MINI-SYMPOSIUM: RECENT ADVANCES IN NEUROIMAGING IN MULTIPLE SCLEROSIS, AND THEIR NEUROPATHLOGICAL SIGNIFICANCE

MRI of cortical lesions and its use in studying their role in MS pathogenesis and disease course

R. Magliozzi[1,2], R. Reynolds[2] and M. Calabrese[1]

[1] Neurology B, Department of Neurological and Movement Sciences, University of Verona, Verona, Italy.
[2] Division of Brain Sciences, Department of Medicine, Imperial College London, London, United Kingdom.

Keywords
grey matter lesions, cortical pathology, atrophy, intrathecal inflammation, meningeal infiltrates

Corresponding author:
R. Magliozzi, Neurology B, Dept. of Neurological and Movement Sciences, University of Verona, Italy (E-mail: rmagliozzi73@gmail.com, roberta.magliozzi@univr.it)

Received 7 June 2018
Accepted 21 June 2018

doi:10.1111/bpa.12642

Abstract

Cortical grey matter (GM) demyelination is present from the earliest stages of multiple sclerosis (MS) and is associated with physical deficits and cognitive impairment. In particular, the rate of disability progression in MS, both in the relapsing and progressive phases, appears to be strictly associated with degenerative GM demyelination and diffuse cortical atrophy. In the last decade, several histopathological studies and advanced radiological methodologies have contributed to better identify the exact involvement/load of cortical pathology in MS, even if the specific inflammatory features and the precise cell and molecular mechanisms of GM demyelination and neurodegeneration in MS remain still not fully understood. It has been proposed that a combined neuropathology, imaging and molecular approach may help to define a more detailed characterization and precise assessment of the heterogeneous features of GM injury and inflammation in MS. This, in turn, will possibly identify specific imaging and biohumoral (cerebrospinal fluid/serum) correlates of cortical pathology that may have an important role in predicting and monitor the disease evolution.

CORTICAL LESIONS IN MULTIPLE SCLEROSIS

Multiple sclerosis (MS) has long been considered a predominantly white matter (WM) disease, based on the obvious demyelination detectable in the WM by histological staining and conventional magnetic resonance imaging (MRI). WM lesions and perivascular inflammation are undoubtedly of major importance in contributing to focal clinical deficits in MS during the relapsing-remitting (RR) disease stage, but neurodegeneration is thought to play a key role in the progressive stage of the disease and to drive clinical disease progression (50).

Although already highlighted in earlier studies that investigated cortical grey matter (GM) pathology in MS (8, 18), it is only during the last 15 years that immunohistochemical and advanced imaging techniques have allowed establishing the true extent and clinical impact of GM involvement in MS. The pathology of progressive MS is characterised by the combined accumulation of chronic demyelinated lesions and axonal loss in the WM, diffuse changes to the normal appearing WM and diffuse and focal cortical GM demyelination and neurodegeneration (16, 26, 29, 33, 37, 43).

Even though demyelinated cortical lesions and brain atrophy are considered as one of the substrates of MS disease progression (12, 23, 25, 51, 65, 66), they have also been shown to be present from the earliest stages of the disease (16,20,40). Furthermore, significant neurodegeneration, including axonal abnormalities and neuronal and synaptic loss, is thought to represent the main pathological substrate of cognitive deterioration at all disease stages (11, 14, 30, 64).

The high prevalence of demyelination in GM areas in MS was detected by a study combining MRI and conventional histology data on 12 post-mortem brains (34). It was hypothesized that the distribution of cortical lesions might be causally related to vascular anatomy, which resulted in the definition of seven distinct types of cortical lesions. Later, another classification system was developed that distinguished between three major cortical lesion types, based on immunohistochemical detection of myelin (47). This is the scoring system that is currently widely used for classification of cortical lesions in MS tissue: type 1 lesions extend across both WM and gray matter and are often called leucocortical lesions; type 2 lesions are contained within the cerebral cortex GM and often occur around a blood vessel; type 3 lesions are subpial and affect the largest cortical area.

In one study type 3 leucocortical lesions represented 18% of lesions and accounted for 14.5% of the demyelinated area, type 2 intracortical lesions represented 17% of GM lesions and accounted for 1.2% of the cortical

lesions area and type 3 subpial lesions represented 60% of the lesions and accounted for 67% of the total cortical demyelinated area (5). A common appearance of type 3 lesions was that of long ribbons of subpial demyelination, often affecting several adjacent gyri. Other type 3 lesions were wedge-shaped, with the base at the surface of the brain. Additionally, a combination of these patterns, with wedge-like lesion areas within bands of more superficial subpial demyelination, was often present.

When this classification system was applied to biopsy samples from patients with early stages of MS, 37% revealed clear evidence of cortical demyelination and general cortical subpial demyelination has been identified as a distinct pattern occurring in a significant subpopulation of MS patients, particularly those with a long and progressive disease course (5). A more recent study of GM and WM demyelination in different regions of the CNS (motor cortex, cingulate, cerebellum, spinal cord and thalamus) found that the area covered by demyelination was greater in the cortex than in the WM at each anatomical site, but in particular GM demyelination was most extensive in the spinal cord and cerebellum (29).

Cortical GM lesions are characterised by the relative absence of lymphocyte infiltration, complement deposition and blood brain barrier (BBB) disruption compared to WM lesions (5, 7, 60), although this distinction is not absolute and significant intracortical lymphocytic infiltrates have been noted in some MS cases (44). Moreover, cortical lesions are thought to be characterized by a dominant effector cell population of ramified microglia (47).

A high frequency of vessels with tight junction abnormalities, which are a sign of BBB compromise, have been shown to be present in the GM, particularly in SPMS cases with long disease duration (39). These changes appeared less severe compared to the WM and appeared not associated with increased cortical inflammation, astrogliosis and complement deposition (60). A striking association between microglia and neurons has been reported in cortical MS lesions. Double-labelling confocal microscopy detected elongated microglia oriented perpendicularly to the pial surface, closely apposed, and often ensheathing apical dendrites and axons in active and chronic active cortical lesions. In addition, other more ramified stellate microglia often extended processes to neuronal perikarya and ensheathed dendrites or axons (21, 59). Unlike microglia/macrophages in WM lesions, which often apposed the terminal ends of transacted axons, microglia in cortical lesions did not consistently associate with the terminal ends of transected neurites.

INTRATHECAL INFLAMMATION AND SUBPIAL CORTICAL DAMAGE

The lack of substantial inflammatory infiltrates, complement deposition and BBB damage in MS cortical lesions led to the initial suggestion that the mechanisms underlying GM and WM pathology may substantially differ (5, 7, 60) and that activated microglia may represent one of the dominant effector cell populations mediating GM damage (47, 61). Only during the last 10 years has it become increasingly clear that immune activation in the meningeal compartment may have a key role in causing damage in the adjacent cerebral cortex. Besides the fact that most cortical lesions are subpial, numerous studies in experimental models and in post-mortem MS brain tissue support this idea (32, 33, 37, 38, 40–42, 49, 55, 58). In particular, it has been shown that secondary progressive MS cases characterized by a high levels of inflammatory infiltrates in the meninges, diffuse or organized in B-cell follicles-like structures, (Figure 1) have more and larger subpial GM lesions, earlier age of disease and disability onset, and earlier age of death (33, 42). The strong association between meningeal inflammation and severity of pathology has been recently substantiated in the spinal cord (2, 19, 32). In addition, increased meningeal inflammation has been shown to be strictly associated with a "surface-in" gradient of neuronal, astrocyte and oligodendrocyte loss accompanied by microglia activation greatest in the most external cortical layers (I-III) close to the CSF surface and decreasing in the most inner ones close to the WM (43). The fact that similar alterations have been found also in the normal appearing GM suggest that the pathology is not only restricted to demyelinated areas but is possibly a diffuse event occurring all over the brain and spinal cord.

At the same time, in type II intracortical lesions focal lymphocytic cuffing has been described in the more superficial GM layers of SPMS cases with prominent meningeal inflammation and extensive cortical pathology either in post-mortem MS cases (44) or in brain biopsies from patients with a recent diagnosis of MS (40).

It has therefore been suggested that while WM demyelination and type II cortical lesions may be preferentially influenced by perivenular inflammation, CSF inflammation may mediate subpial and periventricular demyelination, possibly through a gradient "surface-in" (14,45,49). In order to verify this hypothesis, a combined neuropathology, molecular and imaging study on post-mortem MS cases and in MS patients at the time of diagnosis has been performed demonstrating that a common pattern of intrathecal (meninges and CSF) inflammatory profile strongly correlates with increased cortical pathology, both at time of the diagnosis and of death. In particular, increased expression of pro-inflammatory cytokines (IFNγ, TNF, IL2 and IL22) and molecules related to sustained B-cell activity and lymphoid-neogenesis (CXCL13, CXCL10, LTα, IL6, IL10) was detected in paired meninges and CSF of rapidly progressive post-mortem MS cases with high levels of meningeal inflammation and GM demyelination (45). Significant ($P < 0.0001$) positive correlation was, in fact, detected non only between degree of meningeal inflammation and percentage of cortical demyelination (r = 0.968), but also specifically between degree of meningeal inflammation and CSF levels of CXCL13 (r = 0.943), IFNγ (r = 0.718), IL10 (r = 0.627), CCL22 (r = 0.603), IL16 (r = 0.568) and TNF (r = 0.553) in post-mortem progressive MS patients (Figure 2).

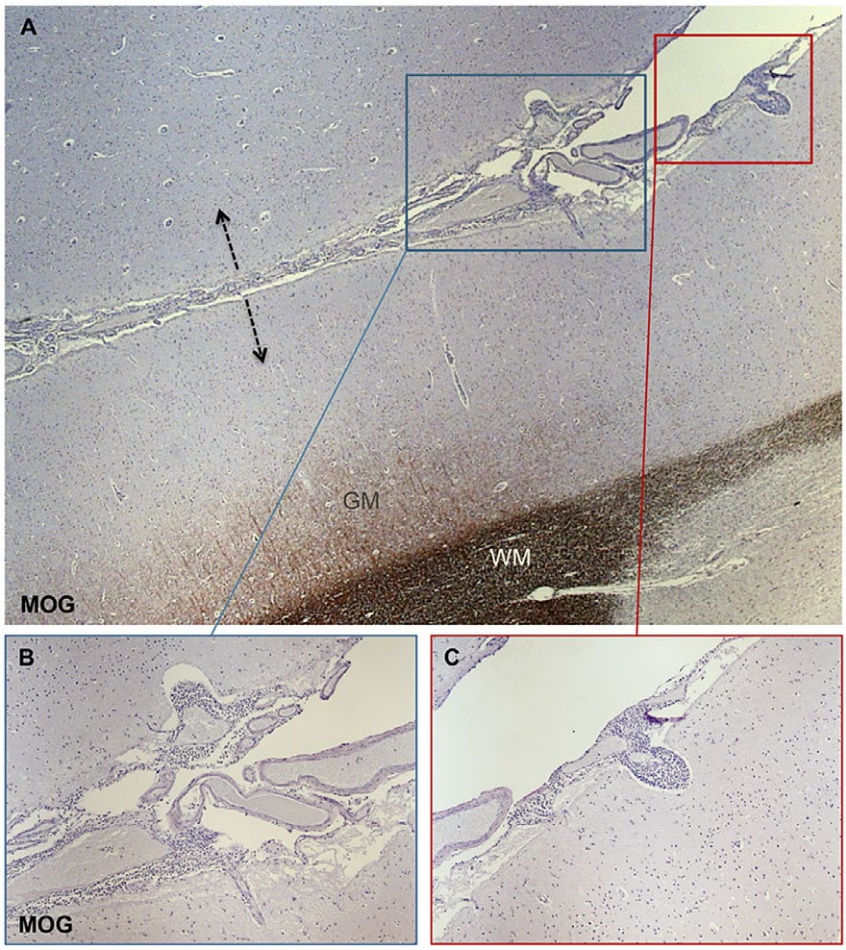

Figure 1. Neuropathology assessment of subpial cortical demyeliantion associated with meningeal inflammatory infiltrates. MOG immunostaining **(A-C)** allows the detection of subpial cortical lesions that are often expanding from both sides (arrows in **A**) of cerebral sulci as specular images. Within the meninges lining on the pial surface numerous extarvasating inflammatory infiltrates are evident (**B** and **C**). Some of them appeared invading the adjacent, superficial cortical layers (**B** and **C**).

This finding corroborates the hypothesis that meningeal infiltrates, diffuse or aggregated in lymphoid-structures, are strongly associated with CSF inflammatory milieu. Similar pro-inflammatory patterns, including increased levels of CXCL13, TNF, IFNγ, CXCL12, IL6, IL8 and IL10, together with high levels of BAFF, APRIL, LIGHT, TWEAK, sTNFR1, sCD163, MMP2 and pentraxin III, were detected in the CSF of MS patients with higher levels of GM lesions detected by 3T double inversion recovery (DIR) imaging at diagnosis (46). Furthermore, cortical lesion load on MRI was found correlated not only with highly inflamed CSF and presence of oligoclonal bands (OCB), but also with elevated CSF levels of neurofilament protein (NF-L), suggesting that intrathecal inflammatory milieu may be one of the key determinants of the rate of cortical demyelination and neurodegeneration in MS (22, 45). In particular, it has been proposed that a specific panel of molecules commonly identified in both neuropathological and clinical parts of the study, including CXCL13, IL10, TNF and IFNγ, might represent a useful prognostic signature of cortical damage and meningeal inflammation, possibly in a subgroup of patients with more rapid and severe disease progression (45). This strongly supports the existence of a "cortical variant" of MS in which the cerebral cortex is predominantly involved.

The finding that cortical pathology can be detected early on *in vivo* is fundamental in order to be able to effectively target the underlying mechanisms of progressive disease before significant disability develops (27, 53). Despite several improvements, including the introduction of GM specific pulse sequences at high field 3T (9) and ultra-high field 7T MRI (35), detailed CSF profiling may give more potential for the development of surrogate biomarkers of cortical GM demyelination/neurodegeneration and compartmentalised meningeal inflammation. This in turn could help to identify those individuals at risk of a more rapid and severe disease course early in the disease course.

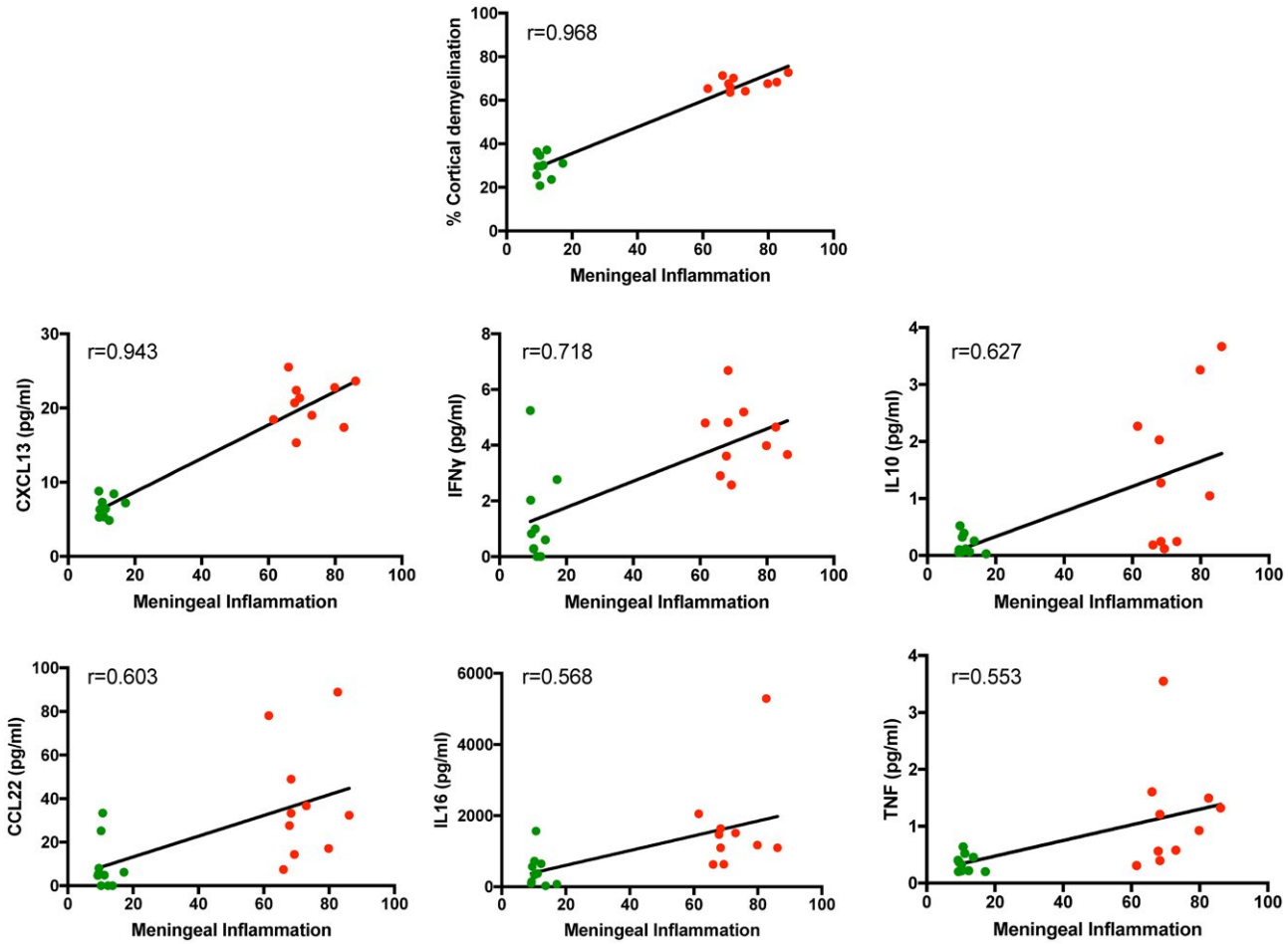

Figure 2. Graphs reporting Pearson correlations between degree of meningeal inflammation and percentage of cortical demyelination (r = 0.968) and CSF levels of CXCL13 (r = 0.943), IFNγ (r = 0.718), IL10 (r = 0.627), CCL22 (r = 0.603), IL16 (r = 0.568) and TNF (r = 0.553) in post-mortem MS patients. All the correlations were significant ($P < 0.001$).

CORTICAL LESION DETECTION BY MAGNETIC RESONANCE

Despite this knowledge and the detailed histological studies, *in vivo* detection of cortical MS lesions using MRI is challenging. A large proportion of cortical lesions go undetected on conventional MRI using standard field strength (17, 27) for several reasons including their small size (53), the anatomical paucity of myelin in the cortex generating little MRI contrast upon demyelination and the partial volume effects from adjacent CSF and WM (34, 47). Furthermore, most of the GM lesions affect the outermost layers of the cortex, in close contact with the sub-arachnoid space, resulting in susceptibility artefact at the interface between cortex and CSF (52). In addition, in contrast to WM lesions, cortical GM lesions show a lack of substantial focal infiltration of blood-derived leukocytes into the cortex (5, 40, 44, 47), complement deposition and BBB damage (5, 7, 60).

Several improvements have been achieved in the last decades, through the introduction of inversion recovery (6) and by developing GM specific pulse sequences such as DIR, DIR (Figure 3) or phase sensitive inversion recovery (9, 28, 54, 56, 57, 62), and by moving to high field 3 T and ultra-high field 7 T MRI systems (12, 24, 35, 63). High field strength analysis of post-mortem MS brain slices has allowed detection of a larger number and a more precise localization of the cortical lesions (3, 29, 36, 52). MP2RAGE and T2*-weighted imaging at 7T have recently been shown to improve detection of leukocortical and juxtacortical lesions in the early disease stages, but not intracortical or subpial lesions (4, 31). However, it still remains unclear whether it could be possible to translate these methods into routine clinical practice.

During the last 10 years, increased focus on the neuroimmunological mechanisms underlying GM pathology has demonstrated that meningeal immune cell accumulation, compartmentalized within the subarachnoid space, as well as altered microglial activity, may have a key role in the pathogenesis of the damage in the cerebral cortex. Similarly, advanced 7 Tesla MRI methodology has been able to show the presence of a gradient of subpial cortical alterations (46), strictly resembling the increased severity of demyelination, neuronal loss and microglia activation

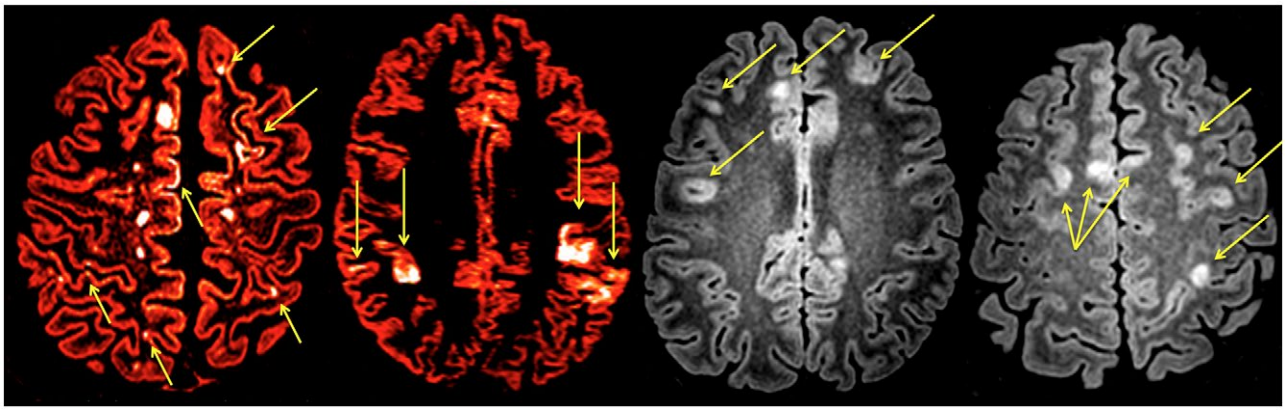

Figure 3. Axial double inversion recovery (DIR) sequences from 4 different subject suffering from relapsing remitting multiple sclerosis (MS). Several cortical lesions are highlighted; intracortical lesions (Yellow arrows), leucocortical lesions (Blu arrows), subpial cortical lesions (White arrow). DIR (**A** and **B**) are red colored to better highlight some cortical lesions.

in the most external cortical layers respect to the inner ones (43). Furthermore, the use of 3D DIR and 3D- Echo Planar Imaging (EPI) SWI has recently shown that CLs susceptibility maps highly reproduce the heterogeneous activity features of GM damage, even at individual level and that QSM hyperintensity edge found in proximity to the pial surface might be due to the subpial gradient of microglial activation (15).

CLINICAL RELEVANCE OF CORTICAL LESIONS

1) Cortical lesions are an early and frequent phenomenon in MS and correlate with disability

In a study conducted in a large patient population, cortical lesions were detected by MRI in the majority (64%) of patients with relapsing remitting (RRMS) and secondary progressive (70%) MS (SPMS), as well as in more than one-third (36.8%) of patients with clinically isolated syndromes (CIS) suggestive of MS (9). In some cases cortical lesions have been detected even before the appearance of WM lesions, thus suggesting that the development of cortical inflammation is an early phenomenon in MS at least partly independent of WM lesions (10).

2) Cortical lesions correlate with physical disability and its progression

The number of cortical lesions convincingly correlate with the severity of the disease: patients with cortical lesions show a higher Expanded Disability Status Scale (EDSS) score, a higher WM T2 lesion volume, a lower brain parenchyma fraction and a higher frequency of CSF IgG oligoclonal bands (IgGOBs), when compared with patients without cortical lesions. A recent study based on 5-year longitudinal observations of more than 300 MS patients with different clinical phenotypes showed that patients with a high cortical lesion load at baseline had the worst clinical evolution and the fastest progression of cortical atrophy after 5 years. Cortical lesion load associated not only with baseline EDSS, but also with the EDSS change and with the % change of GM fraction; such an association being observed in all clinical subsets. Based on the multivariate analysis the authors concluded that CL volume was an independent predictor of disability progression (12). Interestingly, RRMS and SPMS patients were found to accumulate new cortical lesions at a similar rate (0.8/year in RRMS vs. 1.0/year in SPMS), suggesting that, in relapse-onset MS, the medium-term dynamics of cortical lesion evolution may not be influenced by the disease stage. The higher number of cortical lesions observed in SPMS vs. RRMS patients is clearly the consequence of the longer disease duration of the former subgroup, as also indicated by post-mortem studies (37, 42) showing that demyelination and axonal damage in the GM are already present in RRMS patients but become more prominent in the chronic stage of the disease. Finally, the cortical lesion load in the relapsing remitting phase together with the cerebellar volume and age have been suggested as independent markers of the evolution to the secondary progressive phase of the disease (13).

In line with these results are the data coming from patients with the Primary Progressive form of MS (PPMS) (10). In the PPMS population, cortical lesions were observed in up to 80% of the patients examined and were significantly correlated with disease duration, EDSS score, as well as with increasing GM atrophy and disability during the follow up. A multivariate analysis revealed that cortical lesion volume at baseline was an independent predictor of percentage GM volume change and disability accumulation during the subsequent two-year period.

On the contrary, patients with a "benign" MS course (EDSS < 3.0 after 15 years from clinical onset and without any cognitive dysfunction) showed significantly lower cortical lesion number and volume compared to early RRMS patients with the same degree of disability, but much shorter disease duration. After one-year follow-up, "benign"

MS patients did not show an accumulation of cortical lesions compared to early RRMS patients (10). Also in these subgroups the multivariate analysis indicated that cortical lesion number and volume at baseline and their volume change were independent predictors of the clinical status. These findings suggest that the relative sparing of the cortex in benign MS might contribute to the more favorable clinical status in these patients.

3) Cortical lesions as a major substrate of cognitive dysfunction

Cortical pathology may have a significant impact not only on clinical disability but also on cognitive dysfunction in MS. A recent study clearly demonstrated that cortical GM damage significantly contributes to the cognitive decline observed in MS patients. Indeed, patients suffering from RRMS with cognitive deficits had more cortical lesions and atrophy than cognitively normal MS patients. These results are in agreement with neuropathology (28) and imaging (1) studies, which demonstrated a significant cortical volume reduction in cognitive impaired RRMS patients, but neither investigated the role of focal demyelinating lesions in the cortex, nor elucidated how the burden of cortical lesions and the extent of cortical atrophy are interrelated. We observed that cortical lesions number and volume were significantly higher in cognitively impaired RRMS patients compared to those without cognitive deficits (10). Similar results were also obtained when using a conservative approach to the definition of cognitive impairment. Considering that previous studies assessing the relationship between the extent of WM damage and cognitive impairment in MS patients have given conflicting results (1, 51), our data strengthen the notion that the overall burden of WM MRI-visible lesions does not fully account for the severity of cognitive impairment in MS. Such a notion was reinforced further by the results of the multivariate analysis, which revealed that only cortical lesion volume and, even if at a lesser extent, neocortical volume loss are independent predictors of the composite cognitive score (10). On the contrary the evidence is quite strong that early cortical lesions might be helpful in the identification of MS patients at high risk of disability progression (48).

CONCLUSIONS

Despite the exact underlying molecular mechanism are not completely understood, the above mentioned studies suggest that meningeal inflammation and the consequent cortical microglia activation lead to progressive cortical demyelination and neurodegeneration since the MS onset to end-stage of the disease. Such GM pathology has been demonstrated to have a great impact on each clinical parameter, from disability progression to cognitive dysfunction, thus influencing the long-term prognosis of the disease.

For such reason we should improve our ability to detect it since the earliest phases of the disease, we should identify the molecular mechanisms underlying it and finally we should detect specific biomarkers able to highlight those patients at high risk of early neurodegeneration.

Thus, a more detailed characterization and precise assessment of the features and the extent of GM demyelination and of the meningeal and cortical inflammation in MS is required in order to identify imaging and biohumoral (CSF/serum) correlates of cortical pathology that may have an important role in predicting and monitor the disease evolution.

By defining more precisely the substrates of the cerebral cortical damage in MS and their relationship with cortical changes detectable by combined imaging, neuropathological and molecular analysis, this, in turn, will open up an avenue for the development of an effective clinical and therapeutic approach for slowing or stopping the progressive course of MS.

REFERENCES

1. Amato MP, Zipoli V, Portaccio E (2006) Multiple sclerosis-related cognitive changes: a review of cross-sectional and longitudinal studies. *J Neurol Sci* **245**:41–46.
2. Androdias G, Reynolds R, Chanal M, Ritleng C, Confavreux C, Nataf S (2010) Meningeal T cells associate with diffuse axonal loss in multiple sclerosis spinal cords. *Ann Neurol* **68**:465–476.
3. Bagnato F, Yao B, Cantor F, Merkle H, Condon E, Montequin M *et al* (2009) Multisequence-imaging protocols to detect cortical lesions of patients with multiple sclerosis: observations from a post-mortem 3 Tesla imaging study. *J Neurol Sci* **282**:80–85.
4. Beck ES, Sati P, Sethi V, Kober T, Dewey B, Bhargava P *et al* (2018) Improved visualization of cortical lesions in multiple sclerosis using 7T MP2RAGE. *Am J Neuroradiol* **39**:459–466.
5. Bø L, Vedeler C, Nyland H, Trapp B, Mørk S (2003) Intracortical multiple sclerosis lesions are not associated with increased lymphocyte infiltration. *Mult Scler* **9**:323–331.
6. Boggild MD, Williams R, Haq N, Hawkins CP (1996) Cortical plaques visualised by fluid-attenuated inversion recovery imaging in relapsing multiple sclerosis. *Neuroradiology* **38**(Suppl 1):S10–S13.
7. Brink BP, Veerhuis R, Breij ECW, Van Der Valk P, Dijkstra CD, Bö L (2005) The pathology of multiple sclerosis is location-dependent: no significant complement activation is detected in purely cortical lesions. *J Neuropathol Exp Neurol* **64**:147–155.
8. Brownell B, Hughes JT (1962) The distribution of plaques in the cerebrum in multiple sclerosis. *J Neurol Neurosurg Psychiatry* **25**:315–320.
9. Calabrese M, De Stefano N, Atzori M, Bernardi V, Mattisi I, Barachino L *et al* (2007) Detection of cortical inflammatory lesions by double inversion recovery magnetic resonance imaging in patients with multiple sclerosis. *Arch Neurol* **64**:1416–1422.
10. Calabrese M, Gallo P (2009) Magnetic resonance evidence of cortical onset of multiple sclerosis. *Mult Scler* **15**:933–941.
11. Calabrese M, Rinaldi F, Grossi P, Gallo P (2011) Cortical pathology and cognitive impairment in multiple sclerosis. *Exp Rev of Neurother* **11**:425–432.

12. Calabrese M, Poretto V, Favaretto A, Alessio S, Bernardi V, Romualdi C *et al* (2012) Cortical lesion load associates with progression of disability in multiple sclerosis. *Brain* **135**:2952–2961.
13. Calabrese M, Favaretto A, Martini V, Gallo P (2013) Grey matter lesions in MS From histology to clinical implications. *Prion* **7**:20–27.
14. Calabrese M, Magliozzi R, Ciccarelli O, Geurts JJG, Reynolds R, Martin R (2015) Exploring the origins of grey matter damage in multiple sclerosis. *Nat Rev Neurosci* **16**:147–158. Nature Publishing Group.
15. Castellaro M, Magliozzi R, Palombit A, Pitteri M, Silvestri E, Camera V *et al* (2017) Heterogeneity of cortical lesion susceptibility mapping in multiple sclerosis. *Am J Neuroradiol* **38**:1087–1095.
16. Chard D, Miller D (2009) Grey matter pathology in clinically early multiple sclerosis: evidence from magnetic resonance imaging. *J Neurol Sci Elsevier B.V.* **282**:5–11.
17. Daams M, Geurts JJG, Barkhof F (2013) Cortical imaging in multiple sclerosis: recent findings and "grand challenges". *Curr Opin Neurol* **26**:345–352.
18. Dawson JW (1916) The histology of disseminated sclerosis. *Trans R Soc Edinburgh* **50**:517–740.
19. Deluca GC, Alterman R, Martin JL, Mittal A, Blundell S, Bird S *et al* (2013) Casting light on multiple sclerosis heterogeneity: the role of HLA-DRB1 on spinal cord pathology. *Brain* **136**:1025–1034.
20. De Stefano N, Matthews PM, Filippi M, Agosta F, De Luca M, Bartolozzi ML *et al* (2003) Evidence of early cortical atrophy in MS: relevance to white matter changes and disability. *Neurology* **60**:1157–1162.
21. Dutta R, Trapp BD (2007) Pathogenesis of axonal and neuronal damage in multiple sclerosis. *Neurology* **130**(Pt 10):2566–2576.
22. Farina G, Magliozzi R, Pitteri M, Reynolds R, Rossi S, Gajofatto A *et al* (2017) Increased cortical lesion load and intrathecal inflammation is associated with oligoclonal bands in multiple sclerosis patients: a combined CSF and MRI study. *J Neuroinflammation* **14**:40.
23. Filippi M, van den Heuvel MP, Fornito A, He Y, Hulshoff Pol HE, Agosta F *et al* (2013) Assessment of system dysfunction in the brain through MRI-based connectomics. *Lancet Neurol* **12**:1189–1199.
24. Filippi M, Evangelou N, Kangarlu A, Inglese M, Mainero C, Horsfield MA *et al* (2014) Ultra-high-field MR imaging in multiple sclerosis. *J Neurol Neurosurg Psychiatry* **85**:60–6.
25. Fisniku LK, Chard DT, Jackson JS, Anderson VM, Altmann DR, Miszkiel KA *et al* (2008) Gray matter atrophy is related to long-term disability in multiple sclerosis. *Ann Neurol* **64**:247–254.
26. Frischer JM, Bramow S, Dal-Bianco A, Lucchinetti CF, Rauschka H, Schmidbauer M *et al* (2009) The relation between inflammation and neurodegeneration in multiple sclerosis brains. *Brain* **132**(Pt 5):1175–1189.
27. Geurts JJG, Bö L, Pouwels PJW, Castelijns JA, Polman CH, Barkhof F (2005) Cortical lesions in multiple sclerosis: combined postmortem MR imaging and histopathology. *Am J Neuroradiol* **26**:572–577.
28. Geurts JJG, Stys PK, Minagar A, Amor S, Zivadinov R (2009) Gray matter pathology in (chronic) MS: modern views on an early observation. *J Neurol Sci* **282**:12–20.
29. Gilmore CP, Deluca GC, Bö L, Owens T, Lowe J, Esiri MM *et al* (2009) Spinal cord neuronal pathology in multiple sclerosis. *Brain Pathol* **19**:642–9.
30. Giorgio A, De Stefano N (2010) Cognition in multiple sclerosis: relevance of lesions, brain atrophy and proton MR spectroscopy. *Neurol Sci* **31**(SUPPL. 2):S245–S248.
31. Granberg T, Fan Q, Treaba CA, Ouellette R, Herranz E, Mangeat G *et al* (2017) *In vivo* characterization of cortical and white matter neuroaxonal pathology in early multiple sclerosis. *Brain* **140**:2912–2926.
32. Haider L, Zrzavy T, Hametner S, Höftberger R, Bagnato F, Grabner G *et al* (2016)The topograpy of demyelination and neurodegeneration in the multiple sclerosis brain. *Brain* **139**(Pt 3):807–815.
33. Howell OW, Rundle JL, Garg A, Komada M, Brophy PJ, Reynolds R (2010) Activated microglia mediate axoglial disruption that contributes to axonal injury in multiple sclerosis. *J Neuropathol Exp Neurol* **69**:1017–1033.
34. Kidd D, Barkhof F, McConnell R, Algra PR, Allen IV, Revesz T (1999) Cortical lesions in multiple sclerosis. *Brain* **122**:17–26.
35. Kilsdonk ID, De Graaf WL, Soriano AL, Zwanenburg JJ, Visser F, Kuijer JPA *et al* (2013) Multicontrast MR imaging at 7T in multiple sclerosis: highest lesion detection in cortical gray matter with 3D-FLAIR. *Am J Neuroradiol* **34**:791–796.
36. Kilsdonk ID, Jonkman LE, Klaver R, Van Veluw SJ, Zwanenburg JJM, Kuijer JPA *et al* (2016) Increased cortical grey matter lesion detection in multiple sclerosis with 7 T MRI: a post-mortem verification study. *Brain* **139**(Pt 5):1472–1481.
37. Kutzelnigg A, Lucchinetti CF, Stadelmann C, Brück W, Rauschka H, Bergmann M *et al* (2005) Cortical demyelination and diffuse white matter injury in multiple sclerosis. *Brain* **128**(Pt 11):2705–2712.
38. Lassmann H, Brück W, Lucchinetti CF (2007) The immunopathology of multiple sclerosis: an overview. *Brain Pathol* **17**:210–218.
39. Leech S, Kirk J, Plumb J, McQuaid S (2007) Persistent endothelial abnormalities and blood-brain barrier leak in primary and secondary progressive multiple sclerosis. *Neuropathol Appl Neurobiol* **33**:86–98.
40. Lucchinetti CF, Popescu BFG, Bunyan RF, Moll NM, Roemer SF, Lassmann H *et al* (2011) Inflammatory cortical demyelination in early multiple sclerosis. *N Engl J Med* **365**:2188–2197.
41. Magliozzi R, Columba-Cabezas S, Serafini B, Aloisi F (2004) Intracerebral expression of CXCL13 and BAFF is accompanied by formation of lymphoid follicle-like structures in the meninges of mice with relapsing experimental autoimmune encephalomyelitis. *J Neuroimmunol* **148**:11–23.
42. Magliozzi R, Howell O, Vora A, Serafini B, Nicholas R, Puopolo M *et al* (2007) Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. *Brain* **130**(Pt 4):1089–1104.
43. Magliozzi R, Howell OW, Reeves C, Roncaroli F, Nicholas R, Serafini B *et al* (2010) A Gradient of neuronal loss and meningeal inflammation in multiple sclerosis. *Ann Neurol* **68**:477–493.
44. Magliozzi R, Serafini B, Rosicarelli B, Chiappetta G, Veroni C, Reynolds R *et al* (2013) B-cell enrichment and epstein-barr virus infection in inflammatory cortical lesions in secondary progressive multiple sclerosis. *J Neuropathol Exp Neurol* **72**:29–41.

45. Magliozzi R, Howell OW, Nicholas R, Cruciani C, Castellaro M, Romualdi C *et al* (2018) Inflammatory intrathecal profiles and cortical damage in multiple sclerosis. *Ann Neurol* **83**:739–755.
46. Mainero C, Louapre C (2015) Erratum: meningeal inflammation in multiple sclerosis: the key to the origin of cortical lesions. *Neurology* **85**:12–13.
47. Peterson JW, Bö L, Mörk S, Chang A, Trapp BD (2001) Transected neurites, apoptotic neurons, and reduced inflammation in cortical multiple sclerosis lesions. *Ann Neurol* **50**:389–400.
48. Pitteri M, Romualdi C, Magliozzi R, Monaco S, Calabrese M (2017) Cognitive impairment predicts disability progression and cortical thinning in MS: an 8-year study. *Mult Scler* **23**:848–854.
49. Ransohoff RM, Kivisäkk P, Kidd G (2003) Three or more routes for leukocyte migration into the central nervous system. *Nat Rev Immunol* **3**:569–581.
50. Reynolds R, Roncaroli F, Nicholas R, Radotra B, Gveric D, Howell O (2011) The neuropathological basis of clinical progression in multiple sclerosis. *Acta Neuropathol* **122**:155–170.
51. Rovaris M, Comi G, Filippi M (2006) MRI markers of destructive pathology in multiple sclerosis-related cognitive dysfunction. *J Neurol Sci* **245**:111–116.
52. Schmierer K, Parkes HG, So P-W, An SF, Brandner S, Ordidge RJ *et al* (2010) High field (9.4 Tesla) magnetic resonance imaging of cortical grey matter lesions in multiple sclerosis. *Brain* **133**(Pt 3):858–867.
53. Seewann A, Vrenken H, Kooi E, van der Valk P, Knol DL, Polman CH *et al* (2011) Imaging the tip of the iceberg: visualization of cortical lesions in multiple sclerosis. *Mult Scler* **17**:1202–1210.
54. Seewann A, Kooi E-J, Roosendaal SD, Pouwels PJW, Wattjes MP, van der Valk P *et al* (2012) Postmortem verification of MS cortical lesion detection with 3D DIR. *Neurology* **78**:302–308.
55. Serafini B, Rosicarelli B, Magliozzi R, Stigliano E, Aloisi F (2004) Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis. *Brain Pathol* **14**:164–174.
56. Sethi V, Yousry TA, Muhlert N, Ron M, Golay X, Wheeler-Kingshott C *et al* (2012) Improved detection of cortical MS lesions with phase-sensitive inversion recovery MRI. *J Neurol Neurosurg Psychiatry* **83**:877–882.
57. Simon B, Schmidt S, Lukas C, Gieseke J, Träber F, Knol DL *et al* (2010) Improved *in vivo* detection of cortical lesions in multiple sclerosis using double inversion recovery MR imaging at 3 Tesla. *Eur Radiol* **20**:1675–1683.
58. Stadelmann C, Albert M, Wegner C, Brück W (2008) Cortical pathology in multiple sclerosis. *Curr Opin Neurol* **21**:229–234.
59. Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mörk S, Bö L (1998) Axonal transection in the lesions of multiple sclerosis. *N Engl J Med* **338**:278–285.
60. Van Horssen J, Dijkstra CD, De Vries HE (2007) The extracellular matrix in multiple sclerosis pathology. *J Neurochem* **103**:1293–1301.
61. Vercellino M, Merola A, Piacentino C, Votta B, Capello E, Mancardi GL *et al* (2007) Altered glutamate reuptake in relapsing-remitting and secondary progressive multiple sclerosis cortex: correlation with microglia infiltration, demyelination, and neuronal and synaptic damage. *J Neuropathol Exp Neurol* **66**:732–739.
62. Wattjes MP, Lutterbey GG, Gieseke J, Träber F, Klotz L, Schmidt S *et al* (2007) Double inversion recovery brain imaging at 3T: diagnostic value in the detection of multiple sclerosis lesions. *AJNR Am J Neuroradiol* **28**:54–59.
63. Wattjes MP, Barkhof F (2009) High field MRI in the diagnosis of multiple sclerosis: high field-high yield? *Neuroradiology* **51**:279–292.
64. Wegner C, Esiri MM, Chance SA, Palace J, Matthews PM (2006) Neocortical neuronal, synaptic, and glial loss in multiple sclerosis. *Neurology* **67**:960–967.
65. Zivadinov R, Heininen-Brown M, Schirda CV, Poloni GU, Bergsland N, Magnano CR *et al* (2012) Abnormal subcortical deep-gray matter susceptibility-weighted imaging filtered phase measurements in patients with multiple sclerosis. A case-control study. *Neuroimage* **59**:331–339.
66. Zivadinov R, Jakimovski D, Gandhi S, Ahmed R, Dwyer MG, Horakova D *et al* (2016) Clinical relevance of brain atrophy assessment in multiple sclerosis. Implications for its use in a clinical routine. *Exp Rev Neurother* **16**:777–793.