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Magnetisation transfer imaging in multiple sclerosis

Massimo Filippi*,[1] and Marco Rovaris[1]

[1]*Neuroimaging Research Unit, Department of Neuroscience, Scientific Institute Ospedale San Raffaele, Via Olgettina 60, 20132 Milan, Italy*

Magnetisation transfer imaging (MTI) is a magnetic resonance imaging (MRI) technique that has a higher specificity than conventional T2-weighted scans to the heterogeneous pathological substrates of multiple sclerosis (MS) lesions. This review outlines the contribution of MTI in the study of lesion evolution and in the assessment of disease burden in MS. MTI studies of individual MS lesions confirm the pathological heterogeneity of T2-weighted MRI abnormalities and the potential role of unenhanced T1-weighted hypointensities as specific markers of localised severe white matter disruption. Correlative cross-sectional and longitudinal studies using MTI and gadolinium (Gd)-enhanced MRI reveal that MTI findings may vary in lesions with different patterns of enhancement, and that MTI abnormalities are closely related to the onset and recovery of blood-brain barrier disruption in new MS plaques. Measures obtained from MTI scans using whole-brain histogram analysis are highly correlated with the extent of MS abnormalities on conventional MRI scans and predict patients' clinical disability well, since they are sensitive to the amounts of both macro- and microscopic MS disease burden in the whole brain and in specific regions. *Journal of NeuroVirology* (2000) **6,** S115–S120.

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Magnetisation transfer imaging: basic principles

Magnetisation transfer imaging (MTI) is a magnetic resonance imaging (MRI) technique that has recently been applied to the study of multiple sclerosis (MS) (McGowan *et al*, 1998). Low magnetisation transfer ratio (MTR) indicates a reduced capacity of the molecules in the brain tissue matrix to exchange magnetisation with the surrounding (MRI visible) water molecules. Although, in MS, this may be caused either by a reduction in the integrity of macromolecular matrix reflecting damage to the myelin or to the axonal membrane (McDonald *et al*, 1992), or by a dilution of the macromolecules brought about by inflammatory oedema (McDonald *et al*, 1992), studies with animal models (Dousset *et al*, 1992, 1995) reported that MTR reduces only slightly with oedema but more strongly with severe demyelination and axonal loss in lesions of experimental allergic encephalomyelitis (Dousset *et al*, 1992) or lysolecithin-induced demyelination (Dousset *et al*, 1995).

MTI has three main advantages over conventional T2- and T1-weighted MRI in the study of MS. First, it provides both morphological and pathological information with a higher specificity than conventional MRI (McGowan *et al*, 1998). Secondly, it enables us to assess the 'invisible' disease burden in the so-called normal-appearing white matter (NAWM), i.e. the brain tissue which does not show macroscopic abnormalities on conventional MRI (Filippi *et al*, 1995; Loevner *et al*, 1995). Thirdly, with the application of magnetisation transfer (MT) histogram methods (van Buchem *et al*, 1996), it provides, from a single procedure, multiple parameters influenced by both the macro- and microscopic disease burden.

Several metrics can be obtained from MTI scans. The first analysis step is the creation of calculated MT images or MTR maps, which are derived from two sets of images, acquired with and without an off-resonance saturation pulse. MTR maps are derived pixel-by-pixel according to the equation $MTR=(M_0-M_s)/M_0\times 100$ (Dousset *et al*, 1992), in which M_0 is the intensity of a given pixel without

*Correspondence: M Filippi

the saturation pulse, and M_s is the intensity of the same pixel when the saturation pulse is applied. MS lesions, which usually have lower MTR than NAWM (Dousset *et al*, 1992), appear as areas of hypointensity on MTR maps. From these maps, the average MTR for specific regions of interest (ROIs) can be obtained. As a further step, the average lesion MTR for a given patient can be calculated. Moreover, using semi-automated thresholding techniques for lesion segmentation of digital images (Rovaris *et al*, 1997; van Waesberghe *et al*, 1998a), the load of these lesions (i.e., their total volume) can be assessed. From each MTI scan, histograms of MTR values can also be derived, using an image analysis method which was developed by van Buchem *et al* (1996). Histograms of pixel intensity are created from the calculated MT images, after a preliminary manual or semi-automatic image segmentation aimed at excluding all the non-cerebral tissues (e.g., skull, orbital tissue, etc.). To reduce the effects of image noise and also cerebrospinal fluid (CSF) signal, all the pixels with very low MTR (i.e., from 0 to 5–10%) are also excluded from the analysis. Then, the data set of MTR values is displayed as a histogram, which is usually normalised to the total number of brain voxels to allow comparisons of histograms from subjects with different brain volumes. For each histogram, several parameters can be calculated (van Buchem *et al*, 1996): the height and position of the histogram peak (i.e., the most common MTR value in the brain), the average MTR, and the MTR corresponding to the 25th, 50th and 75th percentiles of the histogram (MTR_{25}, MTR_{50} and MTR_{75}), that indicate the MTR at which the integral of the histogram is 25, 50 and 75% of the total, respectively. MT histograms can be obtained both for the whole brain and for specific regions (e.g., frontal lobe, cerebellum, brainstem, etc.), which can be segmented according to standard neuroanatomical references.

MTI in the study of individual MS lesions

Conventional T2-weighted MRI scans have a high sensitivity in revealing MS lesions, but they lack specificity to further characterise the stages of the pathological process in individual lesions (Filippi and Miller, 1996). Oedema, demyelination, gliosis and axonal loss (McDonald *et al*, 1992), all lead to a similar appearance of hyperintensity on T2-weighted images. On the other hand, chronic hypointense areas on unenhanced T1-weighted images show severe tissue disruption (van Walderveen *et al*, 1998), but lesion hypointensity may also occur acutely and transiently in the case of inflammatory oedema and subsequently return to isointensity (van Waesberghe *et al*, 1998b). Finally, gadolinium (Gd)-enhanced T1-weighted images allows active lesions to be separated from inactive lesions (Miller *et al*, 1993), since enhancement occurs as a result of increased blood-brain barrier (BBB) permeability (Kermode *et al*, 1990), but they do not enable us to distinguish purely oedematous active from demyelinating lesions. Therefore, MTI findings have been correlated with conventional MRI abnormalities in several cross-sectional and longitudinal studies aimed at elucidating the variability of pathology in MS lesions and its evolution over time.

Several studies have demonstrated that MTR values for MS lesions visible on T2-weighted MRI are significantly lower than in NAWM, although their range is wide. Dousset *et al* (1992) found an average MTR of 26.3% for MS lesions, with a lower mean value in chronic progressive (23.3%) than in relapsing-remitting (RR) (27.6%) MS patients. Gass *et al* (1994) found that MS lesions had an average MTR lower than that of ischaemic lesions from patients with small-vessel disease, and that the average lesion MTR was significantly higher in benign MS compared to secondary progressive (SP) MS. A recent, longitudinal study (Rocca *et al*, 1999) showed that, over a 3 year follow-up period, new lesions in patients with SPMS presented a more severe and significant MTR reduction than do those in patients with RRMS. Decreased MTR has also been found for NAWM areas that are adjacent to focal T2-weighted MS lesions (Filippi *et al*, 1995; Loevner *et al*, 1995); MTR progressively increased with distance from MS lesions to the cortical gray matter and MTR was lower for patients with more disabling MS courses. The latter findings suggest that the actual size of MS lesions is greater than that visible on T2-weighted images (Filippi *et al*, 1995). All these studies indicate that a wide range of pathological substrates underlie the non-specific conventional MRI signal changes and that T2-weighted MS lesions with lower MTR are expressions of more severe demyelination.

Pathological studies have confirmed that hypointense lesions on T1-weighted MRI correspond to areas of tissue disorganisation due to demyelination and/or axonal loss (van Walderveen *et al*, 1998). The potential of T1-weighted MRI abnormalities as pathologically specific MRI markers of MS severity has been confirmed by studies using MTI. Lower MTR has been reported in hypointense lesions than in lesions that are isointense to NAWM on T1-weighted scans (van Waesberghe *et al*, 1998b; Hiehle *et al*, 1995), and MTR has been found to be inversely correlated with the degree of hypointensity (Hiehle *et al*, 1995). van Waesberghe *et al* (1997) demonstrated that MTR in MS lesions was significantly correlated with their longitudinal relaxation rates and signal intensities normalised to both NAWM and CSF on T1-weighted MRI, thus concluding that these MRI measures may all be considered markers of severe tissue destruction. More recently, the same authors (van Waesberghe *et*

al, 1999) performed a correlative study between MTI findings and post-mortem brain specimens from 17 MS patients and confirmed that both MTR and T1 contrast ratio correlate strongly with axonal density in MS lesions and NAWM. In addition, they found that both MTR and T1 contrast ratio well correlate with the degree of demyelination within MS lesions. van Waesberghe *et al* (1998b) also compared the natural course of active MS lesions on serial unenhanced T1-weighted and MTI scans. They found that the patterns described for active lesions on both baseline and follow-up unenhanced T1-weighted and MTI scans were highly correlated. For MS lesions that changed from hypointense to isointense when Gd enhancement ceased, MTR increased significantly during a 6 month follow-up, whereas a strongly decreased MTR at the time of initial enhancement was predictive of a persistent T1-weighted hypointensity and lower MTR after 6 months.

Gd enhancement of MS lesions may show two typical patterns: homogeneous and ring-like (Kermode *et al*, 1990; Bruck *et al*, 1997). The latter is thought to reflect peripheral inflammation and complete central demyelination (Bruck *et al*, 1997). The relationship between the enhancement patterns of MS lesions and their MTR has been extensively investigated. Petrella *et al* (1996) and Campi *et al* (1996) found that the MTR for homogeneously enhancing lesions was significantly higher than in the central portion of ring-like enhancing lesions. A recent longitudinal study (van Waesberghe *et al*, 1998b) also confirmed that ring-like enhancing lesions had the lowest MTR, both at baseline and at follow-up, after enhancement ceased. Moreover, ring-enhancing lesions had significantly greater enhancement areas than homogeneously enhancing lesions, and all were hypointense on both baseline and follow-up unenhanced T1-weighted scans.

Longitudinal studies correlating MTI and enhanced T1-weighted MRI using monthly (van Waesberghe *et al*, 1998b; Filippi *et al*, 1998b; Dousset *et al*, 1998; Lai *et al*, 1997) or weekly (Silver *et al*, 1998) scanning schedules found that new enhancing lesions all show a reduction of MTR, which may subsequently show a partial or complete recovery. MTR recovery mainly occurs during the first few weeks after new lesion formation, which is consistent with pathological reports of remyelination in nascent MS lesions (Prineas *et al*, 1993). A recent investigation (Filippi *et al*, 1998b) of the correlation between MTR and enhanced MRI used a triple dose (TD) of Gd, a technique which reveals a substantial number of active MS lesions not seen with standard dose (SD) Gd (Filippi *et al*, 1996, 1998c), and showed that new lesions enhancing only after TD had significantly higher MTR than those enhancing with SD (Filippi *et al*, 1998b). Mean MTR in enhancing lesions recovered significantly during a 3 month follow-up period and, at each time point during this follow-up, MTR in TD lesions was significantly higher than in SD lesions (Filippi *et al*, 1998b). These results highlight the pathological heterogeneity of enhancing MS lesions, and indicate a less severe tissue damage in those lesions with less severe blood-brain barrier disruption.

MTI in the assessment of MS disease burden

Measures of MS disease burden that can be derived from MTI scans include MTI lesion load (LL) (Rovaris *et al*, 1997; van Waesberghe *et al*, 1998a) and MTR histogram-derived parameters (van Buchem *et al*, 1996). Several studies (Rovaris *et al*, 1997, 1998; van Waesberghe *et al*, 1998a; Filippi *et al*, 1998a) have shown that MTI, T2- and T1-weighted LL differ considerably, and the measurement reproducibilities also differ. One possible explanation for these conflicting findings is that the identification of MS lesions on MTI scans is rather subjective, albeit supported by the presence of corresponding abnormalities on T2-weighted images. The volume of hypointense lesions on MTI scans would seem not to be a reliable MRI measure of disease burden in MS, as also indicated by its modest correlations with clinical disability (Rovaris *et al*, 1997, 1998), that are similar or even lower than for T2-weighted LL (van Waesberghe *et al*, 1998a; Filippi *et al*, 1998a).

A more convenient way to assess global MS disease burden on MTI scans is to use MT histogram analysis (van Buchem *et al*, 1996). The data from two recent studies (Philips et *et al*, 1998; Rovaris *et al*, 1999a) showed that a reduction of MT histogram peak height is strongly correlated with both an increasing volume of MS lesions seen on T2-weighted MRI and a greater degree of brain atrophy. As expected, T2- and T1-weighted lesion volumes also influence the average brain MTR. The robust correlations between MS disease burden on conventional MRI and MT histogram metrics confirm that the latter technique can be used as a reliable method to assess disease severity in MS. Moreover, the major influence that brain atrophy has on the MT histogram supports the hypothesis that MT histogram analysis may also provide information about the more severe MS pathological processes (either macro- and microscopic) which leads to loss of brain parenchyma.

Several studies demonstrated that brain MT histogram measures can distinguish MS patients from healthy controls (van Buchem *et al*, 1996; Filippi *et al*, 1999a; Rocca *et al*, 1999). MS patients typically have lower average MTR, histogram peak height and position than normal subjects. However, MT histogram parameters can be different in the various clinical forms of MS, as

demonstrated by Filippi *et al* (1999a)) in a cross-sectional study of 93 MS patients with different clinical phenotypes. Patients with clinically isolated syndromes suggestive of MS have MT histogram-derived metrics similar to those from healthy controls, whereas primary progressive MS patients have signficantly lower histogram peak height with normal peak position and only slightly reduced average MTR. RRMS patients have lower average MTR and peak height than benign MS, whose histograms are similar to those of healthy subjects. Patients with SPMS had the lowest MT measures. On the basis of these results (Filippi *et al*, 1999a), it can be concluded that MT histogram-derived measures can provide insights into the pathogenesis of the different MS phenotypes. For instance, the reported findings suggest that, in primary progressive MS, a subtle but widespread damage of the NAWM seems to be the major contributor to the neurological impairment. Other studies have found that MT histogram metrics are also well correlated with the presence of neuropsychological impairment in MS patients (Rovaris *et al*, 1998, 1999b; van Buchem *et al*, 1998) and that MT histogram parameters from the cerebellum and brainstem of MS patients are significant predictors of disability in these functional systems (Mastronardo *et al*, 1998).

Since, in all the aforementioned studies, slabs of whole brain tissue were used to create MT histograms, the relative contributions of macroscopic lesions and of subtle NAWM abnormalities to the overall MS disease burden were not clearly disentangled. However, the macroscopic lesions segmented on T2-weighted images can be superimposed onto the co-registered MTR maps and the areas corresponding to the segmented lesions can be nulled out, thus obtaining MTR maps of normal-appearing brain tissue (NABT) (Tortorella *et al*, 2000). Recent studies using such an approach demonstrated that NABT MT histogram measures are different in the different MS clinical phenotypes (Tortorella *et al*, 2000). Using a multivariate analysis of several MRI and MTI variables, Filippi *et al* (1999c) found that average MTR of the NABT was the only factor that significantly predicted cognitive impairment in a group of 20 MS patients.

Recent advances in MTI acquisition techniques lead to an improved quality for MT-calculated images of the cervical spinal cord. Using cervical cord MT histogram analysis, it has been reported that, in MS patients, MT histogram measures correlate well with locomotor disability (Filippi *et al*, 1999b) and do not differ from those obtained in patients with Devic's neuromyelitis optica (Filippi *et al*, 2000).

References

Bruck W, Bitsch A, Kolenda H, Bruck Y, Stiefel M, Lassmann H (1997). Inflammatory central nervous system demyelination: correlation of magnetic resonance imaging findings with lesion pathology. *Ann Neurol* **42:** 783–793.

Campi A, Filippi M, Comi G, Scotti G, Gerevini S, Dousset V (1996). Magnetization transfer ratios of contrast-enhancing and nonenhancing lesions in multiple sclerosis. *Neuroradiology* **38:** 115–119.

Dousset V, Brochet B, Vital A, Gross C, Benazzouz A, Boullerne A, Bidabe AM, Gin AM, Caille JM (1995). Lysolecithin-induced demyelination in primates: preliminary in vivo study with MR and magnetization transfer. *AJNR Am J Neuroradiol* **16:** 225–231.

Dousset V, Gayou A, Brochet B, Caille JM (1998). Early structural changes in acute MS lesions assessed by serial magnetization transfer studies. *Neurology* **51:** 1150–1155.

Dousset V, Grossman RI, Ramer KN, Schnall MD, Young LH, Gonzalez-Scarano F, Lavi E, Cohen JA (1992). Experimental allergic encephalomyelitis and multiple sclerosis: lesion characterization with magnetization transfer imaging. *Radiology* **182:** 483–491.

Filippi M, Miller DH (1996). MRI in the differential diagnosis and monitoring the treatment of multiple sclerosis. *Curr Opin Neurol* **9:** 178–186.

Filippi M, Bozzali M, Horsfield MA, Rocca MA, Sormani MP, Iannucci G, Colombo B, Comi G (2000). A conventional and magnetization transfer MRI study of the cervical cord in patients with multiple sclerosis. *Neurology* **54:** 207–213.

Filippi M, Campi A, Dousset V, Baratti C, Martinelli V, Canal N, Scotti G, Comi G (1995). A magnetization transfer imaging study of normal-appearing white matter in multiple sclerosis. *Neurology* **45:** 478–482.

Filippi M, Iannucci G, Tortorella C, Minicucci L, Horsfield MA, Colombo B, Sormani MP, Comi G (1999a). Comparison of MS clinical phenotypes using conventional and magnetization transfer MRI. *Neurology* **52:** 588–594.

Filippi M, Rocca MA, Horsfield MA, Comi G (1998a). A one year study of new lesions in multiple sclerosis using monthly gadolinium-enhanced MRI: Correlations with changes of T2 and magnetization transfer lesion loads. *J Neurol Sci* **158:** 203–208.

Filippi M, Rocca MA, Moiola L, Martinelli V, Ghezzi A, Capra R, Salvi F, Comi G (1999b). MRI and MTI changes in the brain and cervical cord from patients with Devic's neuromyelitis optica. *Neurology* **53:** 1705–1710.

Filippi M, Rocca MA, Rizzo G, Horsfield MA, Rovaris M, Minicucci L, Colombo B, Comi G (1998b). Magnetization transfer ratios in MS lesions enhancing after different doses of gadolinium. *Neurology* **50:** 1289–1293.

Filippi M, Rovaris M, Capra R, Gasperini C, Yousry TA, Sormani MP, Prandini F, Horsfield MA, Martinelli V, Bastianello S, Kuhne I, Pozzilli C, Comi G (1998c). A multi-center longitudinal study comparing the sensitivity of monthly MRI after standard and triple dose gadolinium-DTPA for monitoring disease activity in multiple sclerosis: implications for phase II clinical trials. *Brain* **121:** 2011–2020.

Filippi M, Tortorella C, Rovaris M, Bozzali M, Possa F, Iannucci G, Comi G (1999c). Changes in the normal appearing brain tissue and cognitive impairment in multiple sclerosis. *J Neurol* **246** (Suppl 1): I/63.

Filippi M, Yousry T, Campi A, Kandziora C, Colombo B, Voltz R, Martinelli V, Spuler S, Bressi S, Scotti G, Comi G (1996). Comparison of triple dose versus standard dose gadolinium-DTPA for detection of MRI enhancing lesions in patients with MS. *Neurology* **46:** 379–384.

Gass A, Barker GJ, Kidd D, Thorpe JW, MacManus DG, Brennan A, Tofts PS, Thompson AJ, McDonald WI, Miller DH (1994). Correlation of magnetization transfer ratio with disability in multiple sclerosis. *Ann. Neurol* **36:** 62–67.

Hiehle JF, Grossman RI, Ramer KN, Gonzalez-Scarano F, Cohen JA (1995). Magnetization transfer effects in MR-detected multiple sclerosis lesions: comparison with gadolinium-enhanced spin-echo images and non-enhanced T1-weighted images. *AJNR Am J Neuroradiol* **16:** 69–77.

Kermode AG, Thompson AJ, Tofts PS, MacManus DG, Kendall BE, Kingsley DPE, Moseley IF, Rudge P, McDonald WI (1990). Heterogeneity of blood-brain barrier changes in multiple sclerosis: an MRI study with gadolinium-DTPA enhancement. *Neurology* **40:** 229–235.

Lai HM, Davie CA, Gass A, Barker GJ, Webb S, Tofts PS, Thompson AJ, McDonald WI, Miller DH (1997). Serial magnetization transfer ratios in gadolinium-enhancing lesions in multiple sclerosis. *J Neurol* **244:** 308–311.

Loevner LA, Grossman RI, Cohen JA, Lexa FJ, Kessler D, Kolson DL (1995). Microscopic disease in normal-appearing white matter on conventional MR imaging in patients with multiple sclerosis: assessment with magnetization-transfer measurements. *Radiology* **96:** 511–515.

Mastronardo G, Iannucci G, Rocca MA, Minicucci L, Rovaris M, Rodegher M, Comi G, Filippi M (1998). Correlations between clinical and MRI cerebellar involvement in multiple sclerosis: assessment with T2, T1 and MT histograms. *Neurology* **50** (Suppl 4): 108.

McDonald WI, Miller DH, Barnes D (1992). The pathological evolution of multiple sclerosis. *Neuropathol Appl Neurobiol* **18:** 319–334.

McGowan JC, Filippi M, Campi A, Grossman RI (1998). Magnetisation transfer imaging: theory and application to multiple sclerosis. *J Neurol Neurosurg Psychiatry* **64** (Suppl 1): S66–S69.

Miller DH, Barkhof F, Nauta JJP (1993). Gadolinium enhancement increased the sensitivity of MRI in detecting disease activity in MS. *Brain* **116:** 1077–1094.

Petrella JR, Grossman RI, McGowan JC, Campbell G, Cohen JA (1996). Multiple sclerosis lesions: Relationship between MR enhancement pattern and magnetization transfer effect. *AJNR Am J Neuroradiol* **17:** 1041–1049.

Phillips MD, Grossman RI, Miki Y, Wei L, Kolson DL, van Buchem MA, Polansky M, McGowan JC, Udupa JK (1998). Comparison of T2 lesion volume and magnetization transfer ratio histogram analysis and of atrophy and measures of lesion burden in patients with multiple sclerosis. *AJNR Am J Neuroradiol* **19:** 1055–1060.

Prineas JW, Barnard RO, Kwon EE, Shorer LL, Cho ES (1993). Multiple sclerosis: remyelination of nascent lesions. *Ann Neurol* **33:** 137–151.

Rocca MA, Mastronardo G, Rodegher M, Comi G, Filippi M (1999). Long-term changes of magnetization transfer-derived measures from patients with relapsing-remitting and secondary progressive multiple sclerosis. *AJNR Am J Neuroradiol* **20:** 821–827.

Rovaris M, Bozzali M, Rodegher M, Tortorella C, Comi G, Filippi M (1999a). Brain MRI correlates of magnetization transfer imaging metrics in patients with multiple sclerosis. *J Neurol Sci* **66:** 58–63.

Rovaris M, Comi G, Possa F, Iannucci G, Santuccio G, Minicucci L, Filippi M (1999b). Multiparametric brain MRI study of frontal lobe dementia in multiple sclerosis. *Proc Intl Soc Magn Reson Med* **7:** 614.

Rovaris M, Filippi M, Calori G, Rodegher M, Campi A, Colombo B, Comi G (1997). Intra-observer reproducibility in measuring new putative MR markers of demyelination and axonal loss in multiple sclerosis: a comparison with T2-weighted images. *J Neurol* **244:** 266–270.

Rovaris M, Filippi M, Falautano M, Minicucci L, Rocca MA, Martinelli V, Comi G (1998). Relation between MR abnormalities and patterns of cognitive impairment in multiple sclerosis. *Neurology* **50:** 1601–1608.

Silver NC, Lai M, Symms MR, Barker GJ, McDonald WI, Miller DH (1998). Serial magnetization transfer imaging to characterize the early evolution of new MS lesions. *Neurology* **51:** 758–764.

Tortorella C, Viti B, Bozzali M, Sormani MP, Rizzo G, Gilardi MF, Comi G, Filippi M (2000). A magnetization transfer histogram study of normal appearing brain tissue in multiple sclerosis. *Neurology* **54:** 186–193.

van Buchem MA, Grossman RI, Armstrong C, Polansky M, Miki Y, Heyning FH, Boncoeur-Martel MP, Wei L, Udupa JK, Grossman M, Kolson DL, McGowan JC (1998). Correlation of volumetric magnetization transfer imaging with clinical data in MS. *Neurology* **50:** 1609–1617.

van Buchem MA, McGowan JC, Kolson DL, Polansky M, Grossman RI (1996). Quantitative volumetric magnetization transfer analysis in multiple sclerosis: estimation of macroscopic and microscopic disease burden. *Magn Reson Med* **36:** 632–636.

van Waesberghe JHTM, Castelijns JA, Scheltens P, Truyen L, Lycklama à Nijeholt GJ, Hoogenraad FG, Polman CH, Valk J, Barkhof F (1997). Comparison of four potential MR parameters for severe tissue destruction in multiple sclerosis lesions. *Magn Reson Imaging* **15:** 155–162.

van Waesberghe JHTM, Kamphorst W, De Groot CJA, van Walderveen MAA, Castelijns JA, Ravid R, Lycklama à Nijeholt GJ, van der Valk P, Polman CH, Thompson AJ, Barkhof F (1999). Axonal loss in MS lesions: MRI insights into substrates of disability. *Ann Neurol* **46:** 747–754.

van Waesberghe JHTM, van Buchem MA, Filippi M, Castelijns JA, Rocca MA, van der Boom R, Polman CH, Barkhof F (1998a). MR outcome parameters in multiple sclerosis: comparison of surface-based thresholding segmentation and magnetization transfer ratio histographic analysis in relation to disability (a preliminary note). *AJNR Am J Neuroradiol* **19:** 1857–1862.

van Waesberghe JHTM, van Walderveen MAA, Castelijns JA, Scheltens P, Lycklama à Nijeholt GJ, Polman CH, Barkhof F (1998b). Patterns of lesion development in multiple sclerosis: longitudinal observations with T1-weighted spin-echo and magnetization MR. *AJNR Am J Neuroradiol* **19:** 675–683.

van Walderveen MAA, Kamphorst W, Scheltens P, van Waesberghe JHTM, Ravid R, Valk J, Polman Ch, Barkhof F (1998). Histopathologic correlate of hypointense lesions on T1-weighted spin-echo MRI in multiple sclerosis. *Neurology* **50:** 1282–1288.