MULTIPLE SCLEROSIS AND THE GREY MATTER

R. Tanasescu[1†], Marina Ticmeanu[1], Inimioara Cojocaru[1], Cecilia Burcin[1], Gabriela Mihailescu[1], Alexandra Oprisan[1], Amalia Ene[2], Daniela Anghel[3], Adriana Nicolau[4]

[1] Neurology Department, "Colentina" Hospital, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

[2]Neurology Department, University Hospital, Bucharest, Romania

[3]Neurology Department, "Fundeni" Hospital, Bucharest, Romania

[4]Internal Medicine Department, "Colentina" Hospital, Bucharest, Romania

[†]Réseau d'Epidémiologie International Francophone (RECIF)

ABSTRACT

Traditionally, multiple sclerosis (MS) has been considered a white matter disease, but MS lesions are known to occur in grey matter. Recent immunohistochemical studies have demonstrated extensive grey matter demyelination in chronic MS, grossly underestimated by standard hystochemical myelin staining methods. Extensive cortical demyelination is associated with the progressive phases of disease, as less cortical demyelination has been detected in relapsing-remitting MS. The pathology of grey matter lesions differs from that of white matter lesions. Significant axonal transection and neuronal loss have been demonstrated in grey matter MS lesions. Current magnetic resonance imaging (MRI) methods are not sensitive for purely cortical MS lesions. Special MRI techniques permit the visualization of grey matter lesions, and in the future more sensitive MRI methods will help to characterize their clinical significance.
Key words: grey matter; multiple sclerosis; demyelination; cerebral cortex; axonal loss.

Although known for a long time as a white matter (WM) disease, multiple sclerosis (MS) is characterized by lesions that can occur in all central nervous system (CNS) parenchymal areas, including cerebral cortex and deep grey matter (GM) (1).

In the last decade, new techniques, derived from conventional magnetic resonance imaging (cMRI, consisting in dual-echo and post contrast T1-weighted scans), have proved the existence of the GM lesions in MS patients (2). For the clinician, these lesions are important to be known not only because they can explain a part of the neurological deficits (for example, the cognitive impairment), but this allow the correct evaluation of the whole CNS lesion load. In this respect, the assessment of the lesion type and total lesion burden (in the WM *and* GM) during the evolution of the disease, offers data about the disease activity and can be studied in relation with the clinical MS form.

The correlation between the location and size of the GM lesions and the clinical changes is still poorly understood. From the clinical point of vue, some of the MS clinical features were reconsidered in the nineties (cognitive impairment, symptoms with cortical origin, fatigue and mood disorders). Meanwhile, the improvements of the radiological and hystopathological techniques have facilitated the understanding of some of the morphological changes at the origin of the clinical symptoms. However, the knowledge of each particular item needs still to be refined.

We will further review the data regarding the GM damage in MS.

The approach can be structured at three levels: anatomical, structural and functional.

ANATOMICAL CHANGES

The GM impairment in MS patients is not a recent discovery. Classical pathology studies using brain biopsy samples, dating from 19th century and early 20th century (Sander 1889, Dinkler 1904, Schob 1907, Dawson 1916), report findings of extensive cortical demyelination. In some of studied patients, the majority of MS lesions were cortical.

In the next period, the GM damage has been neglected in favour of extensive studies about the WM lesions, being recovered in the last years, mainly because of the progress of the MRI techniques.

The characteristic histological changes in MS consist of discrete areas of myelin, olygodendrocites and axonal loss, so called „plaques"(3). Although

they are located more often in the WM, they also can be found in the central and peripheral GM of the cerebral cortex, thalamus, striatum, protuberance, cerebellum and spinal cord. 5% of them are estimated to be cortical, especially in the frontal lobe, 4% involving the deep GM. 17% of the hemispheric lesions are located at the cortico-subcortical joint. These lesions are underestimated by the macroscopic examination and can be revealed by techniques using myelin staining or, better, by immunohistochemistry.

The plaques involving the inner cortex are the most difficult to be seen by the eye, the estimated frequency being 5% from the whole hemispheric lesions (4). Using myelin staining methods they appear much more frequent; some authors reporting up to 30 lesions on a single sample of frontal lobe parenchyma (5). In other MS patients, GM plaques are described in a greater number then those in the WM (6).

Special techniques for tissue staining are necessary in order to reveal the GM lesions because of their different pattern versus the WM ones: GM lesions have less inflammatory cells, the extent of macrophage and lymphocyte infiltration is low, perivascular cells are lacking and macrophages are present in a smaller number than is WM lesions.

Interestingly, in MS purely cortical lesions T-cell density is not increased and a similar distribution of B cells and T-cell subsets is observed, when compared with non-demyelinated areas in MS patients and controls (7). This finding could suggest that the presence of local lymphocyte infiltration in MS lesions is dependent on lesion location (GM or WM). Complement activation in MS lesions may also be location-dependent, as it was detected in chronic MS autopsy material in WM lesions, but not in GM cortical ones (8).

Another distinguishable feature concerns the major histocompatibility complex antigens: the MHC class II-positive cells in GM lesions have the features of activated microglia, while in the WM lesions MHC class II-positive cells mainly have the morphology of phagocytic macrophages (7,9).

Even classically the most frequent location of the GM lesions was considered to be the border between GM and WM, recent studies using immunohistochemical techniques have identified the subpial regions as the most common location of cortical SM lesions (9,10,11,12).

Recently, Bö et al. used myelin basic protein (MBP) immunohistochemistry to study the extent and distribution of GM demyelination in a brain autopsy material from 20 chronic MS patients, examining 4 samples from each brain, with or without macroscopic changes. The percentage of demyelinated area in the cortex was 25% (median 14%), whereas in the subcortical and periventricular WM was 5% (median 0%). The cortical lesions have been classified in a descriptive manner according to their distribution in the cerebral cortex and subcortical WM (9). Four different cortical lesion types were identified. The most common type, affecting the largest cortical extent and accounting for 67% of the total demyelinated area, was a subpial lesion (type 3). These type 3 lesions commonly appear as long ribbons of subpial demyelination, often affecting several adjacent gyri. In 25% of the patients, this subpial demyelination was extensive in all the four widely spaced tissue blocks selected, indicating a widespread superficial loss of cortical myelin, that was termed by Bö as „general cortical subpial demyelination“ (GSD). Apparently paradoxical, in these patients there was not found an increase in the „lesion burden“ of the WM (7,12). This lack of quantitative correlation between the two types of lesions (in the WM and the GM respectively), is revealed also by other studies which are emphasising the presence of GSD especially in patients with progressive forms of MS (13,14).

Usually, the cortical/WM junction lesions are small in size and, in contrast with the periventricular plaques of the WM, without tendency to increase their volume. These junction lesions are round or ovoid in shape, including both the U fibres and the profound layer of the cortex (6). This latter characteristic is another distinguishable feature when comparing with the WM plaques, which usually spear the U fibres.

Morphologically, if we don't take to account the cortex-WM junction lesions and the giant plaques from Schindler disease that can touch sometimes the GM, one can describe four types of cortical plaques:

- conic shape, with pial basement,
- like a circle, surrounding a vessel,
- „in the mirror“, located on a each side of a sulcus (sometimes surrounding a leptomeningeal vessel),
- confluent, *"moth-eaten-like",* like *"cortical caries"*.

Cortical cerebellar plaques are the same in shape as the ones above.

The brain stem GM plaques are developing in the same manner, the superficial ones usually conic in shape, and the inner ones usually spherical surrounding a vessel. The most affected areas are the interapeductal GM and the ceiling of the IVth

ventricle. At the level of the median raphe, pontine olives, spinal cord and the emergence point of the cranial nerves, especially the Vth and the VIIIth pairs, the lesions may be bilateral, more or less symmetrical.

It is accepted that 86% of the MS patients present GM lesions. In these patients, the GM lesions can reach 26% from the entire damaged cerebral parenchyma (11)

Since axonal degenerescence is certitude in MS, new imunohistochemistry techniques have developed for the study of axonal damage, detecting neurophilaments or A-beta precursor protein (APP) which is transported by the axonal flux (15,16,17). A disruption in the axonal flux can determine the accumulation of APP. There are many recent studies using this method that demonstrate the importance and precocity of axonal damage in MS, as well as the secondary axonal lesions evolving by retrograde degenerescence from a initially demyelinated zone (18,19,20).

The neuronal density correlates with the extent of cortical demyelination, decreasing with 18-23% in MS patients with extensive cortical demyelination (14). In more profound structures as thalamus, the axonal loss can reach about 35% when compared to healthy people (21).

Regarding the optic pathway, the correlation between axonal loss of the optic nerve and morphological derangement in the parvocellular layer of the lateral geniculate nucleus is suggesting that the GM neuronal damage would be the consequence of the WM axonal lesions (22). This is emphasized by the decrease in the mitochondrial functions in the non-demyelinated cortical neurons, possibly secondary to the downstream WM demyelination (23).

Neuronal apoptosis is also involved in the genesis of the GM lesions, playing a role in the demyelination pathway rather than in the inflammatory process (9). The most of the apoptotic neurons are large and pyramidal, and located in the cortical layers 3 and 5. By confocal double-labelling microscopy, it was found that microglia extended processes to ensheathed neuritic elements and apoptotic neuronal cell bodies (9).

The lesional process, whose etio-pathogenic mechanisms are not fully understood, must not be seen as a unidirectional one. The immunohistochemical techniques reveal elements suggesting the regenerative capacity of the neurons neighbouring the subaccute plaques. This regenerative activity is also sustained by the expression of the transcription factors, like Jun (24).

As a conclusion, the morphologic changes of GM are extensive, especially in progressive MS, the subpial lesions being the most frequent type of cortical lesions.

Although the GM damage has been described in most of the studies before the 2000's as a selective process of myelin loss with less inflammation and cyto-architecture derangements than in the WM lesions, the existence of neuronal and axonal damage occurring in the middle of the cortical lesion areas must be emphasized.

MACROSCOPIC FEATURES IN IRM

It has to be said from the start that conventional MRI has a low sensitivity for GM lesions, and GM can look normal even if affected. This is the mainly reason why the extension of the cortical damage is difficult to be established in vivo (11,25).

Indeed, the GM hypersignals in T2-weighted images are difficult to be seen because of their small size and the partial volume effect given by the subarachnoid spaces, but also secondary to the fact that relaxation characteristics offer a low contrast with the surrounding GM (11). This lesions are better visualized in FLAIR sequences T2-wieghted which annihilate the CSF signal (26,27,28).

The frequency of the GM lesions estimated by cMRI is variable, Gadolinium (Gd) - enhanced T1-wieghted images allow one to reveal but the mean is 9,6 lesions/patient, located mainly in the frontal lobe (29). The sensitivity of the cMRI for GM cortical lesions is 3-5%, and bigger for the more profound lesions: 10-40% (25,30). With 140% more lesions comparing with the T2-weighted ones, 16% of them cortical and 26% sub- or juxta-cortical (10). T2-weighted hypo-intensity has been described recently in the central grey nuclei, so called *black*-T2 or BT2 (31). This kind of image can also be found in other cerebral structures where the GM prevails, as the red nucleus and thalamus. Interestingly, it was never described in locus niger. It seem to be connected to iron deposits secondary to neuronal degenerescence, the latter one as a consequence of the toxic free radicals generated by electron transfer, a mechanism similar to those in neurodegenerative diseases (2).

STRUCTURAL CHANGES

New MRI techniques, including magnetization transfer MRI (MT-MRI) and diffusion-weighted MRI (DW-MRI), allow a better evaluation of the structural damage in MS (32).

MT-MRI is based on the interactions between protons in a relatively free environment with those which motion is restricted (bounded to the myelin macromolecules and the cell membranes). Off-resonance irradiation is applied, which saturates the magnetization of the less mobile protons, thus reducing the signal intensity from the observable magnetization. Thus, a low MT ratio (MTR) indicates a reduced capacity of macromolecules in the CNS to exchange magnetization with the surrounding water molecules, reflecting damage to myelin or to the axonal membrane. Decreased MTR correlates with axonal and myelin loss (33).

Diffusion in the microscopic random translational motion of molecules in a fluid system. In the CNS, diffusion is influenced by the micro-structural components of tissue, including cell membranes and organelles. The diffusion coefficient of biological tissues (which can be measured in vivo by MRI) is, therefore, lower than that of the water and, for this reason, it is named as apparent diffusion coefficient (ADC). Pathological processes, resulting in a loss or increased permeability of „restricting" barriers, can determine an increase of the ADC. Since some cellular structures are aligned on the scale of an image pixel, the measurement of diffusion is also dependent on the direction in which diffusion is measured. As a consequence, diffusion measurements can give information about the size, shape, integrity and orientation of the tissues. A measure of diffusion, which is dependent of the orientation of structures, is provided by the mean diffusivity (MD), the average of the ADCs measured in three orthogonal directions. A full characterization of diffusion can be obtained in terms of a tensor, a 3x3matrix which accounts for the correlation existing between molecular displacements along orthogonal directions. From the tensor, it is possible to derive MD, equal to the one-third of its trace, and some other dimensionless indices of anisotropy, one of the most used being fractional anisotropy (FA). The MD is isotropic in the GM and very anisotropic in the WM.

The decrease of MTR and the increase of MD in normal-appearing-MRI GM are signs of the loss of tissue integrity and the structural damage of the barriers for water movement (34). These changes can be secondary to the GM lesions and/or the wallerian degenerescence in the fibres that make the connection with the WM. One can establish which of the two hypotheses the real one at a time is for one patient by comparing the lesion burden in T2 on one side, and MTR and MD on the other side. If there are not any lesions in the WM but MTR and MD are abnormal, there is a high possibility of GM lesions secondary to the disease but not to the WM lesions. The FA is decreased in the WM at both levels, supra- and infra-tentorial, but increased in the grey nuclei in MS patients (35).

CLINICAL CORRELATIONS

There is a significant correlation, especially for secondary progressive SM patients, between the cortical and juxta-cortical anomalies detected in the FLAIR sequences and the age of the disease (29). Naturally, the frequency of the cortical hypersignals in FLAIR in a certain region correlates with the presence of the cortical atrophy in that region.

It is a fact that the extensive cortical damage is associated with chronic and advanced forms of the disease and, meanwhile, it is present in a lesser extend in remittent forms (12,13,14).

As we have said before, the IRM techniques show better the presence and the extension of the juxtacortical lesions, with a higher sensitivity than for the cortical ones. For some authors, the presence of this lesions correlates with the cognitive impairment, depression, affective disorders, cortical symptoms and epilepsy in MS patients (36,37,38,39).

The same correlations have been described, however inconstantly, as regarding the cortical lesions, which are associated with memory troubles, cortical symptoms, depression and affective disorders (36). Other studies suggest that the anomalies of the cortex and the deep GM, representing 5,7% and 4,6% respectively from the total lesion burden in MS, don't correlate nor with the cognitive impairment or the EDSS (40). On the other hand, the indirect markers of the neuronal loss (the black wholes in T1 sequences and the cerebro-medullar atrophy) seem to correlate with the clinical deficit (41,42,43). Other association was found between the T2 lesion burden and the cognitive impairment and the decrease in the cerebral metabolism, measured by positron emission tomography (44).

The T2-weighted hypointensity images (BT2) deserve to be treated separately. BT2 could be explained by the wallerian degenerscence prolonging the T2 but not the T1 sequences, and by the iron deposition. Their presence seems to be correlated with the degree of the handicap and the clinical evolution, but not with the age of the disease or the cerebral atrophy (31). BT2 are highly predictive (more than the T2 lesion burden and the T1 black wholes) for the degree of handicap and the clinical evolution. Moreover, the BT2 situated in the nucleus

caudatum are highly predictive for the enlargement of the third ventricle. This cerebral atrophy marker can predict the degree of handicap (31). Interestingly, the BT2 allow differentiating the remittent forms from the secondary progressive forms of MS, but correlate neither with the lesion activity nor with black holes. Finally, the localisation of BT2 in thalamus, nucleus caudatum and putamen is considered predictive for clinical evolution, and the presence of BT2 in globus pallidus correlates with the global lesion burden in T2 (31). Very recent data suggest that BT2 lesions detected in the globus pallidus and the caudate nucleus with 3T MRI correlate significantly with EDSS and therefore be a useful MRI measure associated with disability in MS and warrants further study (57).

Going back to the MRI imagistic parameters mentioned above (mean diffusivity – MD, magnetization transfer ratio – MTR and fractional anisotropy - FA), it has been proved that the cognitive impairment correlates with the increase of MD and the decrease of MTR in the GM (34). Furthermore, MD seems to correlate with EDSS and the age of the disease, and a decrease of MTR in the normal appearing GM is found especially in recurrent-remittent forms of MS (35,45). As for FA, it diminishes in the WM but is increased in the region of the grey nuclei, especially in putamen and thalamus (35).

Finally, an emphasis must be placed on the heterogeneity of the imagistic examination parameters for which correlations with the clinical features were described. In the future, a standardisation of these parameters is necessary prior to include them in the MS diagnostic algorithms.

FUNCTIONAL CONSEQUENCES

The GM damage in MS can be direct (due to lesions), or indirect, secondary to WM lesions.

The physiopathology of MS implies tissue destruction and degenerescence with focal irreversible lesions of the WM, diffuse axonal loss and cerebral atrophy (17,46). The cerebral atrophy is precocious and a consequence of either the WM lesions alone or the WM and GM damage together (47). The axonal-neuronal damage is involved in the appearance and worsening of the clinical handicap.

Spectroscopy MR (SMR) shows a decrease of N-acetyl-aspartate (NAA) in acute plaques. NAA is a marker of axonal integrity and it's decrease is reversible and correlated with the clinical deficit (48,49). In normal appearing WM, the NAA is irreversible decreased and correlates with the increase of handicap in MS patients. This type of changes seems to have topographic specificity (50). The axonal loss is more severe in secondary progressive forms and correlates with disease age and clinical handicap. However, the speed of NAA decrease is higher in secondary progressive forms of MS (49,51). A decrease of NAA has also been observed in the normal appearing GM in recent MS patients, with a disease start under 3 years.

There is a correlation between the decrease of MTR and NAA, thus between myelin and axonal damage in the plaques, especially in the secondary-progressive forms (52).

The degree of the neuroplasticity and the functional adaptative capacity of the axons are more important in the brain than the spinal cord. Combined studies including functional-MRI (fMRI) and SMR have shown an important increase of the neuronal activation, of almost 5 times, in the sensory-motor cortex, that correlates with a decrease of NAA. Thus, the increase of this neuronal activation helps the functional preservation in the presence of axonal damage. The remission of the deficit precedes that of the axonal damage in the lesion area, and is accompanied by an increase of the recruitment in the motor ipsilateral cortex (53). In this respect, fMRI can be a method to follow-up the recovery after the attack, the reorganization of the neuronal circuits, and thus to estimate the adaptative cortical changes provoqued by the presence of the lesion (54,55,56,59).

THERAPY AND GM IN MS

It is uncertain whether the immunopathogenic mechanisms underlying GM damage in MS are limited by currently available disease-modifying therapies (58). It is also unknown if GM involvement has any relationship with the proposed classification of WM MS lesion subtypes (60). Focal new WM lesions are associated with blood-brain barrier damage, inflammation, and acute axonal injury both in the lesion and distal to the lesion site due to Wallerian degeneration. This type of injury is likely to be limited by immunomodulatory and immunosuppressant drugs. However, diffuse global brain injury including GM involvement is likely associated with a localised inflammatory response that occurs typically behind an intact blood–brain barrier in the absence of ongoing focal WM demyelination. The limited benefit of anti-inflammatory or immunomodulatory therapy in the chronic slowly progressive phase of MS may in part be explained by this pattern of compartmentalization

of the inflammatory reaction in the CNS. Additional studies targeting GM damage are needed to better understand the pathogenic mechanisms and to elaborate more effective therapeutic strategies.

CONCLUSION

Known, but unfair neglected, the GM damage in MS is confirmed by recent IRM data. The neuronal-axonal damage is precocious; the accumulation of axonal loss can explain the progression of the handicap. In response to the GM lesion there is a degree of cortical adjustment, precocious as well as the lesion. Further studies are necessary to establish if these adaptative changes are much more important in recurrent-remitting forms of MS than in progressive and advanced ones. A better knowledge about the GM damage in MS is important at anatomic, structural and functional levels, not only to understand the MS as a complex pathogenic entity, but because of the potential therapeutic emerging approaches.

REFERENCES

1. **Bo L, Geurts JJG, Mork SL et al** – Grey mater pathology in multiple sclerosis. *Acta Neurol Scand* 2006,113: 48-50.
2. **Tourbah A, Seihlean D** – SEP et substance grise. Neurosciences Internationales, *Sclérose en plaques,* 2002, 9:10-13.
3. **Prineas JW, McDonald WI, Franklin RJM** – Demyelinating diseases. In: Graham DI, Lantos PL, eds. *Greenfield's neuropathology.* London: Arnold, 2002;471-550.
4. **Dinkler M** – Zur Kasuistik der multiplen Herdsklerose des Gehirns und Ruckenmarks. *Deuts Zeits f Nervenheilk* 1904;26:233-47.
5. **Schob F** – Ein Beitrag zur patologischen Anatomie der multiplen Sklerose. *Monatschrift Psychiatrie Neurol* 1907;22:62-87.
6. **Prineas J, McDonald W** – Demyelinating diseases. In: Graham D, Lantos P, eds; *Greenfield's neuropathology.*1997, Arnold, London:813-96.
7. **Bö L,Vedeler CA, Nyland H, Trapp BD, Mörk SJ** – Intracortical multiple sclerosis lesions are not associated with increased lymphocyte infiltration. *Multiple Sclerosis* 2003;9:323-31.
8. **Brink BP, Veerhuis R, Breij ECW, van der Valk P, Dijkstra CD, Bö L** – The pathology of multiple sclerosis is location-dependent: No significant complement activation is detected in purely cortical lesions. *J Neuropathol Exp Neurol* 2005; 64:147-55.
9. **Peterson JW, Bö L, Mörk SJ, Chang A, Trapp BD** – Transected neuritis, apoptotic neurons, and reduced inflammation in cortical multiple sclerosis lesions. *Ann Neurol* 2001; 50: 389-400.
10. **BrownellB, Hughes JT** – The distribution of plaques in the cerebrum in multiple sclerosis. *J Neurol Neurosurg Psychiatry* 1962; 25:315-20.
11. **Kidd D, Barkhof f, McConnell R, Algra PR, Allen IV, Revesz T** – Cortical lesions in multiple sclerosis. *Brain* 1999; 122:17-26.
12. **Bö L, Vedeler CA, Nyland HI, Trapp BD, Mörk SJ** – Subpial demyelination in the cerebral cortex of multiple sclerosis patients. *J Neuropathol Exp Neurol* 2003; 62:723-32.
13. **Kutzelnigg A, Lucchinetti CF, Stadelmann C et al** – Cortical demyelination and diffuse white matter injury in multiple sclerosis. *Brain* 2005; 128:2705-2712.
14. **Vercellino M, Plano F, Votta B, Mutani R, Giordana MT, Cavalla P** – Grey matter pathology in multiple sclerosis. *J Neuropathol Exp Neurol* 2005; 64:1101-7.
15. **Ferguson B, Matyszak M, Esiri M, Perry V** – Axonal damage in acute multiple sclerosis lesions. *Brain* 1997; 120:393-9.
16. **Lovas G, Szilagyi N, Majtenyi K et al** – Axonal changes in chronic demyelinated cervical spinal cord plaques. *Brain* 2000;123:308-17.
17. **Trapp B, Peterson J, Ransohoff Ret al** – Axonal transection in the lesions of multiple sclerosis. *N Engl J Med* 1998; 338:278-85.
18. **Lassmann H, Suchanek G, Ozawa K** – Histopathology and the blood-cerebrospinal fluid barrier in multiple sclerosis. *Ann Neurol* 1994; 36:S42-S46.
19. **Ozawa G, Suchanek H, Breishopf H et al** – Patterns of oligodendroglia pathology in multiple sclerosis. *Brain* 1994; 117:1311-22.
20. **Youl B, Kermode A, Thompson A et al** – Destructive lesions in demyelinating disease. *J Neurol Neurosurg Psychiatry* 1991; 54:288-92.
21. **Cifelli A, Arridge M, Jezzard P, Esiri MM, Palace J, Matthews PM** – Thalamic neurodegeneration in multiple sclerosis. *Ann Neurol* 2002; 52:650-3.
22. **Evangelou N, Konz D, Esiri MM, Smith S, Palace J, Matthews PM** – Size-selective neuronal changes in the anterior optic pathways suggest a differential susceptibility to injury in multiple sclerosis. *Brain* 2001; 124:1813-20.
23. **Dutta R, McDonough J, Yin X et al** – Mitochondrial dysfunction as a cause of axonal degeneration in multiple sclerosis patients. *Ann Neurol* 2006; 59:478-89.
24. **Martin G, Segui J, Diaz-Villoslada P et al** – Jun expresion is found in neurons located in the vicinity of subacute plaques in patients with multiple sclerosis. *Neurosci Lett* 1996; 212:95-8.
25. **Newcombe J, Hawkins CP, Henderson CL et al** – Histopathology of multiple sclerosis lesions detected by magnetic resonance imaging in unfixed postmortem central nervous system tissue. *Brain* 1991; 114:1013-23.
26. **Tourbah A, Deschamps R, Stievenart JL et al** – Magnetic Resonance Imaging using FLAIR pulse sequence in White matter diseases. *J Neuradiol* 1996; 23:217-22.
27. **Filippi M, Yousry TA, Baratti C et al** – Quantitative assessment of MR lesion load in multiple sclerosis:a comparison of conventional spin-echo with fast fluid-attenuated inversion recovery. *Brain* 1996; 119:1349-55.
28. **Gawne-Cain ML, O'Riordan JI, Thompson AJ et al** – Multiple sclerosis lesion detection in the brain: a comparison of fast fluid-attenuated inversion recovery and conventional T2-weighted dual spin-echo. *Neurology* 1997; 49:364-70.
29. **Bakshi R, Ariyaratana BS, Banadict RHB, Jacobs L** – Fluid-attenuated inversion recovery magnetic resonance imaging detects cortical and juxtacortical multiple sclerosis lesions. *Arch Neurol* 2001; 58:742-8.
30. **GeurtsJJ, Bö L, Pouwels PJ, Castelijns JA, Polman CH, Barkhof F** – Cortical Lesions in Multiple Sclerosis: Combined Postmortem MR Imaging and Histopathology. *AJNR Am J Neuroradiol* 2005; 26:572-7.
31. **Bakshi R, Banadict RHB, Bermel RA et al** – T2 hypointensity in the deep gray matter of patients with multiple sclerosis. A quantitative magnetic resonance imaging study. *Arch Neurol* 2002; 59:62-8.
32. **Filippi M, Grossman R** – MRI techniques to monitor MS evolution. The present and the future. *Neurology* 2002; 58:1147-53.
33. **Van Waesberghe JH, Kamphorst W, De Groot CJ et al** – Axonal loss in multiple sclerosis lesions: magnetic resonance imaging insights into substrates of disability. *Ann Neurol* 1999; 46:747-54.
34. **Cercignani M, Bozzali M, Iannucci G et al** – Magnetisation transfer ratio and mean diffusivity of normal appearing white and gray matter from patients with multiple sclerosis. *J Neurol Neurosurg Psychiatry* 2001; 70:311-7.
35. **Ciccareli O, Werring DJ, Wheeler-Kingshott CAM et al** – Investigation of MS normal-aooearing brain using diffusion tensor MRI with clinical correlations. *Neurology* 2001; 56:926-33.

36. **Moriarty DM, Blackshaw A, Talbot PR et al** – Memory dysfunction in multiple sclerosis corresponds to juxtacortical lesion load on by fast fluid-attenuated inversion recovery MR images. *Am J Neuroradiol* 1999; 20:1956-62.
37. **Lazeron RH, Langdon DW, Filippi M et al** – Neuropsychological impairment in multiple sclerosis patients: the role of (juxta) cortical lesion on FLAIR. *Mult Scler* 2000; 6:280-5.
38. **Rovaris M, Filippi M, Minicucci L et al** – Cortical/subcortical disease burden and cognitive impairment in patients with multiple sclerosis. *AJNR Am J Neuroradiol* 2000; 21:402-8.
39. **Feinstein A, Roy P, Lobaugh N, Feinstein K, O'Connor P, Black S** – Structural brain abnormalities in multiple sclerosis patients with major depresion. *Neurology* 2004; 62:586-90.
40. **Catala I, Fulton JC, Zhang Xet al** – Mr imaging quantitation of gray matter involvement in multiple sclerosis an dits correlation with disability measures and neurocognitive testing. *Am J Neuroradiol* 1999; 20:1613-8.
41. **Losseff NA, Wang L, Lai HM et al** – Progressive cerebral atrophy in multiple sclerosis: a serial MRI study. *Brain* 1996: 2009-19.
42. **Losseff NA, Webb SL, O'Riordan JI et al** – Spinal cord atrophy and disability in multiple sclerosis: a new reproducible and sensitive MRI method with potential to monitor disease progression. *Brain* 1996; 119:701-8.
43. **VanWalderveen MA, Lycklama A, Nijeholt GJ et al** – Hypointense lesions on T1-wieghted spin-echo magnetic resonance imaging: relation to clinical characteristics in subgroups of patients with multiple sclerosis. *Arch Neurol* 2001; 58:76-81.
44. **Blinkenberg M, Jensen CV, Holm S et al** – A longitudinal study of cerebral glucose metabolism, MRI, and disability in patients with MS. *Neurology* 1999; 53:149-53.
45. **Ge Y, Grossman RI, Udupa JK et al** – Magnetization transfer ratio histogram analysis of gray matter in relapsing-remitting multiple sclerosis. *Am J Neuroradiol* 2001; 22:470-5.
46. **Truyen L, van Waesberghe JHTM, van Walderveen MAA et al** – Accumulation of hypointense lesions („black holes") on T1 spin-echo MR correlates with disease progression in multiple sclerosis. *Neurology* 1997; 47:1469-76.
47. **Rudick RA, Fisher E, Lee JC et al** – Use of the brain parenchymal fraction to measure whole brain atrophy in relapsing-remitting MS. *Neurology* 1999; 53:1698-04.
48. **De Stefano N, Matthews PM, Fu L et al** – Axonal damage correlates with disability in patients with relapsing-remitting multiple sclerosis: results of a longitudinal magnetic resonance spectroscopy study. *Brain* 1998; 121:1469-77.
49. **De Stefano N, Narayanan S, Francis GS et al** – Evidence of axonal damage in early stages of multiple sclerosis an dits relevance to disability. *Arch Neurol* 2001; 58:65-70.
50. **Lee M, Blamire AM, Pendlebury S et al** – Axonal injury or loss in the internal capsule and motor impairment in multiple sclerosis. *Arch Neurol* 2000;57:65-70.
51. **Tourbah A, Stievenart JL, Gout O et al** – Localized proton magnetic resonance spectroscopy in relapsing-remitting versus secondary progressive MS. *Neurology* 1999; 53:1091-7.
52. **Davie CA, Silver NC, BarkerGL et al** – Does the extent of axonal loss and demyelination from chronic lesions in multiple sclerosis correlate with clinical subgroup? *J Neurol Neurosurg Psychiatry* 1999; 67:710-5.
53. **Reddy H, Narayanan S, Arnoutelis R et al** – Evidence for adaptative functional changes in the cerebral cortex with axonal injury from multiple sclerosis. *Brain* 2000; 123:2314-20.
54. **Lee M, Reddy H, Johansen-BergH et al** – The motor cortex shows adaptive functional changes to brain injury from multiple sclerosis. *Ann Neurol* 2000; 47:606-13.
55. **Rocca MA, Falini A, Colombo B et al** – Adaptive functional changes in the cerebral cortex of patients with non disabling MS correlate with the extent of brain structural damage. *Ann Neurol* 2002; 51:330-9.
56. **Rocca MA, Matthews PM, Caputo D et al** – Evidence for widespread movement-associated functional MRI changes in patients with PPMS. *Neurology* 2002; 58:866-72.
57. **Zhang Y, Zabad RG, Wei X et al** – Deep grey matter „black T2" on 3 tesla magnetic resonance imaging correlates with disability in multiple sclerosis. *Multiple Sclerosis* 2007; 13,7:880-883.
58. **Pirko I, Lucchinetti CF, Sriram S et al** – Gray matter involvement in multiple sclerosis. *Neurology,* 2007; 68: 634-642.
59. **Niepel G, Tench CR, Morgan PS et al** – Deep gray matter and fatigue in MS: a T1 relaxation time study. *J Neurol* 2006; 253:896-902
60. **Lucchinetti CF, Bruck W, Rodriguez M et al** – Distinct patterns of multiple sclerosis pathology indicates heterogeneity on pathogenesis. *Brain Pathol* 1996; 6:259-274