Canadian Journal of Medicine 1(2019) 20-28

Canadian Journal of Medicine

WWW.CIKD.CA

Recent Developments of Diagnostic Criteria in Multiple Sclerosis

Massoud Houshman[1,2]*, Fawziah M. Mohammed[3]

[1]Research Center, Knowledge University, Erbil, Kurdistan Region, Iraq

[2]Department of Medical Genetics, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran

[3]Department of Medical Laboratory Sciences. Faculty of Allied Health Sciences. Kuwait University, Kuwait

Keywords:
Multiple sclerosis, Magnetic resonance imaging, Neuropathology, Imaging, Diagnosis, Brain, Spinal

Received
15 July 2019
Received in revised form
23 September 2019
Accepted
20 December 2019

Correspondence:
Massoudh@nigeb.ac.ir

ABSTRACT

This review describes the more important developments of the neuroimaging of multiple sclerosis (MS) in recent years, and provides a discussion of advanced MR imaging techniques with regard to current findings, clinical correlations, and future directions. MS pathology is originally defined by the presence of focal white matter lesions, characterized by inflammatory/demyelinating, axonal loss, edema, blood brain barrier break-down, and neurodegenerative processes that occur earlier in life, which usually affects the gray and white matter, brainstem, cerebellum, spinal cord and optic nerve. In recent years, the use of MRI techniques represents as a powerful tool to non-invasively study different pathological substrates of lesions and microscopic tissue changes. Techniques such as T2-weighted and gadolinium-enhanced T1-weighted MRI are very sensitive in detecting lesions and, thus, increase the level of certainty of MS diagnosis. In this review, we summarize the main evidence supporting the use of advanced MRI techniques provide a better understanding of the neuropathologic processes that most likely are related to disease activity and clinical progression in MS. Such metrics are able to reveal a range of tissue changes that include inflammation, demyelination, axonal loss, reactive glial scaring, neurodegeneration and neuroinflammation. In conclusion, MRI has had a major impact on diagnosing MS, understanding the condition, and monitoring the effects of clinical treatments.

Introduction

Multiple sclerosis (MS) is a common central nervous system (CNS) disease characterised pathologically by the development of multifocal inflammatory demyelinating white matter lesions. The etiology behind the disease is idiopathic, but multiple genetic and environmental factors are presumably involved (Atkins, Amor, & Fletcher, 2012). Although the pathogenesis in its entirety has not been outlined, the disease process involves peripheral activation of T cells by antigen-presenting cells (APCs), transmigration of activated pro-inflammatory T cells through the blood–brain barrier (BBB), and propagation of the immune response directed at myelin sheaths, oligodendrocytes, and nerve axons, leading to demyelination, axon loss, and nerve death due to excite-toxicity (Atkins et al., 2012),moreover, MS is a chronic, inflammatory demyelinating disease of the CNS (Charcot, 1880). Remyelination by oligodendrocyte lineage cells can be identified pathologically by sharply demarcated areas of uniformly thin myelin sheaths (Atkins et al., 2012; Charcot, 1880; Prineas & Connell, 1979). On the other hand, MS produces both inflammatory and degenerative pathology of the brain and spinal cord. The degenerative process has been demonstrated by both histopathology and neuroimaging. Histologically, there is neuronal loss in the cortical and deep gray matter (GM), axonal loss in white matter (WM) and similar changes in the cervical and thoracic spinal cord (Bruck, Kuhlmann, & Stadelmann , 2003; Cifelli, Arridge, Jezzard, Esiri, Palace, & Matthews, 2006; DeLuca, Ebers, & Esiri , 2004; Evangelou, DeLuca, Owens, & Esiri , 2005; Gilmore, DeLuca et al., 2009; Patrikios et al., 2006). Magnetic resonance imaging (MRI) volumetric studies have demonstrated correlations between disability and atrophy of the whole brain, cerebral WM, cerebral GM, and cervical spinal cord (Fisher et al., 2002; Filippi et al., 1996; Furby et al., 2008; 1996; Lin, Blumhardt, & Constantinescu, 2003; Peterson, Bö, Mörk, Chang, & Trapp, 2001; Vogt et al., 2009).

Conventional structural MRI continues to play a vital role in diagnosing and in monitoring disease progression and treatment efficacy in MS; however, it lacks the capability to recognize gray matter lesions or diffuse changes in the so-called normal-appearing brain tissue. Advanced structural MRI techniques such as diffusion tensor imaging (DTI), magnetization transfer imaging (MTI), magnetic resonance spectroscopy (MRS), and relaxometry have evolved as superior tools to examine injury in both lesions and normal-appearing brain tissue with more specificity and sensitivity (Sanfilipo, Benedict, Sharma, Weinstock-Guttman, & Bakshi , 2005).

Advanced MRI techniques, such MTI, DTI, MRS, and relaxometry, have enriched our understanding of pathological correlates and the natural evolution of MS lesions. The appearance of WM lesions on conventional MRI has been related to different stages and the severity of tissue damage. In addition, it is apparent that the damage seen in lesions is higher than normal-appearing white matter (NAWM), but their mutual relationship is not well understood. However, visualization of cortical lesions in MS with standard MRI techniques remains difficult. More advanced MRI techniques, like double inversion recovery (DIR), enabled substantial improvement of cortical lesion detection (Geurts et al., 2005; Polman et al., 2011; Stevenson et al., 1998), and showed that cortical lesions are associated with clinic-cognitive impairment (Calabrese et al., 2007; Calabrese et al., 2009; Simon et al., 2010).The aim of this review article was to the evaluate of advanced imaging tools to investigate multiple sclerosis pathology and the importance of magnetic resonance imaging in its early diagnosis in patients.

Pathogenesis of Multiple Sclerosis

MS probably begins with the activation of auto-reactive CD4+ T helper type 1 (Th1) cells directed against CNS antigens in the periphery. The activated immune cells upregulate surface cell adhesion molecules and cytokine receptors and secrete pro-inflammatory cytokines such as interleukin-2 (IL-2), interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α) as well as chemokines and matrix metalloproteinases (MMPs) which provoke reciprocal changes in endothelial cells, enable adherence of the activated cells to the endothelium and facilitate their migration across the BBB (Calabrese et al., 2009).

Comprehensive Neuropathologic Analysis of Multiple Sclerosis in the Brain and Spinal Cord

MS plaques can occur anywhere in the CNS, in the WM around the ventricles, optic nerves and tracts, cerebellar peduncles, long tracts, corpus callosum and subpial areas of the spinal cord and brainstem, but also in the gray matter. These plaques are composed of perivenular infiltrates of mononuclear inflammatory cells (T-lymphocytes, monocytes/ macrophages, B and plasma cells), demyelinated axons, reduced number of oligodendrocytes, transected axons, and astrocyte roliferation with resultant gliosis. MS lesions may be divided into acute, chronic active and chronic silent. They show profound heterogeneity in the structural and immunopathological patterns of demyelination and oligodendrocyte pathology between different MS patients (Roosendaal et al., 2009), suggesting that MS is a neurologic syndrome rather than a single disease. Traditionally, MS has been considered a T-cell mediated autoimmune disease. It has become apparent recently that its pathogenesis is far more complex, involving all arms of the innate and adoptive immune system with slowly progressive neurodegeneration in addition to acute inflammation (Lucchinetti et al., 2000; Razavi et al., 2015). In progressive MS, it is believed that the inflammatory process becomes increasingly compartmentalized within the CNS and within adjacent meninges. In this MS, NAWM displays persistent microglial activation, infiltration by individual T cells and signs of ongoing axonal injury (McFarland & Martin, 2007). Increasing axonal and neuronal loss are crucial factors contributing to disease progression in MS. Widespread GM involvement not only leads to neuronal loss but also to marked as well as subtle synaptic abnormalities which may also play a role in the progression of the disease (Kutzelnigg et al., 2005; Wegner, Esiri, Chance, Palace, & Matthews, 2006).

Brain Regions Involved and Neuropathology of Neurons in Multiple Sclerosis

Post-mortem studies in MS provided evidence for significant neuronal loss in GM lesions in the thalamus, neocortex, archecortex, cerebellum and spinal cord (Cifelli et al., 2002; Gilmore, DeLuca et al., 2009; Kutzelnigg et al., 2007; Papadopoulos et al., 2009; Schirmer et al., 2009; Wegner et al., 2006). Furthermore, pronounced synaptic loss of up to 47% was found in neocortical and hippocampal lesions in MS (Kutzelnigg et al., 2005; Wegner et al., 2006). Neuronal loss has not only been observed in lesional GM, but also in the normal-appearing GM (NAGM) in the thalamus (Dutta et al., 2006) and also in the neo-cortex in a subset of progressive MS patients (Magliozzi et al., 2006). Neuronal expression of several nuclear encoded mitochondrial genes was significantly decreased in NAGM (Magliozzi et al., 2006) and mitochondrial deletions were also found in normal appearing cortex (Campbell et al., 2011). These widespread neuronal changes in lesional and non-lesional GM are likely to contribute to GM atrophy observedin MS.

Brain Regions Involved and Neuropathology of Grey Matter in Multiple Sclerosis

Recent pathological investigations using more sensitive immunohistochemical methods confirmed substantial demyelination in the GM in MS (Geurts & Barkhof, 2008). The proportion of GM demyelination was even reported to exceed that of WM demyelination in several regions including the neo-cortex, cerebellum, spinal cord and thalamus (Gilmore, Donaldson, Bö, Owens, Lowe, & Evangelou, 2009). GM demyelination is present in the majority of patients with chronic MS (Wegner et al., 2006), but can also be observed in approximately one third of the patients with early MS (Lucchinetti et al., 2011). Similar to WM plaques, GM lesions display demyelination with a sharp border to the surrounding myelinated normal-appearing GM. Early GM lesions also exhibit inflammatory cell infiltration mainly consisting of T cells and macrophages (Lucchinetti et al., 2011). Chronic GM lesions appear relatively hypocellular with respect to white matter lesions (Evangelou et al., 2005). They show microglial activation and axonal transections (Evangelou et al., 2005), but less astrogliosis than chronic WM plaques.

Brain Regions Involved and Neuropathology of White Matter in Multiple Sclerosis

WM lesions are sharply demarcated areas preferentially localized in the periventricular WM, spinal cord, optic nerves, medulla oblongata, pons and cerebellum. As evidenced by early pathological examination (Dawson, 1916) and recent high-resolution MRI (Ge, Zohrabian, & Grossman, 2008), MS lesions typically develop around centrally located veins, these lesions in are characterized by demyelination, inflammation, axonal damage and gliosis. The distinct histopathological features of MS WM plaques depend on the age of the lesion, which is reflected in the amount of inflammation and glial scarring. Recent studies on early actively demyelinating lesions indicate that distinct pathological mechanisms of demyelination may be present in different patients (Lucchinetti et al., 2000). As the lesion develops over time, the plaque tends to grow centrifugally. About 40–60% of chronic WM lesions from patients with late-stage disease show signs of remyelination (Barkhof et al., 2003; Goldschmidt, Antel, König, Bruck, & Kuhlmann, 2009). The remyelination capacity within WM lesions appears to decrease over time as the disease progresses since a higher proportion of WM lesions (80%) from patients with early MS displays pronounced remyelination (Goldschmidt et al., 2009). This decrease of remyelination in chronic disease might be related to a differentiation failure of oligodendroglial progenitor cells in patients with chronic MS (Kuhlmann et al., 2008).

Magnetic Resonance Imaging of Multiple Sclerosis Lesions

MRI uses strong magnetic fields and radiofrequency energy to produce images of the central nervous system with exquisite anatomic detail. The ability to noninvasively assess the brain and spinal cord has transformed the approach to the diagnosis and treatment of MS. The evolution of the diagnostic criteria for MS reflects the increasing importance of MRI findings in establishing the likelihood of the diagnosis, from examination-based to the currently used McDonald criteria in which MRI findings can be used to establish dissemination in both time and space (McDonald et al., 2001; Poser et al., 1983; Schumacher et al., 1965).

MRI is the most sensitive test to detect and demonstrate MS lesions. It is used to support the diagnosis, estimate lesion load and disease activity, measure brain atrophy and axonal loss, follow disease progression, provide prognosis, serve as a surrogate marker and provide outcome measures in clinical trials. MS lesions are hyper-intense on T2-weighted, proton density or FLAIR imaging (fluid-attenuated inversion recovery), and hypointense or isointense on T1-weighted imaging. They

are typically ovoid in shape, of small size (3–8 mm on average, although giant plaques may occur), located mainly in the periventricular white matter but are also common in the posterior fossa, spinal cord and in subcortical location. They tend to be perpendicular to the ventricles, involve the corpus callosum and U-fibers, and may enhance with Gd, especially during active inflammation, due to disruption of the BBB. Newer MRI techniques facilitated the detection of both gray matter and white matter microstructural damage, and combined histopathologic-MRI correlation studies helped to clarify pathological specificity and sensitivity of these techniques (Ceccarelli, Bakshi, & Neema, 2012). Furthermore, T1-weighted scans (spin-lattice) produce images in which fat is bright and water is dark, providing good contrast between white and gray matter. In scans that are T2-weighted (spin-spin), fat is dark and water is bright, thus pathologic processes associated with edema will appear bright on T2-weighted scans. FLAIR is a T2-weighted scan with an added inversion pulse that nulls the water signal from cerebrospinal fluid (CSF)-filled spaces such as the ventricles, thus accentuating periventricular lesions and juxtacortical lesions typical of MS (Filippi et al., 1696). Of importance, the adoption of these guidelines requires the cooperation of clinical radiologists who typically have the final say over the details of the imaging protocols that are adapted at a particular center. Neurologists can play an important role in educating their local neuroradiologists on the evolution of diagnostic approaches in MS and the need for consistent scan acquisition in the serial evaluation of patients with MS.

Magnetic Resonance Imaging of Gray Matter Lesions

Cortical lesions are one of the key indicators of GM disease in MS. The application of advanced pulse sequences such as double-inversion recovery (DIR) and the implementation of high-field and ultrahigh- Field (UHF) MRI has notably increased the detectability of MS cortical pathology. Promising techniques developed at UHF have shown increased sensitivity to cortical lesions and improved anatomical localization, in particular to subpial cortical lesions (Nielsen et al., 2012; Tallantyre et al., 2010). It is hypothesized that visibility of cortical lesions on an MRI scan is highly dependent on lesion size rather than a distinctive pathologic process or location (Seewann et al., 2011). Because of its ability to demonstrate longitudinal changes, cortical lesions quantification could become a useful outcome measure in drug trials (Sormani et al., 2011). However, the kinetics of evolution and pathogenic mechanisms behind the formation of cortical lesions remain unclear.

Magnetic Resonance Imaging of White Matter Lesions

Several quantitative MRI techniques have been employed to characterize the diffuse abnormalities in NAWM that escape detection by conventional MRI (Bakshi et al., 2008). For example, the integrity of white matter tracts in specific neuronal circuits can be evaluated using DTI-based tractography and voxel-wise analysis .MTI that quantifies macromolecules can be used to study demyelination and remyelination; MRS can be used to determine the concentration of metabolites, which can serve as indicators of key pathological processes including axonal and neuronal damage; and relaxometry techniques can be utilized to uncover MS pathology owing to the sensitivity of T1 and T2 times to changes in water content and iron deposition. These MRI techniques have consistently shown that damage in NAWM is present in all MS phenotypes, starting from the earliest stage of the disease to more widespread involvement in the progressive phases of MS (Bodini et al., 2011; Dalton et al., 2012).

Magnetic Resonance Imaging of Spinal Cord Lesions

The value of spinal cord MRI in the diagnosis and monitoring of MS has been well documented. Cord lesion detection using conventional sequences at 1.5 T MRI is still difficult, though new sequences and high-field MRI have improved detection sensitivity (White, Zhang, & Healey, 2011). Although assessment of cord atrophy is challenging, it has been described in all MS subtypes yielding improved correlations with disease status (Bonati et al., 2011; Cohen et al., 2012; Okuda et al., 2011; Rocca et al., 2011). Brain involvement and cord involvement as determined by MRI are unrelated both for lesions and atrophy, indicating that damage in the two compartments progress rather independently (Cohen et al., 2012). Quantitative MRI mapping of diffuse damage in the cord has shed light on clinical and pathological correlates of MS. For example, an indirect role of mitochondrial dysfunction using quantification of N-acetylaspartate levels in the cord has been linked to disability. Although progress has been made in spinal DTI, the use of axial and radial diffusivities and their interpretation in terms of underlying myelin and axonal integrity is still not clear (Ciccarelli et al., 2010).

Conclusion

Several MRI paradigms in development hold out the promise of expanding our knowledge in the coming years. Conventional and advanced MRI techniques have significantly enhanced our understanding of tissue damage in MS. Improvements in these MRI techniques have accelerated in the recent past, as summarized in this review. Structural MRI is nowadays part of the routine procedures used for MS diagnosis and follow-up in a clinical setting. Besides clinical detection of MS lesions, there are now a wide range of advanced tools that could contribute to understand MS physiopathology and to design future trials, aimed at enhancing myelin repair and reducing neurodegeneration and neuro-inflammation. The implementation of specific imaging metrics and their application in MS, together with the development of appropriate post-processing methodologies, therefore represent an outstanding field of research that will contribute to improved knowledge and clinical care in the MS field. Despite the incorporation of MRI and other paraclinical evidence into the diagnostic scheme, careful clinical evaluation and judgment, and the principle of "no better explanation" remain the most important aspects of MS diagnosis and should not be replaced by any diagnostic test.

References

Atkins, G. J, Amor, S., & Fletcher, J. (2012). *The biology of multiple sclerosis*. Cambridge: Cambridge University Press.

Bakshi, R., Thompson, A. J., Rocca, M. A., Pelletier, D., Dousset, V., Barkhof, F., ... & Filippi, M. (2008). MRI in multiple sclerosis: current status and future prospects. *The Lancet Neurology*, *7*(7), 615–625.

Barkhof, F., Bruck, W., De Groot, C. J., Bergers, E., Hulshof, S., Geurts, J., ... & van der Valk, P. (2003). Remyelinated lesions in multiple sclerosis: magnetic resonance image appearance. *Archives of Neurology*, *60*(8), 1073–1081.

Bodini, B., Battaglini, M., De Stefano, N., Khaleeli, Z., Barkhof, F., Chard, D., ... & Rovira, A. (2011). T2 lesion location really matters: a 10-year follow-up study in primary progressive multiple sclerosis. *Journal of Neurology, Neurosurgery and Psychiatry*, *82*(1), 72–77.

Bonati, U., Fisniku, L. K., Altmann, D. R., Yiannakas, M. C., Furby, J., Thompson, A. J., ... & Chard, D. T. (2011). Cervical cord and brain grey matter atrophy independently associate with long-term MS disability. *Journal of Neurology, Neurosurgery and Psychiatry*, *82*(4), 471–472.

Bruck, W., Kuhlmann, T., & Stadelmann, C. (2003). Remyelination in multiple sclerosis. *Journal of the Neurological Sciences*, *206*(2), 181–185.

Calabrese, M., Agosta, F., Rinaldi, F., Mattisi, I., Grossi, P., Favaretto, A., ... & Perini, P. (2009). Cortical lesions and atrophy associated with cognitive impairment in relapsing-remitting multiple sclerosis. *Archives of Neurology*, *66*(9), 1144–1150.

Calabrese, M., De Stefano, N., Atzori, M., Bernardi, V., Mattisi, I., Barachino, L., ... & Battistin, L. (2007). Detection of cortical inflammatory lesions by double inversion recovery magnetic resonance imaging in patients with multiple sclerosis. *Archives of Neurology*, *64*(10), 1416–1422.

Campbell, G. R., Ziabreva, I., Reeve, A. K., Krishnan, K. J., Reynolds, R., Howell, O., ... & Mahad, D. J. (2011). Mitochondrial DNA deletions and neurodegeneration in multiple sclerosis. *Annals of Neurology*, *69*(3), 481–492.

Ceccarelli, A., Bakshi, R., & Neema, M. (2012). MRI in multiple sclerosis: a review of the current literature. *Current Opinion in Neurology*, *25*(4), 402–409.

Ciccarelli, O., Toosy, A. T., De Stefano, N., Wheeler-Kingshott, C. A., Miller, D. H., & Thompson, A. J. (2010). Assessing neuronal metabolism in vivo by modeling imaging measures. *Journal of Neuroscience*, *30*(45), 15030–15033.

Charcot, J. M. (1880). *Lectures on diseases of the nervous system*. London: New Sydenham Society.

Cifelli, A., Arridge, M., Jezzard, P., Esiri, M. M., Palace, J., & Matthews, P. M. (2002). Thalamic neurodegeneration in multiple sclerosis. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society*, *52*(5), 650–653.

Cohen, A. B., Neema, M., Arora, A., Dell'Oglio, E., Benedict, R. H., Tauhid, S., ... & Stankiewicz, J. M. (2012). The relationships among MRI□defined spinal cord involvement, brain involvement, and disability in multiple sclerosis. *Journal of Neuroimaging*, *22*(2), 122–128.

Dalton, C. M., Bodini, B., Samson, R. S., Battaglini, M., Fisniku, L. K., Thompson, A. J., ... & Chard, D. T. (2012). Brain lesion location and clinical status 20 years after a diagnosis of clinically isolated syndrome suggestive of multiple sclerosis. *Multiple Sclerosis Journal*, *18*(3), 322–328.

Dawson, J. W. (1916). The histology of disseminated sclerosis. *Edinburgh Medical Journal*, *17*(5), 311.

DeLuca, G. C., Ebers, G. C., & Esiri, M. M. (2004). Axonal loss in multiple sclerosis: a pathological survey of the corticospinal and sensory tracts. *Brain*, *127*(5), 1009–1018.

Dutta, R., McDonough, J., Yin, X., Peterson, J., Chang, A., Torres, T., ... & Rudick, R. (2006). Mitochondrial dysfunction as a cause of axonal degeneration in multiple sclerosis patients. *Annals of Neurology*, *59*(3), 478–489.

Evangelou, N., DeLuca, G. C., Owens, T., & Esiri, M. M. (2005). Pathological study of spinal cord atrophy in multiple sclerosis suggests limited role of local lesions. *Brain*, *128*(1), 29–34.

Filippi, M., Campi, A., Colombo, B., Pereira, C., Martinelli, V., Baratti, C., & Comi, G. (1996). A spinal cord MRI study of benign and secondary progressive multiple sclerosis. *Journal of Neurology*, *243*(7), 502–505.

Fisher, E., Rudick, R. A., Simon, J. H., Cutter, G., Baier, M., Lee, J. C., ... & Simonian, N. A. (2002). Eight-year follow-up study of brain atrophy in patients with MS. *Neurology*, *59*(9), 1412–1420.

Furby, J., Hayton, T., Anderson, V., Altmann, D., Brenner, R., Chataway, J., ... & Kapoor, R. (2008). Magnetic resonance imaging measures of brain and spinal cord atrophy correlate with clinical impairment in secondary progressive multiple sclerosis. *Multiple Sclerosis Journal*, *14*(8), 1068–1075.

Ge, Y., Zohrabian, V. M., & Grossman, R. I. (2008). Seven-Tesla magnetic resonance imaging: new vision of microvascular abnormalities in multiple sclerosis. *Archives of Neurology*, *65*(6), 812–816.

Geurts, J. J., & Barkhof, F. (2008). Grey matter pathology in multiple sclerosis. *The Lancet Neurology*, *7*(9), 841–851.

Geurts, J. J., Pouwels, P. J., Uitdehaag, B. M., Polman, C. H., Barkhof, F., & Castelijns, J. A. (2005). Intracortical lesions in multiple sclerosis: improved detection with 3D double inversion-recovery MR imaging. *Radiology*, *236*(1), 254–260.

Gilmore, C. P., DeLuca, G. C., Bö, L., Owens, T., Lowe, J., Esiri, M. M., & Evangelou, N. (2009). Spinal cord neuronal pathology in multiple sclerosis. *Brain pathology*, *19*(4), 642–649.

Gilmore, C. P., Donaldson, I., Bö, L., Owens, T., Lowe, J., & Evangelou, N. (2009). Regional variations in the extent and pattern of grey matter demyelination in multiple sclerosis: a comparison between the cerebral cortex, cerebellar cortex, deep grey matter nuclei and the spinal cord. *Journal of Neurology, Neurosurgery & Psychiatry*, *80*(2), 182–187.

Goldschmidt, T., Antel, J., König, F. B., Bruck, W., & Kuhlmann, T. (2009). Remyelination capacity of the MS brain decreases with disease chronicity. *Neurology*, *72*(22), 1914–1921.

Kutzelnigg, A., Faber□Rod, J. C., Bauer, J., Lucchinetti, C. F., Sorensen, P. S., Laursen, H., ... & Lassmann, H. (2007). Widespread demyelination in the cerebellar cortex in multiple sclerosis. *Brain Pathology*, *17*(1), 38–44.

Kutzelnigg, A., Lucchinetti, C. F., Stadelmann, C., Bruck, W., Rauschka, H., Bergmann, M., ... & Lassmann, H. (2005). Cortical demyelination and diffuse white matter injury in multiple sclerosis. *Brain*, *128*(11), 2705–2712.

Lin, X., Blumhardt, L. D., & Constantinescu, C. S. (2003). The relationship of brain and cervical cord volume to disability in clinical subtypes of multiple sclerosis: a three□dimensional MRI study. *Acta Neurologica Scandinavica*, *108*(6), 401–406.

Lucchinetti, C., Bruck, W., Parisi, J., Scheithauer, B., Rodriguez, M., & Lassmann, H. (2000). Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society*, *47*(6), 707–717.

Lucchinetti, C. F., Popescu, B. F., Bunyan, R. F., Moll, N. M., Roemer, S. F., Lassmann, H., ... & Weigand, S. D. (2011). Inflammatory cortical demyelination in early multiple sclerosis. *New England Journal of Medicine*, *365*(23), 2188–2197.

Magliozzi, R., Howell, O. W., Reeves, C., Roncaroli, F., Nicholas, R., Serafini, B., ... & Reynolds, R. (2010). A gradient of neuronal loss and meningeal inflammation in multiple sclerosis. *Annals of Neurology*, *68*(4), 477–493.

McDonald, W. I., Compston, A., Edan, G., Goodkin, D., Hartung, H. P., Lublin, F. D., ... & Sandberg□Wollheim, M. (2001). Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society*, *50*(1), 121–127.

McFarland, H. F., & Martin, R. (2007). Multiple sclerosis: a complicated picture of autoimmunity. *Nature Immunology*, *8*(9), 913-919.

Nielsen, A. S., Kinkel, R. P., Tinelli, E., Benner, T., Cohen□Adad, J., & Mainero, C. (2012). Focal cortical lesion detection in multiple sclerosis: 3 tesla DIR versus 7 tesla FLASH□T2. *Journal of Magnetic Resonance Imaging*, *35*(3), 537–542.

Okuda, D. T., Mowry, E. M., Cree, B. A. C., Crabtree, E. C., Goodin, D. S., Waubant, E., & Pelletier, D. (2011). Asymptomatic spinal cord lesions predict disease progression in radiologically isolated syndrome. *Neurology*, *76*(8), 686–692.

Papadopoulos, D., Dukes, S., Patel, R., Nicholas, R., Vora, A., & Reynolds, R. (2009). Substantial archaeocortical atrophy and neuronal loss in multiple sclerosis. *Brain Pathology*, *19*(2), 238–253.

Patrikios, P., Stadelmann, C., Kutzelnigg, A., Rauschka, H., Schmidbauer, M., Laursen, H., ... & Lassmann, H. (2006). Remyelination is extensive in a subset of multiple sclerosis patients. *Brain*, *129*(12), 3165–3172.

Peterson, J. W., Bö, L., Mörk, S., Chang, A., & Trapp, B. D. (2001). Transected neurites, apoptotic neurons, and reduced inflammation in cortical multiple sclerosis lesions. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society*, *50*(3), 389–400.

Polman, C. H., Reingold, S. C., Banwell, B., Clanet, M., Cohen, J. A., Filippi, M., ... & Lublin, F. D. (2011). Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Annals of Neurology*, *69*(2), 292–302.

Poser, C. M., Paty, D. W., Scheinberg, L., McDonald, W. I., Davis, F. A., Ebers, G. C., ... & Tourtellotte, W. W. (1983). New diagnostic criteria for multiple sclerosis: guidelines for research protocols. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society*, *13*(3), 227–231.

Prineas, J. W., & Connell, F. (1979). Remyelination in multiple sclerosis. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society*, *5*(1), 22–31.

Razavi, S., Nazem, G., Mardani, M., Esfandiari, E., Salehi, H., & Esfahani, S. H. Z. (2015). Neurotrophic factors and their effects in the treatment of multiple sclerosis. *Advanced Biomedical Research, 17*(4), 53.

Rocca, M. A., Horsfield, M. A., Sala, S., Copetti, M., Valsasina, P., Mesaros, S., ... & Comi, G. (2011). A multicenter assessment of cervical cord atrophy among MS clinical phenotypes. *Neurology*, *76*(24), 2096–2102.

Roosendaal, S. D., Moraal, B., Pouwels, P. J. W., Vrenken, H., Castelijns, J. A., Barkhof, F., & Geurts, J. J. G. (2009). Accumulation of cortical lesions in MS: relation with cognitive impairment. *Multiple Sclerosis Journal*, *15*(6), 708–714.

Sanfilipo, M. P., Benedict, R. H., Sharma, J., Weinstock-Guttman, B., & Bakshi, R. (2005). The relationship between whole brain volume and disability in multiple sclerosis: a comparison of normalized gray vs. white matter with misclassification correction. *Neuroimage*, *26*(4), 1068–1077.

Schirmer, L., Albert, M., Buss, A., Schulz□Schaeffer, W. J., Antel, J. P., Bruck, W., & Stadelmann, C. (2009). Substantial early, but nonprogressive neuronal loss in multiple sclerosis (MS) spinal cord. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society*, *66*(5), 698–704.

Schumacher, G. A., Beebe, G., Kibler, R. F., Kurland, L. T., Kurtzke, J. F., McDowell, F., ... & Willmon, T. L. (1965). Problems of experimental trials of therapy in multiple sclerosis: report by the panel on the evaluation of experimental trials of therapy in multiple sclerosis. *Annals of the New York Academy of Sciences*, *122*(1), 552–568.

Seewann, A., Vrenken, H., Kooi, E. J., Van Der Valk, P., Knol, D. L., Polman, C. H., ... & Geurts, J. J. (2011). Imaging the tip of the iceberg: visualization of cortical lesions in multiple sclerosis. *Multiple Sclerosis Journal*, *17*(10), 1202–1210.

Simon, B., Schmidt, S., Lukas, C., Gieseke, J., Träber, F., Knol, D. L., ... & Wattjes, M. P. (2010). Improved in vivo detection of cortical lesions in multiple sclerosis using double inversion recovery MR imaging at 3 Tesla. *European Radiology*, *20*(7), 1675–1683.

Sormani, M. P., Calabrese, M., Signori, A., Giorgio, A., Gallo, P., & De Stefano, N. (2011). Modeling the distribution of new MRI cortical lesions in multiple sclerosis longitudinal studies. *PLoS One*, *6*(10), e26712.

Stevenson, V. L., Leary, S. M., Losseff, N. A., Parker, G. J. M., Barker, G. J., Husmani, Y., ... & Thompson, A. J. (1998). Spinal cord atrophy and disability in MS: a longitudinal study. *Neurology*, *51*(1), 234–238.

Tallantyre, E. C., Morgan, P. S., Dixon, J. E., Al□Radaideh, A., Brookes, M. J., Morris, P. G., & Evangelou, N. (2010). 3 Tesla and 7 Tesla MRI of multiple sclerosis cortical lesions. *Journal of Magnetic Resonance Imaging*, *32*(4), 971–977.

Vogt, J., Paul, F., Aktas, O., Müller□Wielsch, K., Dörr, J., Dörr, S., ... & Raine, C. S. (2009). Lower motor neuron loss in multiple sclerosis and experimental autoimmune encephalomyelitis. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society*, *66*(3), 310–322.

Wegner, C., Esiri, M. M., Chance, S. A., Palace, J., & Matthews, P. M. (2006). Neocortical neuronal, synaptic, and glial loss in multiple sclerosis. *Neurology*, *67*(6), 960–967.

White, M. L., Zhang, Y., & Healey, K. (2011). Cervical spinal cord multiple sclerosis: evaluation with 2D multi-echo recombined gradient echo MR imaging. *The Journal of Spinal Cord Medicine*, *34*(1), 93–98.