Biomarkers for multiple sclerosis: an update for 2014

Óscar Fernández, Roland Martin, Àlex Rovira, Sara Llufriu, Ángela Vidal-Jordana, Victoria E. Fernández-Sánchez, José C. Álvarez-Cermeño, Guillermo Izquierdo, Rafael Arroyo-González, Alfredo Rodríguez-Antigüedad, Bonaventura Casanova-Estruch, Xavier Montalban

Summary. Multiple sclerosis is a chronic, demyelinating and inflammatory disease of the central nervous system that mainly affects young adults. It is characterised by processes involving inflammation, demyelination and axonal destruction, and as a result the pathogenic aspects and response to treatment of the disease vary widely. It is therefore difficult to establish a prognosis for these patients or to determine the effectiveness of the different drugs that are employed. Current clinical research into the development of new biomarkers has advanced a great deal in recent years, especially in the early stages of the disease. Yet, it is essential to further our knowledge about novel markers of the disease, and not only in the more advanced stages, so as to be able to stop disability from progressing and to establish new therapy regimens in these patients. This review presents an update on the information available about the biomarkers that are currently validated and used in multiple sclerosis, together with the possible candidates for utilisation in routine clinical practice.

Key words. Attacks. Biomarkers. Cerebrospinal fluid. Disability. Multiple sclerosis. Update.

Hospital Regional Universitario Carlos Haya; Málaga (O. Fernández, V.E. Fernández-Sánchez). Hospital Universitario de Zúrich; Suiza (R. Martin). Hospital Universitari Vall d'Hebron; Barcelona (A. Rovira, A. Vidal-Jordana, X. Montalban). Hospital Clínic; Barcelona (S. Llufriu). Hospital Universitario Ramón y Cajal; Madrid (J.C. Álvarez-Cermeño). Hospital Universitario Virgen Macarena; Sevilla (G. Izquierdo). Hospital Clínico San Carlos; Madrid (R. Arroyo-González). Hospital de Basurto; Bilbao (A. Rodríguez-Antigüedad). Hospital Universitari i Politècnic La Fe; Valencia, Spain (B. Casanova-Estruch).

Corresponding author:
Dr. Óscar Fernández Fernández. Instituto de Neurociencias Clínicas. Hospital Regional Universitario de Málaga. Instituto de Investigaciones Biomédicas de Málaga (IBIMA). Universidad de Málaga. Avda. Carlos Haya, s/n. E-29010 Málaga.

E-mail:
oscar.fernandez.sspa@juntadeandalucia.es

Funding:
This work has received unconditioned financial support for medical education from Teva Pharma España.

Conflicts of interest:
O.F. has received honoraria as a consultant, advisor, speaker or moderator and has participated in clinical trials and other research projects promoted by Biogen-Idec, Bayer-Schering, Merck Serono, Teva, Novartis, Genzyme, Almirall and Allergan. The remaining authors declare no conflicts of interest concerning this article.

Accepted:
13.05.14.

How to cite this paper:
Fernández O, Martin R, Rovira A, Llufriu S, Vidal-Jordana A, Fernández-Sánchez VE, et al. Biomarkers for multiple sclerosis: an update for 2014. Rev Neurol 2014; 58: 553-70.

→

General characteristics of multiple sclerosis

Multiple sclerosis (MS) is a chronic and disabling demyelinating disease primarily affecting young adults (20-40 years-old) [1]. It is a complex disease that involves independent processes of inflammation, demyelination, neurodegeneration, remyelination and axonal repair. This entire process is initially mediated by T cell activity [2]. Although the pathogenesis of MS has not been completely elucidated, there are findings that suggest it is a multifactorial disease involving both genetic (HLA-DRB1*15:01; DRB5*01:01, IL7RA, etc.) and environmental factors (viral infection by the Epstein-Barr virus, vitamin D deficiency and other possibilities) that favour the dysregulation of the immune system [3].

Biomarkers: general concepts

Because knowledge of the pathogenesis, course and treatment of MS are limited, the identification of biomarkers for MS will contribute to a better understanding of the disease processes. A biomarker is a characteristic that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a particular therapeutic intervention [4]. Some working definitions are as follows:

- *Type 0:* biomarker that defines the natural course of the disease and correlates longitudinally with known clinical indices.
- *Type 1:* biomarker that reflects the clinical effects of a particular therapeutic intervention, according to its mechanism of action.
- *Type 2:* biomarker or surrogate value that is intended to serve as a substitute for an important clinical variable and is expected to predict the effect of a therapeutic intervention.
- *Clinical Variable:* clinically relevant measure of how the patient feels, functions or survives.

Biomarkers may be classified according to the processes taking place in MS: markers of the characteristics of the disease, markers of the disease state, markers of disease activity, markers predictive of long-term progression and markers of treatment effect, the dosage or the combination treatment. Likewise, in MS, in response to a disturbance of the immune system, cytokines could be considered biomarkers, as could their receptors, antibodies (e.g., similar to the aquaporin-4 antibodies in neuromyelitis optica (NMO)), the frequency of antigen specific T cells, adhesion molecules and biomolecules related to apoptosis and the cell cycle. Other possible classifications relate to specific processes of MS, such as biomarkers of breakdown of the blood-brain barrier (BBB), de-

Versión española disponible en www.neurologia.com

myelination, oxidative stress, axonal damage, gliosis, remyelination and repair.

Blood-brain barrier breakdown biomarkers

Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMP) were identified as biomarkers of BBB breakdown, including MMP-2 (collagenase A), MMP-9 (gelatinase B), MMP-3 (stromelysin) and MMP-1 (collagenase). Increased MMP-9 and decreased TIMP-1 in the cerebrospinal fluid (CSF) of MS patients have been correlated with increased magnetic resonance (MR) activity [5].

In addition to the activity of MMP, neutral proteases may be potential biomarkers of the breakdown of the BBB, according to research conducted by Cuzner et al [6], which found increased levels of proteases in the CSF during MS attacks.

All these findings support the results of the investigation led by Trojano [5], in which it was observed that treatment with beta interferon (IFN β) increased the level of intercellular adhesion molecule type 1 and decreased the level of MMP-9. Meanwhile, Bielekova et al [8] demonstrated that daclizumab did not directly affect the BBB; however, it did induce an immunomodulatory change, reducing by more than 80% the number of gadolinium-enhancing lesions (Gd+).

Radiological biomarkers

Conventional magnetic resonance: cerebral atrophy

Since its introduction, MRI has become the most important paraclinical technique not only for the diagnosis of MS but also as a prognostic marker in the initial stage of the disease, in relation to both the prediction of clinical relapses and the severity of future disability. Furthermore, it has contributed to a better understanding of the natural history of the disease and evaluation of the effectiveness of new treatments.

Currently, it is known that the number and volume of T_2 weighted visible lesions progress by approximately 5-10% per year; in fact, this measure has been used as a secondary measure in most clinical trials in recent years [9].

However, the problem is that the volumes of T_2, despite their correlation with disability and mortality in recent studies, are a measure that only minimally reflects disease neuropathology. In fact, the density of axonal injury observed in T_2 can be highly variable, with ranges from 0 to 100%, based on studies of *post mortem* tissue samples obtained in Amsterdam [10]. Furthermore, it has been reported that in progressive forms of the disease, the relationship of T_2 lesions to disability is particularly weak due to a plateau effect. This effect was first described by Li et al [11], who, after six years of follow-up, observed in more than 1,300 patients with MS that although there was a progression of disability, it did not correlate with the progression in T_2 lesion volume.

Currently, it is known that cerebral atrophy begins in the initial stages of MS but has greater relevance in the later stages. There are several other pathological mechanisms involved in cerebral atrophy, which occurs in both the white matter and the grey matter and is partially associated with the lesions observed in the T_2 weighted sequences [12]. Similarly, measures of cerebral atrophy are more strongly associated with cognitive impairment and physical disability of patients [13,14] than the measurements obtained from the T_2 sequences, although it must be considered that the detection of cerebral atrophy can be masked by the more obvious inflammatory effect of the disease itself in the early stages; furthermore, it can also be falsely accelerated by the anti-inflammatory effect of certain drugs.

Moreover, it is noteworthy that cerebral atrophy occurs in MS patients more quickly than in healthy patients. In fact, it has been reported that the annual rate of progression of cerebral atrophy is roughly on the order of 0.5-1% in MS patients, markedly higher than in young adults, where it ranges from 0.1-0.43% per year. Therefore, it has been suggested that cerebral atrophy could be a biomarker to assess significant neurodegeneration in MS patients [15]; therefore, it is being used in several clinical trials to assess the degree of neuroprotection afforded by the different drugs studied [16].

In this context, the findings of the investigation led and recently published by Popescu [17] support the claim that neurodegeneration is clinically associated with cerebral atrophy and global atrophy as a biomarker predictor of disability at 10 years in patients with MS. Meanwhile, the work by Goodin et al [18] analyses cerebral atrophy using this parameter in patients with MS, correlated not only with the progression of the disease but also with death in these patients.

Cerebral atrophy occurs in the early stages of the disease, as has been observed in research led by Pérez-Miralles [19], which showed how during the first nine months after a clinically isolated syndrome (CIS), there is a significant decrease in brain volume

in patients who had a second clinical episode and, therefore, converted to clinically definite MS, compared to patients who did not present until after a follow up period of several years. De Stefano et al [20] emphasise that the progression of cerebral atrophy is similar in different clinical phenotypes of the disease when brain volume values are normalised to the initial brain volume (Fig. 1). Further, in a transversal study with 95 patients with CIS, 657 with relapsing-remitting MS (RRMS), 124 with secondary progressive MS (SPMS) and 50 with primary progressive MS (PPMS), Roosendaal et al [21] stressed that the regional cerebral atrophy in grey matter is the parameter that correlates best with clinical disability and cognitive impairment of the disease, as well as being related to T_1 and T_2 lesion volumes.

Currently, it is known that different immunomodulatory drugs available in the therapeutic arsenal for the treatment of MS have led to controversial results with regard to their effects on reducing cerebral atrophy (Table I) [22,23], some of which show that this effect only becomes apparent in the second year after starting treatment. This phenomenon is known as the pseudoatrophy effect, explained by the anti-inflammatory effect of the drugs, which cause a decrease in brain volume and thus a false degree of acceleration of atrophy. In this context, the study by Vidal-Jordana et al [24] showed an acceleration of the decline in brain volume attributable to the effect of pseudoatrophy, after a year of treatment with natalizumab in patients who initially presented with active gadolinium lesions, while these differences did not exist in patients from the second year of treatment. The most unique findings of this study were that the effect of natalizumab induced pseudoatrophy that selectively affected the cerebral white matter but not the grey matter.

Other studies of SPMS and PPMS have been inconclusive regarding the effect of preventing cerebral atrophy [25,26]. However, recently developed drugs have demonstrated more encouraging results, as they are able not only to prevent cerebral atrophy but also to reduce the degree of disability progression [27].

Cerebral atrophy measures are attractive for measuring the neurodegeneration component of the disease; however, there are a number of intermediate quantification processes in which different errors may exist throughout the course of the analysis, which must be considered when seeking to use this biomarker in clinical trials.

In short, cerebral atrophy is present from the early stages of the disease. Although it is not a measure that is used in daily clinical practice, it is an attractive measure to assess the neurodegenerative component of the disease and as a prognostic marker of disability progression, especially when selectively analysing the grey matter.

Figure 1. Change in brain volume in the different forms of multiple sclerosis. Adapted from [20]. PPMS: primary progressive multiple sclerosis; RRMS: relapsing-remitting multiple sclerosis; SPMS: secondary progressive multiple sclerosis; PCBV: percentage change in brain volume; CIS: clinically isolated syndrome; NCV: normal cerebral volume.

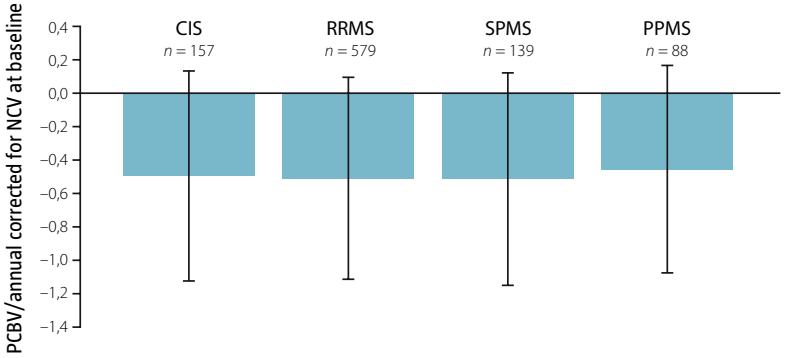


Table I. Summary of studies associated with immunomodulatory drugs to reduce cerebral atrophy.

	n	Drug	Reduction of cerebral atrophy
Cohen et al [145]	1,292	Fingolimod	Yes
Kappos et al [146]	1,272	Fingolimod	Yes
Calabresi et al [147]	1,083	Fingolimod	Yes
Rudick et al [148]	172	IFN β-1a intramuscular	No (1st year) / Yes (2nd year)
Jones et al [149]	519	IFN β-1a subcutaneous	No
Miller et al [150]	942	Natalizumab	No (1st year) / Yes (2nd year)
Rovaris et al [151], Sormani et al [152]	227	Glatiramer acetate	No (first nine months) / Yes (from nine months)

IFN β: interferon beta.

Non-conventional magnetic resonance

Non-conventional MRI offers a number of advantages over conventional MRI. First, it provides quantitative information and identifies specific pathological changes in both the normal-appearing white matter and grey matter. It allows us to perform both global and regional analysis, is more re-

producible and is an automated technique. However, non-conventional MRI techniques also have a number of disadvantages compared to conventional techniques: these techniques still require standardisation and optimisation and are missing normative reference values; the processing is difficult, and there is variability among different observers and centres [16,28]. Their features make them promising techniques, but they lack the actual capacity to differentiate among patient phenotypes and predict disease progression or response to treatment.

The various non-conventional MRI techniques available are described below.

Diffusion tensor imaging

Diffusion tensor imaging is based on the movement of water molecules in tissue. As the directionality of movement of molecules can be altered in some lesions, the directionality of this study informs us about the integrity of brain tissue. It is possible to obtain a series of indices such as fractional anisotropy or mean diffusivity, which can be translated as demyelination events, axonal damage or gliosis; radial diffusivity is more specific for myelin damage and the axial diffusivity of axonal damage [29]. It also allows for the study of the white matter tracts via tractography and the consequences of MS in connectivity between different brain regions. Studies have associated disability values, both physical and cognitive, with the integrity of brain tissue, particularly at the regional level [30-32]. Thus, the diffusion values of certain white matter tracts and specific regions of grey matter were significantly associated with performance in cognitive tasks [33]. At the predictive level, this technique has presented inconclusive results, but it can anticipate the progression of disability in the disease and even cerebral atrophy [30,32].

Magnetisation transfer

Magnetisation transfer allows us to obtain the magnetisation transfer ratio (MTR), which is associated with decreased myelin damage [16,34]. In the normal-appearing white matter and grey matter, a decrease is observed in this *ratio* in MS patients, which has been shown to worsen over time and in more severe stages of the disease. A decrease in the MTR appears in acute and chronic injury and may even precede its appearance in T_2 sequences. The MTR then increases as remyelination occurs. In addition, low MTR values have been associated with a worse long-term prognosis.

The study by Arnold et al describes an optimisation technique that can quantify the longitudinal changes of the MTR in acute lesions. In this study, patients were treated with bone marrow transplantation and improved clinically –in terms of the score obtained in Kurtzke's Expanded Disability Status Scale (EDSS)– remyelinating better than patients who were stable, suggesting that variations in the MTR can be of great importance when assessing remyelination in clinical trials [35].

Spectroscopy

Spectroscopy allows us to calculate the concentration of different metabolites in brain tissue. The most commonly monitored metabolites include N-acetyl-aspartate (NAA), which is a marker of axonal damage; myo-inositol, which increases in astrogliosis; and glutamate, which is associated with neurotoxicity.

It appears that the increase in myo-inositol (astrogliosis) may precede the NAA or axonal damage; such damage, in turn, precedes cerebral atrophy [36]. It has also been observed that an increase in myo-inositol favours the conversion of CIS to clinically definite MS [16]. The *ratio* between myo-inositol (as a marker of astrogliosis) and white matter NAA can predict the progression of disability and cerebral atrophy over the ensuing four years [37].

The study by Kirov et al [38] was designed to characterise the metabolic changes of the white matter and grey matter in patients with RRMS treated with immunomodulatory agents using spectroscopy. The authors of this study concluded that spectroscopy can help monitor response to treatment, as was observed in this group of patients, who sustained increased levels of myo-inositol; however, the axonal alterations of NAA seemed to improve. Therefore, these values could be potential markers of the evolution of the response to treatment of patients with MS.

Magnetic susceptibility is a technique for detecting levels of paramagnetic substances, such as accumulated iron or deoxyhaemoglobin, and diamagnetic substances such as accumulated calcium. An increase in iron deposits has been observed in the basal ganglia in patients diagnosed with MS, especially in advanced forms of the disease, and this increase is associated with the degree of disability [39]. In longitudinal studies, it has been observed that this accumulation of iron appears as a halo around the lesions, which may persist over time [40].

Double inversion recovery

Another non-conventional MRI technique is double inversion recovery. This sequence suppresses the signals of the white matter and CSF. As a result,

the white matter appears hypointense, allowing a greater contrast with demyelinating lesions (hyperintense). This technique also allows the visualisation of cortical lesions, but only 18% of the lesions observed in pathological anatomy can be visualised. The limitation of this technique is that it produces a large number of false positives, reducing its specificity [41]. However, we can improve the specificity of this technique by combining it with other sequences, such as *Stage Sensitive Inversion Recovery* [42].

Positron emission tomography

Positron emission tomography permits the detection of activated microglia. Politis et al [43] observed an increase in tracer PK11195 (a marker of activated microglia) binding to the grey matter in the cortex of both RRMS patients and SPMS patients, showing a very strong correlation between the amount of the tracer and the patient's disability, especially in the secondary progressive form. The same team recently presented data from a study showing that in patients who started treatment with natalizumab, the union with the tracer in the cortex decreases [44].

Optical coherence tomography

Optical coherence tomography (OCT) is a non-invasive imaging technique that allows us to visualise the fundus *in vivo* and thus access the retina and its different layers. OCT is based on the optical principle of interferometry, which combines light beams from different receivers to generate a higher resolution image in real time. The operating principle of OCT is similar to the principle of ultrasound, with the difference that instead of using ultrasonic waves, near-infrared light is used, which is reflected by the tissue being studied, in this case the retina. Thus, the light beam travels through the different layers of the retina and, depending on the properties of each tissue, a new light beam is reflected back to the receiver, which is analysed using built-in computer *software*. Two OCT measures are primarily used in observing MS: the first is the measurement of the thickness of the nerve fibre layer of the retina (RNFL), measured quantitatively around the optic nerve head (μm). The second is the macular volume (MV), which is measured around the macula, allowing the inclusion of the total MV, and the thickness of the different layers (after post-processing), also expressed quantitatively (μm). The OCT analysis program incorporates a standardised database by age and sex, which allows us to evaluate these results not only quantitatively but also qualitatively, according to the distribution within the normal range.

OCT in MS is rapid, non-invasive, quantitative, accurate and reproducible [45], with high rates of inter-and intra-observer correlation. OCT also correlates well with clinic patients and is sensitive to changes over time. Finally, it could assist with the differential diagnosis of neuromyelitis optica and optic neuropathies in MS [46-48].

Numerous cross-sectional studies published in recent years show the differences in RNFL thickness between MS patients and healthy controls. The recent meta-analysis by Petzold et al [49] showed that the greatest loss of volume of the RNFL occurs in patients affected with MS with a previous history of optic neuritis. The average difference between eyes with optic neuritis and healthy controls, based on all eyes tested, was –20.38 μm, with a 95% confidence interval (CI 95%) = –22.86 to –17.91. Moreover, this decrease in RNFL thickness was also observed to a lesser extent in the eyes of patients with MS without optic neuritis when compared to healthy controls (–7.08 μm; CI 95% = –8.65 to –5.52 μm).

To date, there have been few longitudinal studies evaluating the change in the RNFL in MS patients [50-53]. However, they concluded that there is progressive axonal damage in the RNFL and global MV of patients with MS and that these changes occur more markedly than in healthy controls (Table II).

Perhaps the most interesting aspect of OCT as a biomarker for MS is that OCT measurements were correlated with neurological disability and brain MRI parameters. Thus, overall, it appears that there is a moderate correlation between the different parameters of OCT and disability of the disease, as measured by the EDSS [54-60], and greater loss of RNFL thickness or MV corresponds to a higher score on the EDSS. This result was also correlated with volumetric measures in the brain MRI studies analysed, so that patients with greater axonal loss in the RNFL and the MV suffered a greater loss of brain volume [55,56,59].

New research developments have begun to investigate the relationship between the parameters of OCT and the regional atrophy of both white matter and grey matter. Recently, in a cross-sectional study in patients in the early stages of MS, it was found that both the thickness of the RNFL and the MV are correlated with global cerebral volume and white matter [61].

In addition, OCT allows the investigation of the segmentation of the different layers of the retina and its possible relationship to the pathogenesis and evolution of MS. In this sense, Saidha et al [62]

Table II. Summary of results from longitudinal studies of optical coherence tomography measures.

	Patients with MS	Healthy controls
García-Martín et al [50]	*n* = 81 NFLR: –4.5 µm/year MV: –0.10 µm/year	NA
Herrero et al [51]	*n* = 94 NFLR: –3.7 µm/year MV: –0.18 µm/year	*n* = 50 NFLR: –0.15 µm/year MV: –0.005 µm/year
Sepulcre et al [52]	*n* = 61 NFLR: –2.4 µm/year	*n* = 29 NFLR: –1.1 µm/year
Talman et al [53]	*n* = 299 NFLR: –1.6 µm/year	*n* = 60 NFLR: –0.16 µm/year

NA: not applicable; NFLR: nerve fibre layer of the retina; MV: macular volume.

found that in certain subgroups of patients, there could be primary damage of the retina, referred to as primary macular thinning. OCT segmentation techniques showed that this degeneration occurred predominantly in the inner and outer nuclear layers of the retina. The authors also found that patients with this primary pathology of the retina had greater disease severity as measured by the level of severity of MS.

In this context, Saidha et al [63] also studied the correlation between the different layers of the retina and different brain volumes in patients with MS and detected a correlation of the volumes of the nuclear and axonal retinal layers with the volume of cerebral grey matter, suggesting the existence of different pathological substrates. As in most studies, these associations were detected mainly in eyes with no prior history of optic neuritis.

CSF Biomarkers

The study of CSF in MS is useful for the detection of biomarkers that delineate the course of the disease [64], as it is closely related to the pathology of the central nervous system (CNS) fluid, which facilitates the identification of any type of alteration of the CSF.

Oligoclonal bands

The presence of oligoclonal bands (OCB) of IgG in CSF was the first marker to identify the presence of a demyelinating disease nature. The older accepted criteria to diagnose the existence of MS was the existence of a compatible clinical demonstration of the presence of OCB IgG in CSF or an elevated IgG index, although it must be recognised that the study of CSF has been excluded almost entirely from the current diagnostic criteria, leaving only three minor criteria for the diagnosis of primary progressive MS [65]. Although the presence of OCB IgG in CSF is not specific to MS, it does assist in early differential diagnosis against other demyelinating and non-demyelinating diseases [66].

The study of OCB IgG is particularly necessary and interesting in patients with rare clinical diagnoses that are difficult to distinguish from MS. The study of OCB IgG in CSF is also useful in the diagnosis of MS in non-Caucasian populations of patients, or populations not characteristically affected by MS. For their part, Álvarez-Cermeño and Villar [67], in a recently published research study, concluded that OCB IgG in CSF prevents misdiagnosis in patients with comorbidities that meet the criteria for MS. They allow a more accurate diagnosis and thereby early and effective treatment. Furthermore, the presence of OCB IgG in CSF has significant predictive power of evolution to clinically definite MS in patients with ACS [68] and is regarded as a supplementary parameter to MRI [69].

The presence of OCB IgM in CSF is a biomarker of poor prognosis in patients with MS [70]. A study conducted by Villar et al [71] concluded that the presence of BOC IgM was associated with a more rapid form of PMSCs disease evolution with a greater likelihood of reaching a score of 6 in the EDSS.

Recently, Villar et al [71] studied the possible reactivity of the antigens of myelin lipids. The results showed that the presence of IgM lipid-specific bands formed a more accurate marker of the early development of a second case than bands of total IgM. Approximately 20% of patients with non-lipid-specific OCB IgM showed a similar trend to IgM (–) patients. Furthermore, the synthesis of IgM was a transient response in these patients. Research conducted by the same authors concluded that lipid-specific IgM bands are associated with a more aggressive course of the disease [72].

The results obtained by Thangarajh [73] validated these findings, confirming that the presence of IgM bands (+) in CSF predicts early progression to SPMS. Magraner et al [74] found that the presence of IgM lipid-specific OCB in the first clinical event suggests the presence of demyelination processes and is related to T2 lesion volume from baseline and cerebral atrophy.

The work conducted by Bosca [75] indicated that the presence of IgM lipid-specific OCB could be related to a suboptimal response to treatment with interferon. Meanwhile, Villar et al [76] demonstrated that IgM bands are associated with an optimal response to treatment with natalizumab. The results of this study indicated that the disappearance of IgM bands was not observed in patients who exhibited disease activity despite treatment with natalizumab.

Finally, the research findings of Sadaba et al [77] revealed the presence of IgM on the surface of macrophages and axons, which may suggest that the presence of bands of intrathecal IgM is not merely a marker of poor prognosis but may also be related to the pathophysiology of MS, as in these patients, OCB IgM (+) also increases the number of neurofilaments observed in CSF (submitted for publication).

Neurofilaments

There are several types of neurofilament (NF), which are classified according to their protein: light chain (NFL), medium chain (NFM) and heavy chain (NFH).

A relationship has been observed between the NF and the type of disease. Thus, the study by Salzer et al [78] concluded that there is a correlation between high levels of NFL in CSF and poor outcome, and thus NFL could be used as prognostic biomarker in patients with RRMS and the resulting decision as a marker of therapeutic intervention. Later, Madeddu et al [79], in a recently published study, analysed the levels of NFL and other proteins, such as glial fibrillary acidic protein (GFAP) and isoforms II and III of β-tubulin (β-Tub), in the CSF of patients with MS and other neurological diseases. The results of this study were not very enlightening but seem to indicate that patients with high levels of NFL fare worse. It was also observed that the levels of all proteins in the CSF except β-Tub III are reduced with increasing EDSS score. Therefore, the levels of β-Tub II could be used for the diagnosis of MS, and the levels of β-Tub III could be used as a prognostic biomarker.

Kuhle et al [80] studied the levels of NFH-NFH (SMI35) and its correlation with disability, disease activity or specific stages of MS, and they observed increased levels of NFH (SMI35) in all forms of the disease compared with the control group of healthy patients. It was also noted that there is a correlation between the levels of NFH (SMI35) and the EDSS score in the RRMS and SCA forms.

Teunissen and Khalil [81] studied the presence of NF in the early stages of MS to try to establish a prognosis of patients from the onset of MS, and they concluded that the light subunits of NF better reflect acute axonal damage, which may also serve as a value to estimate the prognosis of SCA conversion to MS and the correlation with disease progression.

Using microarrays, Quintana et al [82] studied the antibody reactivity in paired serum and CSF samples from RRMS patients and control groups with other non-inflammatory neurological diseases compared to 334 different antigens, including lipids, myelin antigens and heat shock proteins. The findings of this study open a new avenue of research for the identification of new biomarkers of disease progression and response to treatment in patients with RRMS.

In line with the results of prognostic value regarding the conversion of SCA to MS, the usefulness of the NF to assess the conversion of SCA to different forms of MS has been reflected in the research recently published by Khalil et al [83], in which an increase in NF levels from the early forms of the disease is related to the observed lesion load.

Finally, Axelsson et al [84] studied the effect of immunosuppressive therapy (mitoxantrone and rituximab) on the levels of markers of cerebral tissue injury and axonal damage (NFL) in the CSF of 35 patients with chronic progressive MS, confirming that the levels of NFL CSF could serve as a potential surrogate marker for the efficacy of a given treatment.

Clinical biomarkers

Attacks against disability

As described previously, the techniques of conventional MRI have low predictive power regarding disability because long-term disability progression is strongly influenced by the process of diffuse inflammation of both the white matter and apparently normal grey matter, of cortical lesions and demyelination processes [85-87].

Leray et al [88] hypothesised that there are two stages in the pathophysiology of MS: the first stage dependent on focal inflammation, and a second stage dependent on diffuse inflammation and neurodegeneration but independent of the focal inflammation. Under this approach, the authors concluded that attacks that occurred at the beginning of the disease (during the first three years) were a predictive factor for the first stage of the disease (EDSS = 3) but not for the second stage or secondary progressive form of the disease (EDSS> 3). In fact, it was observed that the progression of disabil-

ity during the second stage was independent of the duration of the first and that the average length of the second stage ranged from six to nine years in different groups of patients, regardless of the duration of the first stage.

Similarly, the results published by van Scalfari et al [89] indicated that the number of attacks and their frequency during the first two years of the disease significantly influence the course of MS, even allowing prediction up to a secondary progressive form, with a single score of 6, 8 or 10 on the EDSS. However, paradoxically, it was observed that neither attacks after the first two years of the disease nor the total number of attacks suffered during this period of time exert any impact on the progress of the disease towards a secondary progressive form. As a final conclusion, the work suggested that the dissociation between attacks and disease progression reflected that attacks, by themselves, are poor prognostic markers of long-term disability.

The study by De Stefano et al [20] analysed MRI volumetric measurements of the brain in the different subtypes of the disease and concluded that measures of cerebral atrophy are similar in all forms of the disease, both in patients with ACS and patients with RRMS or SPMS. Furthermore, when analysing the percentage change in brain volume normalised after a year, it was observed that the inexorable neurodegeneration follows a similar course throughout the disease regardless of the subtype of MS (Fig. 1) [20,90]. In fact, it is estimated that annual reductions in brain volume from 0.5 to 1.3% occur in MS patients, compared to 0.1 to 0.43% in healthy controls. In this regard, the work of Popescu et al [17] was an excellent translation into clinical practice, as the initial values confirmed cerebral atrophy as a biomarker of poor prognosis and, ultimately, long-term disability.

Thus, the scientific evidence suggests that the process of disability in MS can be explained by the involvement of two different and, in a sense, independent routes, contributing in the same way to neurodegeneration and subsequent disability in RRMS patients. One route would consist of adaptive immunity, mediated by peripheral autoreactive T cells entering the CNS; the other route would consist of innate immunity, involving the activation of resident or endogenous cells in the CNS.

The first route would involve autoreactive T cells (CD4, CD8 and CD17), which would be responsible for producing a cascade of inflammatory events, causing focal inflammation, demyelination and acute axonal damage [91-97]. The focal damage is responsible for attacks and be visible on conventional MRI images [98], characterised by T_2 hyperintense lesions representing both acute and chronic demyelination; gadolinium enhancing lesions that correspond to areas of active inflammation and BHE breakage; and finally, focal lesions that can lead to persistent focal damage observed as T_1 hypointense lesions, 'Black holes', which represent focal areas of permanent axonal loss.

However, the second route is the largest contributor to the process of neurodegeneration. This pathway is dependent on the resident CNS cells [92-97] but also includes an independent pathway partially mediated by peripheral T cells. This second pathway induces the activation of microglia and astrocytes, leading to a diffuse pathology [93, 99]. These astrocytes, activated by a cascade of proinflammatory cytokines, can alter the energy metabolism and cause axonal degeneration in the white matter [100,101]. Axonal density in the apparently normal white matter decreases by 12-45% in specific tracts of the spinal cord and corpus callosum [99,102,103].

This astrocyte activation occurs early and may even precede axonal damage measures, which is reflected in the results of the study by Kirov et al [104], analysing spectroscopy in patients with early RRMS (median duration: 2.3 years) and who were minimally affected (EDSS = 1.4). The authors of this study suggested that astrocyte activation occurs early, even in periods preceding clinical remission and axonal damage, when, in turn, cerebral atrophy is anticipated. In addition, a significant increase in astrogliosis markers, such as creatinine, myo-inositol and choline, was observed in normal-appearing white matter.

These findings are related to the results obtained by De Stefano et al [105], demonstrated by MTR, which can detect diffuse axonal damage in the normal appearing white matter of patients with early MS of short duration and low lesion load. Moreover, in these same patients, there was a decline in the NAA/creatine ratio, considered as a surrogate marker of axonal damage.

Meanwhile Fisher et al [106] noted that long-term disability in MS patients correlated significantly with increasing loss of brain tissue (r = 0.27, p = 0.001). Therefore, treatments that prevent early cerebral atrophy or loss of brain tissue have the potential to reduce long-term disability.

Prognostic clinical biomarkers

Given the clinical heterogeneity of MS, there has been much interest among researchers in identifying new predictors of disease progression.

To date, disability is the only variable that truly marks the course of the disease. However, in an attempt to determine the clinical prognostic variables of MS, there have been two systematic reviews [106,107], which, despite the limitations described and methodological variability of the studies included, defined a set of prognostic variables grouped into demographic (gender and age of onset) or clinical biomarkers (initial evolution activity in attacks, attack sequel gestation).

In the systematic review by Langer-Gould et al [108], gender appeared to be a prognostic factor, as it is a factor associated with disability, although the data were not conclusive.

The age of onset of the disease also appears to have important prognostic value. An earlier age of onset is associated with a slower progression of disability. However, this beneficial effect is fictitious, as patients exhibiting the disease at a younger age take longer to reach disability (EDSS = 4-6) but do so at a similar or younger age than patients with late onset of the disease [109]. Finally, the shift to a secondary progressive evolution is related to the age of the patients, and this change occurs near age 40.

The initial clinical presentation, based on the number of lesions on topography, has been associated with the prognosis of the disease. Thus, the presentation of symptoms attributable to MS with more than one lesion topography correlates with a greater likelihood of reaching disability at an EDSS score of 4, in relation to the presentation at the beginning of topographical injury; however, when impairment is reached at an EDSS score of 6, this difference disappears.

The initial course of MS has been observed to be closely related to the prognosis of disability. Thus, patients beginning with the primary progressive form (PPMS) reach a high level of disability (EDSS = 6) more quickly than patients with RRMS.

As previously discussed, the number of attacks of the disease at the beginning (first year) is a prognostic factor of disability. However, this effect is maintained only until the patient reaches a 4 or 6 disability as measured by the EDSS because, from that moment on, disability progresses continuously and insidiously, regardless of the number of attacks. In this sense, Confavreux et al [110] and Leray et al [88] appreciate the clinical significance of the onset of attacks of disease until the patient reaches disability at an EDSS score of 3 or 4; from that time, disability evolves continuously and irreversibly. Therefore, when considering new therapeutic strategies, all efforts should aim to delay or attenuate the progressive stage of MS [89].

Currently, it is accepted that pregnancy reduces the risk of attacks, although during the postpartum and lactation period the risk increases [111]. Furthermore, motherhood does not condition the prognosis of MS long term, as dissociation between attacks and disability occurs.

Clinical biomarkers of therapeutic response

According to the philosophical movement begun by Jean-Paul Sartre in the mid-20th century, phenomena are simply things as they are, as available to the consciousness. Phenomenology is therefore the sensible appearance of things.

However, in the case of MS, this sensible appearance never coincides with the supposed underlying reality. Therefore, when a patient is evaluated, a series of symptoms are observed, most of which occur as a result of the attacks themselves or can occur insidiously and progressively. The attack will have clinical sequelae, and the insidious onset and progression expressed by these symptoms will produce a progressive disability (Fig. 2). In this context, analysing the results of clinical trials, we studied the increase in disability. However, we did not assess what increasing disability actually means: that is, if it is a sequel to the attack itself or if there really has been a worsening of disability progression in these patients. Therefore, this approach should be established routinely in future daily clinical practice.

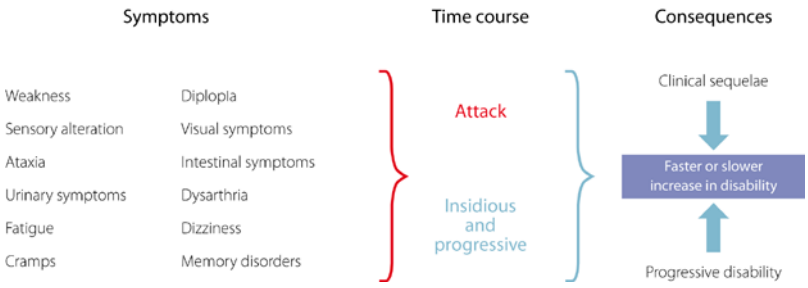
The issues discussed below are the following: the pathological basis and pathogenic attacks; the effect of attacks on disability and the short- or long-term basis of pathological symptoms of progressive evolution; pathogenesis progression off-shoots; and finally, the clinical, pathological and/or pathogenic relationship between attacks and progression.

An attack is a complex phenomenon with a clearly inflammatory base. The mediated leakage of B lymphocytes and T lymphocytes now confirms that there is a breakdown of the BBB, with a pitch of cytokines, resulting in a clinical expression with specific symptomatology. However, as mentioned above, the study by Scalfari et al [89] found that the temporal relationship between attacks and disease progression (from 3 to 6 on the EDSS) is independent of the number of attacks.

Regarding neurodegeneration, Frischer et al [112] showed that in all phenotypes of MS, the involvement of the white matter and apparently normal grey matter has an inflammatory basis, and anti-inflammatory treatment will affect the disease.

All these findings seem to indicate that MS is a difficult and complex disease, most likely because

Figure 2. Clinical phenomenology in multiple sclerosis.



we are not facing a single disease but at least two diseases. Indeed, the results obtained by the investigation led by Khoury [113] stress that atrophy in the cerebral cortex depends critically on the LCR penetrating the cortex and destroying certain neurons. All of these phenomena are a consequence of the presence of lymphoid follicles that produce this type of alteration. Finally, we note that an inflammatory process beginning in the meninges is responsible for brain damage, which subsequently correlates with disability. In fact, the causal relationship between attacks and disability progression, from the point of view of pathology, can identify two separate and distinct stages of the disease [114]. We have a first stage, relapsing-remitting, characterised by disruption of the BBB; and a second stage, in which the BBB is closed and the destruction of apparently normal nervous tissue develops due to the inflammatory process that activates microglia and ultimately causes neuronal destruction.

Confounding factors that have been identified in MS include the following: first, the concept of disability progression. We must assess whether the progression of disability is a result of the aftermath of attacks or is the result of chronic progression unrelated to attacks. Based on this approach, Young et al [115] investigated whether attacks contribute significantly to the development of disability in patients with MS and obtained conflicting results, so we must be cautious in this regard.

Moreover, the patient's age is an important factor when assessing the resilience to an attack, as age is most keenly involved in neuronal plasticity. Furthermore, the dependence of the progressive stage of the disease on age and time is uncertain, and finally, the lack of accurate progression markers make this aspect even more complex.

From a clinical standpoint, the therapeutic response will be measured by the number of relapses and the progression of disability. With regard to attacks, it is necessary to evaluate the number of attacks, the intensity and the aftermath. With regard to disability, it is necessary to evaluate the rate of progression and symptoms of disability, including cognitive symptoms and fatigue.

Biomarkers in the first-line treatment for MS

We can identify different types of biomarkers for a drug according to the approach we seek: if we want to evaluate the biological activity, biomarkers should be directed to the mechanism of drug action. Biomarkers can indicate the presence or absence of response, or can be associated with a specific adverse event.

In the case of IFN β, the action mechanism is quite complex, involving different cells and different effects on each of them, and it is mediated by membrane receptors and the resulting cascade of signalling to the transcription or inhibition of various genes/proteins, which are ultimately responsible for the mechanism of action. There are many studies evaluating the effect of IFN β gene expression in vitro and in vivo. If we were able to comprehend the differences between responders and nonresponders, we could identify and even predict which patients will respond to treatment. Thus, the study published by van Baarsen et al [116] analysed the gene expression patterns of 16 patients with RRMS treated with IFN β. Using microarray analysis in blood samples drawn before treatment and after one month, the study authors identified a subgroup of patients who exhibited the overexpression of 126 genes in response to IFN β and another subgroup of patients in whom there was no difference in gene expression, and these genes were downregulated, concluding that gene expression before treatment with IFN β could be used as a biomarker of differential clinical response.

In the same manner, by characterising responders and nonresponders, Comabella et al [117] analysed the gene expression patterns of 47 RRMS patients using microarrays before treatment with IFN β and after three months of treatment, and these results are compared with the clinical presentation of these patients at 24 months. A patient was considered non-responsive in the event of at least one attack and the progression of disability as measured by the EDSS. After 24 months of treatment with IFN β, a combination of eight genes was identified,

predominantly type I interferons, whose gene expression may predict response to treatment with 80% accuracy induced.

While performing studies on the mechanism of action of glatiramer acetate (GA) [118], Ziemssen et al [119] determined that the change from Th1 to Th2 induced by GA brain-derived neurotrophic factor (BDNF) increased significantly. The authors concluded that the production of GA BDNF could induce neuroprotection and enhance neuronal repair.

Based on these findings, White et al [120], in a small sample of patients (n = 19) who had not received prior treatment for MS, demonstrated increased production of BDNF in patients responding to treatment compared to non-responders. Thus, the authors of the study concluded that the production of BDNF may be related to the clinical response to GA from the start of treatment, but further studies to confirm this finding would be necessary.

In another line of research on the mechanism of action of GA, there is the study conducted by Farina et al [121], where the levels of interleukin-4 (IL-4) and IFN γ were analysed in blood samples from 20 healthy controls, 20 patients with untreated MS and 20 MS patients treated with GA. The authors of this study found that treatment with GA leads to the regulation of cytokines and, in particular, to increased levels of IL-4 and IFN γ in T cells, even at low doses of GA. Consequently, the frequency of producing GA-reactive cytokines is a potential biomarkers to identify T cells.

In connection with these results, Valenzuela et al [122] evaluated whether the clinical response to treatment with GA correlated with the modulation of the expression of IL-4 and IFN in patients with MS. According to the response to treatment, patients were classified as responders or non-responders. The proliferation of T cells producing IL-4 and IFN γ did not differ among the three groups of patients; however, in the responder group, the levels of induction of IL-4 were maintained throughout treatment, whereas in non-responders or hyporesponders, these levels decreased.

Other biomarkers: evoked potentials

In neurophysiology, evoked potentials are the result of the synchronisation of neuronal activity groups or CNS axons generated by exteroceptive stimuli. Evoked potentials are non-invasive techniques that allow us to study CNS function, working, in this sense, with the diagnosis, evaluation and monitoring of MS. According to the modality of the stimulus used, evoked potentials are divided into visual, acoustic, somatosensory and motor. Evoked potentials produce graphs with a specific morphology: latency provides information on the conduction of the nerve impulse and, therefore, on myelin (delayed mean latency demyelination); the axon amplitude is also informative, as a decrease in amplitude may be associated with axonal loss [123].

The main use of evoked potentials in MS is to define the impairment of motor and sensory pathways in the presence of inconclusive symptoms. They can provide information on the pathophysiology of the underlying lesion in the patient. However, as described by Comi et al [124], the only potentials recommended for the diagnosis of MS are visual evoked potentials, with a sensitivity ranging from 50 to 85%, compared with 30 to 65% of evoked potentials in the somatosensory lower limb. This point is also reflected in the systematic review by Gronseth and Ashman [125] on the evidence of class II, in which no studies were included involving motor evoked potentials, and in which the authors conclude that visual evoked potentials can actually be useful for the diagnosis of MS; somatosensory evoked potentials could likely be useful; while the noise of the trunk would be of little use. Thus, the use of a battery of evoked potentials comprising all modalities would improve the sensitivity (60-85%), though not the specificity (50-85%), as the specificity of evoked potentials in the diagnosis of MS is limited by the clinical context.

The use of evoked potentials in the diagnosis of MS has been regulated by the technological advances of MRI. Thus, the diagnostic criteria of Poser et al [126] considered the paraclinical diagnostic evidence; visual evoked potentials were a requirement for the McDonald diagnostic criteria of 2005 [127] but were not considered in the 2010 McDonald criteria [65]. However, it is important to note that evoked potentials are important in detecting subclinical lesions and in confirming doubtful attacks.

The prognostic value of evoked potentials in the conversion of CIS to MS patients remains a much debated point and is, to date, inconclusive [128,129]. It appears that the motor evoked potentials are predictive of motor impairment in 41% of patients with ACS [130], as a correlation has been observed between the scales of evoked potentials and measures of disease disability (EDSS), especially when a global scale is used [131-137].

In recent years, numerous studies have indicated the existence of a clear correlation between evoked potentials and disability, as measured by certain measures of neurodegeneration [49,52,58,62,128,138,

139], specifically, a correlation between the evoked potentials, the normal white matter and certain OCT parameters [58,128,139,140]. In this context, the study by Bejarano et al [141] in 2011 evaluated the correlation between motor evoked potentials using computational classifiers with the EDSS, MRI and other clinical measures, and the measures obtained from the EDSS and motor evoked potentials were best correlated with disability.

Regarding the use of evoked potentials in monitoring response to treatment, a weak correlation exists between conventional MRI measures and disability scores such as the EDSS [142], but the correlations with measures of potential were observed to be more stable [143,144].

Therefore, based on these findings, the use of evoked potentials may be advised in patients with suspected MS to document inconclusive symptoms, to support the diagnosis of dissemination in space [123] and, experimentally, for the prognosis of disease progression.

Conclusions

Since its introduction, MRI has emerged as the most important paraclinical technique in the diagnosis of MS. Cerebral atrophy is presented as an attractive measure to assess neuroprotection and is a prognostic marker for the progression of disability; however, more research is needed to incorporate it into routine clinical practice. Meanwhile, non-conventional MRI techniques are useful for the pathogenic substrate of disability and disease progression and to determine the neuroprotection and remyelination ability of new drugs. However, technological improvements are necessary to gain the ability to provide information at the individual level that is applicable to clinical practice.

Very interesting results are obtained from studies using OCT data. To summarise, OCT data are presented as a potential biomarker of neurodegeneration, which correlates with clinical disability variables and parameters of diffuse axonal injury. However, studies have been conducted with very heterogeneous populations (both inter-and intrapopulation), and the technique cannot yet be used in routine clinical practice.

The study of the CSF in MS is useful for the detection of biomarkers of the course of the disease, as CSF is very similar to the pathology of CNS fluid. The combined study of NF chains in CSF may be useful for detecting forecasts and evolutionary profiles in MS and in the choice of treatment. The NF is presented as a future biomarker of response to treatment, but it is necessary to conduct further studies to bring this approach into the clinic. Moreover, the presence of OCB IgG in the CSF is not specific to MS but can aid in differential diagnosis against other demyelinating diseases and is considered complementary to the results of the RM parameter. In contrast, the presence of IgM BOC is a marker of poor prognosis and is correlated with progression to secondary progressive forms of the disease.

From the clinical perspective, the prognosis of MS is correlated with disability, which is affected by the gender of the patient, the number of attacks at the beginning of the disease and clinical outcome. We also know that there is a dissociation between attacks and disability, so that new treatments should be designed to slow the process of neurodegeneration, which, as we have seen, continues inexorably in MS.

Regarding treatment response biomarkers, note that various biomarkers have been identified in the context of the mechanism of action; however, there is currently no biomarker that reliably discriminates between responders and non-responders to immunomodulatory therapy .

Finally, the development of more precise studies with evoked potentials in MS is working very well, contributing to the assessment of the disability progression of the disease, neurodegeneration and treatment response.

References

1. Sospedra M, Martin R. Immunology of multiple sclerosis. Annu Rev Immunol 2005; 23: 683-747.
2. Sawcer S, Hellenthal G, Pirinen M, Spencer CC, Patsopoulos NA, Moutsianas L, et al. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. Nature 2011; 476: 214-9.
3. Martin R, Sturzebecher CS, McFarland HF. Immunotherapy of multiple sclerosis: where are we? Where should we go? Nat Immunol 2001; 2: 785-8.
4. Bielekova B, Martin R. Development of biomarkers in multiple sclerosis. Brain 2004; 127 :1463-78.
5. Waubant E. Biomarkers indicative of blood-brain barrier disruption in multiple sclerosis. Dis Markers 2006; 22: 235-44.
6. Cuzner ML, Opdenakker G. Plasminogen activators and matrix metalloproteases, mediators of extracellular proteolysis in inflammatory demyelination of the central nervous system. J Neuroimmunol 1999; 94: 1-14.
7. Trojano M, Defazio G, Avolio C, Paolicelli D, Giuliani F, Giorelli M, et al. Effects of rIFN-beta-1b on serum circulating ICAM-1 in relapsing remitting multiple sclerosis and on the membrane-bound ICAM-1 expression on brain microvascular endothelial cells. J Neurovirol 2000; 6 (Suppl 2): S47-51.
8. Bielekova B, Richert N, Howard T, Blevins G, Markovic-Plese S, McCartin J, et al. Humanized anti-CD25 (daclizumab) inhibits disease activity in multiple sclerosis patients failing to respond to interferon beta. Proc Natl Acad Sci U S A 2004; 101: 8705-8.

9. McFarland HF, Stone LA, Calabresi PA, Maloni H, Bash CN, Frank JA. MRI studies of multiple sclerosis: implications for the natural history of the disease and for monitoring effectiveness of experimental therapies. Mult Scler 1996; 2: 198-205.
10. Van Waesberghe JH, Kamphorst W, De Groot CJ, Van Walderveen MA, Castelijns JA, Ravid R, et al. Axonal loss in multiple sclerosis lesions: magnetic resonance imaging insights into substrates of disability. Ann Neurol 1999; 46: 747-54.
11. Li DK, Held U, Petkau J, Daumer M, Barkhof F, Fazekas F, et al. MRI T2 lesion burden in multiple sclerosis: a plateauing relationship with clinical disability. Neurology 2006; 66: 1384-9.
12. Simon JH. Brain atrophy in multiple sclerosis: what we know and would like to know. Mult Scler 2006; 12: 679-87.
13. Minneboo A, Jasperse B, Barkhof F, Uitdehaag BM, Knol DL, De Groot V, et al. Predicting short-term disability progression in early multiple sclerosis: added value of MRI parameters. J Neurol Neurosurg Psychiatry 2008; 79: 917-23.
14. Prinster A, Quarantelli M, Lanzillo R, Orefice G, Vacca G, Carotenuto B, et al. A voxel-based morphometry study of disease severity correlates in relapsing-remitting multiple sclerosis. Mult Scler 2010; 16: 45-54.
15. Zivadinov R, Bakshi R. Central nervous system atrophy and clinical status in multiple sclerosis. J Neuroimaging 2004; 14 (Suppl 3): S27-35.
16. Bakshi R, Thompson AJ, Rocca MA, Pelletier D, Dousset V, Barkhof F, et al. MRI in multiple sclerosis: current status and future prospects. Lancet Neurol 2008; 7: 615-25.
17. Popescu V, Agosta F, Hulst HE, Sluimer IC, Knol DL, Sormani MP, et al. Brain atrophy and lesion load predict long term disability in multiple sclerosis. J Neurol Neurosurg Psychiatry 2013; 84: 1082-91.
18. Goodin DS, Reder AT, Ebers GC, Cutter G, Kremenchutzky M, Oger J, et al. Survival in MS: a randomized cohort study 21 years after the start of the pivotal IFNbeta-1b trial. Neurology 2012; 78: 1315-22.
19. Pérez-Miralles F, Sastre-Garriga J, Tintoré M, Arrambide G, Nos C, Perkal H, et al. Clinical impact of early brain atrophy in clinically isolated syndromes. Mult Scler 2013; 19: 1978-86.
20. De Stefano N, Giorgio A, Battaglini M, Rovaris M, Sormani MP, Barkhof F, et al. Assessing brain atrophy rates in a large population of untreated multiple sclerosis subtypes. Neurology 2010; 74: 1868-76.
21. Roosendaal SD, Bendfeldt K, Vrenken H, Polman CH, Borgwardt S, Radue EW, et al. Grey matter volume in a large cohort of MS patients: relation to MRI parameters and disability. Mult Scler 2011; 17: 1098-106.
22. Bermel RA, Bakshi R. The measurement and clinical relevance of brain atrophy in multiple sclerosis. Lancet Neurol 2006; 5: 158-70.
23. Zivadinov R, Reder AT, Filippi M, Minagar A, Stuve O, Lassmann H, et al. Mechanisms of action of disease-modifying agents and brain volume changes in multiple sclerosis. Neurology 2008; 71: 136-44.
24. Vidal-Jordana A, Sastre-Garriga J, Perez-Miralles F, Tur C, Tintore M, Horga A, et al. Early brain pseudoatrophy while on natalizumab therapy is due to white matter volume changes. Mult Scler 2013; 19: 1175-81.
25. Kappos L. Effect of drugs in secondary disease progression in patients with multiple sclerosis. Mult Scler 2004; 10 (Suppl 1): S46-54.
26. Wolinsky JS, PROMiSe Trial Study Group. The PROMiSe trial: baseline data review and progress report. Mult Scler 2004; 10 (Suppl 1): S65-71.
27. Comi G, Jeffery D, Kappos L, Montalban X, Boyko A, Rocca MA, et al. Placebo-controlled trial of oral laquinimod for multiple sclerosis. N Engl J Med 2012; 366: 1000-9.
28. Ceccarelli A, Bakshi R, Neema M. MRI in multiple sclerosis: a review of the current literature. Curr Opin Neurol 2012; 25: 402-9.
29. Song SK, Sun SW, Ju WK, Lin SJ, Cross AH, Neufeld AH. Diffusion tensor imaging detects and differentiates axon and myelin degeneration in mouse optic nerve after retinal ischemia. Neuroimage 2003; 20: 1714-22.
30. Filippi M, Rocca MA, De Stefano N, Enzinger C, Fisher E, Horsfield MA, et al. Magnetic resonance techniques in multiple sclerosis: the present and the future. Arch Neurol 2011; 68: 1514-20.
31. Inglese M, Bester M. Diffusion imaging in multiple sclerosis: research and clinical implications. NMR Biomed 2010; 23: 865-72.
32. Sbardella E, Tona F, Petsas N, Pantano P. DTI measurements in multiple sclerosis: evaluation of brain damage and clinical implications. Mult Scler Int 2013; 2013: 671730.
33. Llufriu S, Martinez-Heras E, Fortea J, Blanco Y, Berenguer J, Gabilondo I, et al. Cognitive functions in multiple sclerosis: impact of gray matter integrity. Mult Scler 2014; 20: 424-32.
34. Arnold DL. Evidence for neuroprotection and remyelination using imaging techniques. Neurology 2007; 68 (Suppl 3): S83-90.
35. Brown RA, Narayanan S, Arnold DL. Segmentation of magnetization transfer ratio lesions for longitudinal analysis of demyelination and remyelination in multiple sclerosis. Neuroimage 2012; 66C: 103-9.
36. Narayana PA. Magnetic resonance spectroscopy in the monitoring of multiple sclerosis. J Neuroimaging 2005; 15 (Suppl 4): S46-57.
37. Llufriu S, Kornak J, Ratiney H, Oh J, Brenneman D, Sampat M, et al. MR spectroscopy markers of disease progression in multiple sclerosis: a clinical validation study. Neurology 2012; 78 (Meeting Abstracts 1): IN3-1.005.
38. Kirov II, Tal A, Babb JS, Herbert J, Gonen O. Serial proton MR spectroscopy of gray and white matter in relapsing-remitting MS. Neurology 2013; 80: 39-46.
39. Ropele S, de Graaf W, Khalil M, Wattjes MP, Langkammer C, Rocca MA, et al. MRI assessment of iron deposition in multiple sclerosis. J Magn Reson Imaging 2011; 34: 13-21.
40. Bian W, Harter K, Hammond-Rosenbluth KE, Lupo JM, Xu D, Kelley DA, et al. A serial in vivo 7T magnetic resonance phase imaging study of white matter lesions in multiple sclerosis. Mult Scler 2013; 19: 69-75.
41. Zivadinov R, Pirko I. Advances in understanding gray matter pathology in multiple sclerosis: are we ready to redefine disease pathogenesis? BMC Neurol 2012; 12: 9.
42. Sethi V, Yousry TA, Muhlert N, Ron M, Golay X, Wheeler-Kingshott C, et al. Improved detection of cortical MS lesions with phase-sensitive inversion recovery MRI. J Neurol Neurosurg Psychiatry 2012; 83: 877-82.
43. Politis M, Giannetti P, Su P, Turkheimer F, Keihaninejad S, Wu K, et al. Increased PK11195 PET binding in the cortex of patients with MS correlates with disability. Neurology 2012; 79: 523-30.
44. Politis M, Gianetti P, Niccolini F, Su P, Turkheimer F, Waldman A, et al. Decreased microglial activation precedes stabilization of disability in multiple sclerosis patients treated with natalizumab. Neurology 2013; 80 (Meeting Abstracts 1): S21.004.
45. Syc SB, Warner CV, Hiremath GS, Farrell SK, Ratchford JN, Conger A, et al. Reproducibility of high-resolution optical coherence tomography in multiple sclerosis. Mult Scler 2010; 16: 829-39.
46. Brandt AU, Zimmermann H, Kaufhold F, Promesberger J, Schippling S, Finis D, et al. Patterns of retinal damage facilitate differential diagnosis between Susac syndrome and MS. PLoS One 2012; 7: e38741.
47. Chan CK, Cheng AC, Leung CK, Cheung CY, Yung AY, Gong B, et al. Quantitative assessment of optic nerve head morphology and retinal nerve fibre layer in non-arteritic anterior ischaemic optic neuropathy with optical coherence tomography and confocal scanning laser ophthalmoloscopy. Br J Ophthalmol 2009; 93: 731-5.
48. Ratchford JN, Quigg ME, Conger A, Frohman T, Frohman E, Balcer LJ, et al. Optical coherence tomography helps differentiate neuromyelitis optica and MS optic neuropathies. Neurology 2009; 73: 302-8.

49. Petzold A, de Boer JF, Schippling S, Vermersch P, Kardon R, Green A, et al. Optical coherence tomography in multiple sclerosis: a systematic review and meta-analysis. Lancet Neurol 2010; 9: 921-32.
50. Garcia-Martin E, Pueyo V, Martin J, Almarcegui C, Ara JR, Dolz I, et al. Progressive changes in the retinal nerve fiber layer in patients with multiple sclerosis. Eur J Ophthalmol 2010; 20: 167-73.
51. Herrero R, Garcia-Martín E, Almarcegui C, Ara JR, Rodríguez-Mena D, Martín J, et al. Progressive degeneration of the retinal nerve fiber layer in patients with multiple sclerosis. Invest Ophthalmol Vis Sci 2012; 53: 8344-9.
52. Sepulcre J, Murie-Fernández M, Salinas-Alamán A, García-Layana A, Bejarano B, Villoslada P. Diagnostic accuracy of retinal abnormalities in predicting disease activity in MS. Neurology 2007; 68: 1488-94.
53. Talman LS, Bisker ER, Sackel DJ, Long DA Jr, Galetta KM, Ratchford JN, et al. Longitudinal study of vision and retinal nerve fiber layer thickness in multiple sclerosis. Ann Neurol 2010; 67: 749-60.
54. Albrecht P, Frohlich R, Hartung HP, Kieseier BC, Methner A. Optical coherence tomography measures axonal loss in multiple sclerosis independently of optic neuritis. J Neurol 2007; 254: 1595-6.
55. Gordon-Lipkin E, Chodkowski B, Reich DS, Smith SA, Pulicken M, Balcer LJ, et al. Retinal nerve fiber layer is associated with brain atrophy in multiple sclerosis. Neurology 2007; 69: 1603-9.
56. Grazioli E, Zivadinov R, Weinstock-Guttman B, Lincoff N, Baier M, Wong JR, et al. Retinal nerve fiber layer thickness is associated with brain MRI outcomes in multiple sclerosis. J Neurol Sci 2008; 268: 12-7.
57. Henderson AP, Trip SA, Schlottmann PG, Altmann DR, Garway-Heath DF, Plant GT, et al. An investigation of the retinal nerve fibre layer in progressive multiple sclerosis using optical coherence tomography. Brain 2008; 131: 277-87.
58. Pueyo V, Martín J, Fernández J, Almarcegui C, Ara J, Egea C, et al. Axonal loss in the retinal nerve fiber layer in patients with multiple sclerosis. Mult Scler 2008; 14: 609-14.
59. Siger M, Dziegielewski K, Jasek L, Bieniek M, Nicpan A, Nawrocki J, et al. Optical coherence tomography in multiple sclerosis: thickness of the retinal nerve fiber layer as a potential measure of axonal loss and brain atrophy. J Neurol 2008; 255: 1555-60.
60. Toledo J, Sepulcre J, Salinas-Alamán A, García-Layana A, Murie-Fernández M, Bejarano B, et al. Retinal nerve fiber layer atrophy is associated with physical and cognitive disability in multiple sclerosis. Mult Scler 2008; 14: 906-12.
61. Young KL, Brandt AU, Petzold A, Reitz LY, Lintze F, Paul F, et al. Loss of retinal nerve fibre layer axons indicates white but not grey matter damage in early multiple sclerosis. Eur J Neurol 2013; 20: 803-11.
62. Saidha S, Syc SB, Ibrahim MA, Eckstein C, Warner CV, Farrell SK, et al. Primary retinal pathology in multiple sclerosis as detected by optical coherence tomography. Brain 2011; 134: 518-33.
63. Saidha S, Sotirchos ES, Oh J, Syc SB, Seigo MA, Shiee N, et al. Relationships between retinal axonal and neuronal measures and global central nervous system pathology in multiple sclerosis. JAMA Neurol 2013; 70: 34-43.
64. Corcione A, Aloisi F, Serafini B, Capello E, Mancardi GL, Pistoia V, et al. B-cell differentiation in the CNS of patients with multiple sclerosis. Autoimmun Rev 2005; 4: 549-54.
65. Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol 2011; 69: 292-302.
66. Villar LM, Masjuán J, Sadaba MC, González-Porque P, Plaza J, Bootello A, et al. Early differential diagnosis of multiple sclerosis using a new oligoclonal band test. Arch Neurol 2005; 62: 574-7.
67. Álvarez-Cermeño JC, Villar LM. Multiple sclerosis: oligoclonal bands –a useful tool to avoid MS misdiagnosis. Nat Rev Neurol 2013; 9: 303-4.
68. Masjuán J, Álvarez-Cermeño JC, García-Barragán N, Díaz-Sánchez M, Espino M, Sadaba MC, et al. Clinically isolated syndromes: a new oligoclonal band test accurately predicts conversion to MS. Neurology 2006; 66: 576-8.
69. Tumani H, Hartung HP, Hemmer B, Teunissen C, Deisenhammer F, Giovannoni G, et al. Cerebrospinal fluid biomarkers in multiple sclerosis. Neurobiol Dis 2009; 35: 117-27.
70. Villar LM, Masjuán J, González-Porque P, Plaza J, Sadaba MC, Roldán E, et al. Intrathecal IgM synthesis is a prognostic factor in multiple sclerosis. Ann Neurol 2003; 53: 222-6.
71. Villar LM, Sadaba MC, Roldán E, Masjuán J, González-Porque P, Villarrubia N, et al. Intrathecal synthesis of oligoclonal IgM against myelin lipids predicts an aggressive disease course in MS. J Clin Invest 2005; 115: 187-94.
72. Villar L, Garcia-Barragan N, Espino M, Roldan E, Sadaba M, Gomez-Rial J, et al. Influence of oligoclonal IgM specificity in multiple sclerosis disease course. Mult Scler 2008; 14: 183-7.
73. Thangarajh M, Gomez-Rial J, Hedstrom AK, Hillert J, Álvarez-Cermeño JC, Masterman T, et al. Lipid-specific immunoglobulin M in CSF predicts adverse long-term outcome in multiple sclerosis. Mult Scler 2008; 14: 1208-13.
74. Magraner MJ, Bosca I, Simo-Castello M, Garcia-Marti G, Alberich-Bayarri A, Coret F, et al. Brain atrophy and lesion load are related to CSF lipid-specific IgM oligoclonal bands in clinically isolated syndromes. Neuroradiology 2012; 54: 5-12.
75. Bosca I, Villar LM, Coret F, Magraner MJ, Simo-Castello M, Álvarez-Cermeño JC, et al. Response to interferon in multiple sclerosis is related to lipid-specific oligoclonal IgM bands. Mult Scler 2010; 16: 810-5.
76. Villar LM, Garcia-Sanchez MI, Costa-Frossard L, Espino M, Roldan E, Paramo D, et al. Immunological markers of optimal response to natalizumab in multiple sclerosis. Arch Neurol 2012; 69: 191-7.
77. Sadaba MC, Tzartos J, Paino C, Garcia-Villanueva M, Álvarez-Cermeño JC, Villar LM, et al. Axonal and oligodendrocyte-localized IgM and IgG deposits in MS lesions. J Neuroimmunol 2012; 247: 86-94.
78. Salzer J, Svenningsson A, Sundstrom P. Neurofilament light as a prognostic marker in multiple sclerosis. Mult Scler 2010; 16: 287-92.
79. Madeddu R, Farace C, Tolu P, Solinas G, Asara Y, Sotgiu MA, et al. Cytoskeletal proteins in the cerebrospinal fluid as biomarker of multiple sclerosis. Neurol Sci 2012; 34: 181-6.
80. Kuhle J, Leppert D, Petzold A, Regeniter A, Schindler C, Mehling M, et al. Neurofilament heavy chain in CSF correlates with relapses and disability in multiple sclerosis. Neurology 2011; 76: 1206-13.
81. Teunissen CE, Khalil M. Neurofilaments as biomarkers in multiple sclerosis. Mult Scler 2012; 18: 552-6.
82. Quintana FJ, Farez MF, Izquierdo G, Lucas M, Cohen IR, Weiner HL. Antigen microarrays identify CNS-produced autoantibodies in RRMS. Neurology 2012; 78: 532-9.
83. Khalil M, Enzinger C, Langkammer C, Ropele S, Mader A, Trentini A, et al. CSF neurofilament and N-acetylaspartate related brain changes in clinically isolated syndrome. Mult Scler 2013; 19: 436-42.
84. Axelsson M, Malmestrom C, Gunnarsson M, Zetterberg H, Sundstrom P, Lycke J, et al. Immunosuppressive therapy reduces axonal damage in progressive multiple sclerosis. Mult Scler 2014; 20: 43-50.
85. DeLuca GC, Ebers GC, Esiri MM. Axonal loss in multiple sclerosis: a pathological survey of the corticospinal and sensory tracts. Brain 2004; 127: 1009-18.
86. Evangelou N, DeLuca GC, Owens T, Esiri MM. Pathological study of spinal cord atrophy in multiple sclerosis suggests limited role of local lesions. Brain 2005; 128: 29-34.
87. Kutzelnigg A, Lucchinetti CF, Stadelmann C, Bruck W, Rauschka H, Bergmann M, et al. Cortical demyelination and diffuse white matter injury in multiple sclerosis. Brain 2005; 128: 2705-12.

88. Leray E, Yaouanq J, Le PE, Coustans M, Laplaud D, Oger J, et al. Evidence for a two-stage disability progression in multiple sclerosis. Brain 2010; 133: 1900-13.
89. Scalfari A, Neuhaus A, Degenhardt A, Rice GP, Muraro PA, Daumer M, et al. The natural history of multiple sclerosis: a geographically based study 10: relapses and long-term disability. Brain 2010; 133: 1914-29.
90. Martola J, Bergstrom J, Fredrikson S, Stawiarz L, Hillert J, Zhang Y, et al. A longitudinal observational study of brain atrophy rate reflecting four decades of multiple sclerosis: a comparison of serial 1D, 2D, and volumetric measurements from MRI images. Neuroradiology 2010; 52: 109-17.
91. De Jager PL, Hafler DA. New therapeutic approaches for multiple sclerosis. Annu Rev Med 2007; 58: 417-32.
92. Lassmann H. Models of multiple sclerosis: new insights into pathophysiology and repair. Curr Opin Neurol 2008; 21: 242-7.
93. Markiewicz I, Lukomska B. The role of astrocytes in the physiology and pathology of the central nervous system. Acta Neurobiol Exp (Wars) 2006; 66: 343-58.
94. Nair A, Frederick TJ, Miller SD. Astrocytes in multiple sclerosis: a product of their environment. Cell Mol Life Sci 2008; 65: 2702-20.
95. Van der Walt A, Butzkueven H, Kolbe S, Marriott M, Alexandrou E, Gresle M, et al. Neuroprotection in multiple sclerosis: a therapeutic challenge for the next decade. Pharmacol Ther 2010; 126: 82-93.
96. Weiner HL. A shift from adaptive to innate immunity: a potential mechanism of disease progression in multiple sclerosis. J Neurol 2008; 255 (Suppl 1): S3-11.
97. Zhang D, Hu X, Qian L, O'Callaghan JP, Hong JS. Astrogliosis in CNS pathologies: is there a role for microglia? Mol Neurobiol 2010; 41: 232-41.
98. Bermel RA, Fox RJ. MRI in multiple sclerosis. Continuum (Minneap Minn) 2010; 16 (5 Multiple Sclerosis): 37-57.
99. Filippi M, Rocca MA, Barkhof F, Bruck W, Chen JT, Comi G, et al. Association between pathological and MRI findings in multiple sclerosis. Lancet Neurol 2012; 11: 349-60.
100. Cambron M, D'Haeseleer M, Laureys G, Clinckers R, Debruyne J, De Keyser J. White-matter astrocytes, axonal energy metabolism, and axonal degeneration in multiple sclerosis. J Cereb Blood Flow Metab 2012; 32: 413-24.
101. Sofroniew MV. Molecular dissection of reactive astrogliosis and glial scar formation. Trends Neurosci 2009; 32: 638-47.
102. Bjartmar C, Kinkel RP, Kidd G, Rudick RA, Trapp BD. Axonal loss in normal-appearing white matter in a patient with acute MS. Neurology 2001; 57: 1248-52.
103. Evangelou N, Esiri MM, Smith S, Palace J, Matthews PM. Quantitative pathological evidence for axonal loss in normal appearing white matter in multiple sclerosis. Ann Neurol 2000; 47: 391-5.
104. Kirov II, Patil V, Babb JS, Rusinek H, Herbert J, Gonen O. MR spectroscopy indicates diffuse multiple sclerosis activity during remission. J Neurol Neurosurg Psychiatry 2009; 80: 1330-6.
105. De Stefano N, Narayanan S, Francis SJ, Smith S, Mortilla M, Tartaglia MC, et al. Diffuse axonal and tissue injury in patients with multiple sclerosis with low cerebral lesion load and no disability. Arch Neurol 2002; 59: 1565-71.
106. Fisher E, Rudick RA, Simon JH, Cutter G, Baier M, Lee JC, et al. Eight-year follow-up study of brain atrophy in patients with MS. Neurology 2002; 59: 1412-20.
107. Degenhardt A, Ramagopalan SV, Scalfari A, Ebers GC. Clinical prognostic factors in multiple sclerosis: a natural history review. Nat Rev Neurol 2009; 5: 672-82.
108. Langer-Gould A, Popat RA, Huang SM, Cobb K, Fontoura P, Gould MK, et al. Clinical and demographic predictors of long-term disability in patients with relapsing-remitting multiple sclerosis: a systematic review. Arch Neurol 2006; 63: 1686-91.
109. Confavreux C, Vukusic S. Age at disability milestones in multiple sclerosis. Brain 2006; 129: 595-605.
110. Confavreux C, Vukusic S, Adeleine P. Early clinical predictors and progression of irreversible disability in multiple sclerosis: an amnesic process. Brain 2003; 126: 770-82.
111. Confavreux C, Hutchinson M, Hours MM, Cortinovis-Tourniaire P, Moreau T. Rate of pregnancy-related relapse in multiple sclerosis. Pregnancy in Multiple Sclerosis Group. N Engl J Med 1998; 339: 285-91.
112. Frischer JM, Bramow S, Dal-Bianco A, Lucchinetti CF, Rauschka H, Schmidbauer M, et al. The relation between inflammation and neurodegeneration in multiple sclerosis brains. Brain 2009; 132: 1175-89.
113. Khoury S, Bakshi R. Cerebral pseudoatrophy or real atrophy after therapy in multiple sclerosis. Ann Neurol 2010; 68: 778-9.
114. Bradl M, Lassmann H. Progressive multiple sclerosis. Semin Immunopathol 2009; 31: 455-65.
115. Young PJ, Lederer C, Eder K, Daumer M, Neiss A, Polman C, et al. Relapses and subsequent worsening of disability in relapsing-remitting multiple sclerosis. Neurology 2006; 67: 804-8.
116. Van Baarsen LG, Vosslamber S, Tijssen M, Baggen JM, Van der Voort LF, Killestein J, et al. Pharmacogenomics of interferon-beta therapy in multiple sclerosis: baseline IFN signature determines pharmacological differences between patients. PLoS One 2008; 3: e1927.
117. Comabella M, Lunemann JD, Rio J, Sanchez A, Lopez C, Julia E, et al. A type I interferon signature in monocytes is associated with poor response to interferon-beta in multiple sclerosis. Brain 2009; 132: 3353-65.
118. Farina C, Weber MS, Meinl E, Wekerle H, Hohlfeld R. Glatiramer acetate in multiple sclerosis: update on potential mechanisms of action. Lancet Neurol 2005; 4: 567-75.
119. Ziemssen T, Kumpfel T, Klinkert WE, Neuhaus O, Hohlfeld R. Glatiramer acetate-specific T-helper 1- and 2-type cell lines produce BDNF: implications for multiple sclerosis therapy. Brain-derived neurotrophic factor. Brain 2002; 125: 2381-91.
120. Blanco Y, Moral EA, Costa M, Gomez-Choco M, Torres-Peraza JF, Alonso-Magdalena L, et al. Effect of glatiramer acetate (Copaxone) on the immunophenotypic and cytokine profile and BDNF production in multiple sclerosis: a longitudinal study. Neurosci Lett 2006; 406: 270-5.
121. Farina C, Then BF, Albrecht H, Meinl E, Yassouridis A, Neuhaus O, et al. Treatment of multiple sclerosis with Copaxone (COP): Elispot assay detects COP-induced interleukin-4 and interferon-gamma response in blood cells. Brain 2001; 124: 705-19.
122. Valenzuela RM, Costello K, Chen M, Said A, Johnson KP, Dhib-Jalbut S. Clinical response to glatiramer acetate correlates with modulation of IFN-gamma and IL-4 expression in multiple sclerosis. Mult Scler 2007; 13: 754-62.
123. Fernandez V, Valls-Sole J, Relova JL, Raguer N, Miralles F, Dinca L, et al. Recommendations for the clinical use of motor evoked potentials in multiple sclerosis. Neurologia 2013; 28: 408-16.
124. Comi G, Leocani L, Medaglini S, Locatelli T, Martinelli V, Santuccio G, et al. Evoked potentials in diagnosis and monitoring of multiple sclerosis. Electroencephalogr Clin Neurophysiol Suppl 1999; 49: 13-8.
125. Gronseth GS, Ashman EJ. Practice parameter: the usefulness of evoked potentials in identifying clinically silent lesions in patients with suspected multiple sclerosis (an evidence-based review): report of the Quality Standards Subcommittee of the American Academy of Neurology. Neurology 2000; 54: 1720-5.
126. Poser CM, Paty DW, Scheinberg L, McDonald WI, Davis FA, Ebers GC, et al. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Ann Neurol 1983; 13: 227-31.
127. Polman CH, Reingold SC, Edan G, Filippi M, Hartung HP, Kappos L, et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the 'McDonald Criteria'. Ann Neurol 2005; 58: 840-6.
128. Klistorner A, Arvind H, Nguyen T, Garrick R, Paine M, Graham S, et al. Fellow eye changes in optic neuritis correlate with the risk of multiple sclerosis. Mult Scler 2009; 15: 928-32.
129. Pelayo R, Montalban X, Minoves T, Moncho D, Rio J, Nos C, et al. Do multimodal evoked potentials add information to MRI in clinically isolated syndromes? Mult Scler 2010; 16: 55-61.
130. Rico A, Audoin B, Franques J, Eusebio A, Reuter F, Malikova I,

et al. Motor evoked potentials in clinically isolated syndrome suggestive of multiple sclerosis. Mult Scler 2009; 15: 355-62.
131. Jung P, Beyerle A, Ziemann U. Multimodal evoked potentials measure and predict disability progression in early relapsing-remitting multiple sclerosis. Mult Scler 2008; 14: 553-6.
132. Kale N, Agaoglu J, Onder G, Tanik O. Correlation between disability and transcranial magnetic stimulation abnormalities in patients with multiple sclerosis. J Clin Neurosci 2009; 16: 1439-42.
133. Kalkers NF, Strijers RL, Jasperse MM, Neacsu V, Geurts JJ, Barkhof F, et al. Motor evoked potential: a reliable and objective measure to document the functional consequences of multiple sclerosis? Relation to disability and MRI. Clin Neurophysiol 2007; 118: 1332-40.
134. Kallmann BA, Fackelmann S, Toyka KV, Rieckmann P, Reiners K. Early abnormalities of evoked potentials and future disability in patients with multiple sclerosis. Mult Scler 2006; 12: 58-65.
135. Leocani L, Rovaris M, Boneschi FM, Medaglini S, Rossi P, Martinelli V, et al. Multimodal evoked potentials to assess the evolution of multiple sclerosis: a longitudinal study. J Neurol Neurosurg Psychiatry 2006; 77: 1030-5.
136. O'Connor P, Marchetti P, Lee L, Perera M. Evoked potential abnormality scores are a useful measure of disease burden in relapsing-remitting multiple sclerosis. Ann Neurol 1998; 44: 404-7.
137. Schlaeger R, D'Souza M, Schindler C, Grize L, Kappos L, Fuhr P. Combined evoked potentials as markers and predictors of disability in early multiple sclerosis. Clin Neurophysiol 2012; 123: 406-10.
138. Fisher JB, Jacobs DA, Markowitz CE, Galetta SL, Volpe NJ, Nano-Schiavi ML, et al. Relation of visual function to retinal nerve fiber layer thickness in multiple sclerosis. Ophthalmology 2006; 113: 324-32.
139. Naismith RT, Tutlam NT, Xu J, Shepherd JB, Klawiter EC, Song SK, et al. Optical coherence tomography is less sensitive than visual evoked potentials in optic neuritis. Neurology 2009; 73: 46-52.
140. Fernandez V, Postigo MJ, Urbaneja P, Leon A, Alonso A, Guerrero M, et al. Comparing objective measures of neurodegeneration in multiple sclerosis (MS). J Neurol 2013; 260 (Suppl 1): S229.
141. Bejarano B, Bianco M, Gonzalez-Moron D, Sepulcre J, Goñi J, Arcocha J, et al. Computational classifiers for predicting the short-term course of Multiple sclerosis. BMC Neurol 2011; 11: 67.
142. Filippi M, Rocca MA. MR imaging of gray matter involvement in multiple sclerosis: implications for understanding disease pathophysiology and monitoring treatment efficacy. AJNR Am J Neuroradiol 2010; 31: 1171-7.
143. Feuillet L, Pelletier J, Suchet L, Rico A, Ali CA, Pouget J, et al. Prospective clinical and electrophysiological follow-up on a multiple sclerosis population treated with interferon beta-1 a: a pilot study. Mult Scler 2007; 13: 348-56.
144. Nuwer MR, Packwood JW, Myers LW, Ellison GW. Evoked potentials predict the clinical changes in a multiple sclerosis drug study. Neurology 1987; 37: 1754-61.
145. Cohen JA, Barkhof F, Comi G, Hartung HP, Khatri BO, Montalban X, et al. Oral fingolimod or intramuscular interferon for relapsing multiple sclerosis. N Engl J Med 2010; 362: 402-15.
146. Kappos L, Radue EW, O'Connor P, Polman C, Hohlfeld R, Calabresi P, et al. A placebo-controlled trial of oral fingolimod in relapsing multiple sclerosis. N Engl J Med 2010; 362: 387-401.
147. Calabresi PA, Bohnen NI. Can PET imaging tell us what's the matter with the gray matter in multiple sclerosis? Neurology 2012; 79: 496-7.
148. Rudick RA, Fisher E, Lee JC, Simon J, Jacobs L. Use of the brain parenchymal fraction to measure whole brain atrophy in relapsing-remitting MS. Multiple Sclerosis Collaborative Research Group. Neurology 1999; 53: 1698-704.
149. Jones CK, Riddehough A, Li DKB, Zhao G, Paty DW, and the PRISMS Study Group. MRI cerebral atrophy in relapsing-remitting MS: results from the PRISMS trial [abstract]. Neurology 2001; 56 (Suppl 3): S379.
150. Miller DH, Soon D, Fernando KT, MacManus DG, Barker GJ, Yousry TA, et al. MRI outcomes in a placebo-controlled trial of natalizumab in relapsing MS. Neurology 2007; 68: 1390-401.
151. Rovaris M, Comi G, Rocca MA, Wolinsky JS, Filippi M. Short-term brain volume change in relapsing-remitting multiple sclerosis: effect of glatiramer acetate and implications. Brain 2001; 124: 1803-12.
152. Sormani MP, Rovaris M, Valsasina P, Wolinsky JS, Comi G, Filippi M. Measurement error of two different techniques for brain atrophy assessment in multiple sclerosis. Neurology 2004; 62: 1432-4.

Biomarcadores en la esclerosis múltiple: puesta al día 2014

Resumen. La esclerosis múltiple es una enfermedad crónica, desmielinizante e inflamatoria del sistema nervioso central, que afecta principalmente a adultos jóvenes. Se caracteriza por procesos de inflamación, desmielinización y destrucción axonal, que confieren a esta enfermedad una gran variabilidad en los aspectos patogénicos y de respuesta al tratamiento. Por ello es muy difícil establecer el pronóstico de estos pacientes, así como la eficacia de los diferentes fármacos. La investigación clínica actual en el desarrollo de nuevos biomarcadores ha experimentado un gran avance en los últimos años, especialmente al inicio de la enfermedad. Sin embargo, es prioritario avanzar en el conocimiento de nuevos marcadores de la enfermedad, no sólo en la fase más avanzada, con el objetivo de prevenir la progresión de la discapacidad y establecer nuevas pautas terapéuticas en estos pacientes. Esta revisión presenta una actualización de la información acerca de los biomarcadores actualmente validados y utilizados en la esclerosis múltiple, así como de los posibles candidatos de utilización en la práctica clínica habitual.

Palabras clave. Actualización. Biomarcadores. Brotes. Discapacidad. Esclerosis múltiple. Líquido cefalorraquídeo.