokines in multiple sclerosis: CXCL12 and CXCL13 up-regulation is differentially linked to CNS immune cell recruitment. Brain : a journal of neurology 2006; 129(Pt 1): 200-11.
Kutzelnigg A, Lucchinetti CF, Stadelmann C, Bruck W, Rauschka H, Bergmann M*, et al.* Cortical demyelination and diffuse white matter injury in multiple sclerosis. Brain : a journal of neurology 2005; 128(Pt 11): 2705-12.
Lassmann H. Review: the architecture of inflammatory demyelinating lesions: implications for studies on pathogenesis. Neuropathol Appl Neurobiol 2011; 37(7): 698-710.
Liu Z, Pardini M, Yaldizli O, Sethi V, Muhlert N, Wheeler-Kingshott CA*, et al.* Magnetization transfer ratio measures in normal-appearing white matter show periventricular gradient abnormalities in multiple sclerosis. Brain : a journal of neurology 2015; 138(Pt 5): 1239-46.
Lovato L, Willis SN, Rodig SJ, Caron T, Almendinger SE, Howell OW*, et al.* Related B cell clones populate the meninges and parenchyma of patients with multiple sclerosis. Brain : a journal of neurology 2011; 134(Pt 2): 534-41.
Lucchinetti CF, Popescu BF, Bunyan RF, Moll NM, Roemer SF, Lassmann H*, et al.* Inflammatory cortical demyelination in early multiple sclerosis. N Engl J Med 2011; 365(23): 2188-97.
Magliozzi R, Fadda G, Bar-Or A, Brown R, Mazziotti V, Nicholas R*, et al.* Substantial 'subependymal-in' gradient of thalamic damage in progressive multiple sclerosis. Multiple Sclerosis Journal 2018a; 24(S2): 94-5.
Magliozzi R, Howell O, Vora A, Serafini B, Nicholas R, Puopolo M*, et al.* Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. Brain : a journal of neurology 2007; 130(Pt 4): 1089-104.
Magliozzi R, Howell OW, Durrenberger P, Arico E, James R, Cruciani C*, et al.* Meningeal inflammation changes the balance of TNF signalling in cortical grey matter in multiple sclerosis. J Neuroinflammation 2019; 16(1): 259.

Magliozzi R, Howell OW, Nicholas R, Cruciani C, Castellaro M, Romualdi C*, et al.* Inflammatory intrathecal profiles and cortical damage in multiple sclerosis. Annals of neurology 2018b; 83(4): 739-55.
Magliozzi R, Howell OW, Reeves C, Roncaroli F, Nicholas R, Serafini B*, et al.* A Gradient of neuronal loss and meningeal inflammation in multiple sclerosis. Annals of neurology 2010; 68(4): 477-93.
Mahajan KR, Nakamura K, Cohen JA, Trapp BD, Ontaneda D. Intrinsic and Extrinsic Mechanisms of Thalamic Pathology in Multiple Sclerosis. Annals of neurology 2020; 88(1): 81-92.
Mainero C, Louapre C, Govindarajan ST, Gianni C, Nielsen AS, Cohen-Adad J*, et al.* A gradient in cortical pathology in multiple sclerosis by in vivo quantitative 7 T imaging. Brain : a journal of neurology 2015; 138(Pt 4): 932-45.
Martinez Sosa S, Smith KJ. Understanding a role for hypoxia in lesion formation and location in the deep and periventricular white matter in small vessel disease and multiple sclerosis. Clin Sci (Lond) 2017; 131(20): 2503-24.
Meinl E, Krumbholz M, Derfuss T, Junker A, Hohlfeld R. Compartmentalization of inflammation in the CNS: a major mechanism driving progressive multiple sclerosis. Journal of the neurological sciences 2008; 274(1-2): 42-4.
Menard A, Pierig R, Pelletier J, Bensa P, Belliveau J, Mandrand B*, et al.* Detection of a gliotoxic activity in the cerebrospinal fluid from multiple sclerosis patients. Neurosci Lett 1998; 245(1): 49-52.
Moreau T, Thorpe J, Miller D, Moseley I, Hale G, Waldmann H*, et al.* Preliminary evidence from magnetic resonance imaging for reduction in disease activity after lymphocyte depletion in multiple sclerosis. Lancet 1994; 344(8918): 298-301.
Oppenheimer DR. The cervical cord in multiple sclerosis. Neuropathol Appl Neurobiol 1978; 4(2): 151-62.
Papadopoulou A, Menegola M, Kuhle J, Ramagopalan SV, D'Souza M, Sprenger T*, et al.* Lesion-to-ventricle distance and other risk factors for the persistence of newly formed black holes in relapsing-remitting multiple sclerosis. Multiple sclerosis (Houndmills, Basingstoke, England) 2014; 20(3): 322-30.
Pardini M, Gualco L, Bommarito G, Roccatagliata L, Schiavi S, Solaro C*, et al.* CSF oligoclonal bands and normal appearing white matter periventricular damage in patients with clinically isolated syndrome suggestive of MS. Mult Scler Relat Disord 2019; 31: 93-6.
Pardini M, Sudre CH, Prados F, Yaldizli O, Sethi V, Muhlert N*, et al.* Relationship of grey and white matter abnormalities with distance from the surface of the brain in multiple sclerosis. Journal of neurology, neurosurgery, and psychiatry 2016.
Patrikios P, Stadelmann C, Kutzelnigg A, Rauschka H, Schmidbauer M, Laursen H*, et al.* Remyelination is extensive in a subset of multiple sclerosis patients. Brain : a journal of neurology 2006; 129(Pt 12): 3165-72.
Poirion E, Bodini B, Benoit C, Tonietto M, Bera G, Giannesini C*, et al.* A gradient of periventricular microglial activation suggests a CSF-derived neurodegenerative component in MS. Multiple Sclerosis Journal 2017; 23(3_suppl): 80.
Prineas JW, Kwon EE, Cho ES, Sharer LR, Barnett MH, Oleszak EL*, et al.* Immunopathology of secondary-progressive multiple sclerosis. Annals of neurology 2001; 50(5): 646-57.
Prineas JW, Wright RG. Macrophages, lymphocytes, and plasma cells in the perivascular compartment in chronic multiple sclerosis. Lab Invest 1978; 38(4): 409-21.
Reali C, Magliozzi R, Roncaroli F, Nicholas R, Howell OW, Reynolds R. B cell rich meningeal inflammation associates with increased spinal cord pathology in multiple sclerosis. Brain pathology (Zurich, Switzerland) 2020.

Reboldi A, Coisne C, Baumjohann D, Benvenuto F, Bottinelli D, Lira S*, et al.* C-C chemokine receptor 6-regulated entry of TH-17 cells into the CNS through the choroid plexus is required for the initiation of EAE. Nat Immunol 2009; 10(5): 514-23.
Ribbert H. Ueber multiple sclerose des Gehirns und Rückenmarks; 1882.
Rossi S, Furlan R, De Chiara V, Motta C, Studer V, Mori F*, et al.* Interleukin-1beta causes synaptic hyperexcitability in multiple sclerosis. Annals of neurology 2012; 71(1): 76-83.
Rossi S, Motta C, Studer V, Barbieri F, Buttari F, Bergami A*, et al.* Tumor necrosis factor is elevated in progressive multiple sclerosis and causes excitotoxic neurodegeneration. Multiple sclerosis (Houndmills, Basingstoke, England) 2014; 20(3): 304-12.
Samson RS, Brownlee WJ, Cardoso M, J. , Brown JWL, Pardini M, F. PC*, et al.* Outer and inner cortical MTR abnormalities in clinically isolated syndromes. Multiple Sclerosis Journal 2016; 22(3 (Supp)): 30.
Samson RS, Cardoso MJ, Muhlert N, Sethi V, Wheeler-Kingshott CA, Ron M*, et al.* Investigation of outer cortical magnetisation transfer ratio abnormalities in multiple sclerosis clinical subgroups. Multiple sclerosis (Houndmills, Basingstoke, England) 2014; 20(10): 1322-30.
Schmierer K, Scaravilli F, Altmann DR, Barker GJ, Miller DH. Magnetization transfer ratio and myelin in postmortem multiple sclerosis brain. Annals of neurology 2004; 56(3): 407-15.
Schmierer K, Wheeler-Kingshott CA, Boulby PA, Scaravilli F, Altmann DR, Barker GJ*, et al.* Diffusion tensor imaging of post mortem multiple sclerosis brain. NeuroImage 2007; 35(2): 467-77.
Seewann A, Kooi EJ, Roosendaal SD, Pouwels PJ, Wattjes MP, van der Valk P*, et al.* Postmortem verification of MS cortical lesion detection with 3D DIR. Neurology 2012; 78(5): 302-8.
Tallantyre EC, Morgan PS, Dixon JE, Al-Radaideh A, Brookes MJ, Evangelou N*, et al.* A comparison of 3T and 7T in the detection of small parenchymal veins within MS lesions. Investigative radiology 2009; 44(9): 491-4.
Varga AW, Johnson G, Babb JS, Herbert J, Grossman RI, Inglese M. White matter hemodynamic abnormalities precede sub-cortical gray matter changes in multiple sclerosis. Journal of the neurological sciences 2009; 282(1-2): 28-33.
Vidaurre OG, Haines JD, Katz Sand I, Adula KP, Huynh JL, McGraw CA*, et al.* Cerebrospinal fluid ceramides from patients with multiple sclerosis impair neuronal bioenergetics. Brain : a journal of neurology 2014; 137(Pt 8): 2271-86.
Vovenko EP. Transmural oxygen tension gradients in rat cerebral cortex arterioles. Neurosci Behav Physiol 2009; 39(4): 363-70.
Vrenken H, Geurts JJ, Knol DL, Polman CH, Castelijns JA, Pouwels PJ*, et al.* Normal-appearing white matter changes vary with distance to lesions in multiple sclerosis. AJNR American journal of neuroradiology 2006; 27(9): 2005-11.
Wang J, Wang J, Sun J, Gong X. Evaluation of the anatomy and variants of internal cerebral veins with phase-sensitive MR imaging. Surg Radiol Anat 2010; 32(7): 669-74.
Wattjes MR, A; Miller, D; Yousry TA; Sormani, MP; de Stefano, N; Tintoré, M; Auger, C; Tur, C; Filippi, M; Rocca, MA; Fazekas, F; Kappos, L; Polman, C; Barkhof, F; Montalban, X. Evidence-based guidelines: MAGNIMS consensus guidelines on the use of MRI in multiple sclerosis—establishing disease prognosis and monitoring patients. Nature Reviews Neurology 2015; 11: 597–606.
Wentling M, Lopez-Gomez C, Park HJ, Amatruda M, Ntranos A, Aramini J*, et al.* A metabolic perspective on CSF-mediated neurodegeneration in multiple sclerosis. Brain : a journal of neurology 2019.
Wuerfel J, Haertle M, Waiczies H, Tysiak E, Bechmann I, Wernecke KD*, et al.* Perivascular spaces--MRI marker of inflammatory activity in the brain? Brain : a journal of neurology 2008; 131(Pt 9): 2332-40.

Zivadinov R, Ramasamy DP, Vaneckova M, Gandhi S, Chandra A, Hagemeier J*, et al.* Leptomeningeal contrast enhancement is associated with progression of cortical atrophy in MS: A retrospective, pilot, observational longitudinal study. Multiple sclerosis (Houndmills, Basingstoke, England) 2017; 23(10): 1336-45.
Zurawski J, Lassmann H, Bakshi R. Use of Magnetic Resonance Imaging to Visualize Leptomeningeal Inflammation in Patients With Multiple Sclerosis: A Review. JAMA neurology 2017; 74(1): 100-9.
Zurawski J, Tauhid S, Chu R, Khalid F, Healy BC, Weiner HL*, et al.* 7T MRI cerebral leptomeningeal enhancement is common in relapsing-remitting multiple sclerosis and is associated with cortical and thalamic lesions. Multiple sclerosis (Houndmills, Basingstoke, England) 2020; 26(2): 177-87.

## Figure Legends

### Figure 1

Brain segmentation into concentric bands between the ventricular walls and pial surfaces. **Inset A**: periventricular extralesional white matter (WM) MTR in each band comparing people with RRMS, SPMS, PPMS and healthy controls. Mean ± 2 standard errors. MTR is given in per cent units (pu). **Inset B**: periventricular extralesional WM MTR in each band comparing healthy controls to patients 4.6 months after a clinically isolated optic neuritis (ON) divided into those who did convert to clinically definite MS (CDMS) (yellow) and those that did not (green). **Inset C:** periventricular grey matter (GM) MTR in each band comparing patients with RRMS, SPMS, PPMS and healthy controls. **Inset D:** periventricular lesional white matter MTR in each band in patients with RRMS, SPMS and PPMS. **Inset E**: cortical GM MTR in each band comparing people with relapsing-remitting multiple sclerosis (RRMS), secondary progressive multiple sclerosis (SPMS), primary progressive multiple sclerosis (PPMS) and healthy controls.

### Figure 2

Gradient of demyelination (**A, D, G**), microglia activation (**C, E, H**) and axonal loss (**F**), evident in the motor cortex (**A-C**), thalamus (**D-F**) and hippocampus (**G, H**) in post-mortem progressive MS. Extensive subpial cortical demyelination (**A**) is often associated with increased meningeal inflammation, diffuse or aggregated in lymphoid-like aggregates (**B**). Substantial increase of MHC-class II+ activated microglia/macrophages density is observed in the external cortical layers in close proximity to the pial surface (**C**) but not in the internal cortical layers, close to the white matter, according to a surface-in gradient.

Similar gradient of demyelination (**D, G**) and increased density of MHC-class II+ activated microglia/macrophages is seen in the thalamus (**D-F**) and hippocampus (**G-H**), in close proximity to the ependyma surface. Surface-in gradient of axonal loss (**F**) as visualized by Bielschosky staining, is also observed in a thalamic lesion (**D**) in the external areas close to the ependyma.

### Figure 3

While meningeal and ependymal factors may explain local gradients in pathology, alone they do not readily link gradients seen in both grey and white matter. Instead, proximity to CSF provides a more plausible unifying explanation, but also means that anything that can diffuse into the CSF may be relevant, including constituents arising from the brain's extracellular fluid or blood (via the choroid plexus). It also complicates the search for a cause for meningeal and ependymal inflammation, as this

may not necessarily be the source of the factor responsible for surface-in gradients in brain pathology, but instead be another consequence of it.