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ACCURACY OF MULTIPLE SCLEROSIS DIAGNOSTIC CRITERIA FOR DETECTING PERIVENULAR DEMYELINATION VISUALIZED BY MRI AND FREQUENCY OF MS-MIMICKING DISEASES

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ABSTRACT

ACCURACY OF MULTIPLE SCLEROSIS DIAGNOSTIC CRITERIA FOR DETECTING PERIVENULAR DEMYELINATION VISUALIZED BY MRI AND FREQUENCY OF MS-MIMICKING DISEASES

Objective:

Perivenular lesions (PVL) are a cardinal pathological feature of multiple sclerosis (MS) that now can non-invasively be detected by brain MRI. High PVL frequency/patient (PVL-f) is specific of MS and can therefore be used as a gold standard to evaluate accuracy and predictive values of the MS diagnostic criteria in distinct types of MS and the frequency of possible MS misdiagnosis, in particular in MS patients fulfilling the MS diagnostic criteria but carrying also red flags (clinical laboratory or MRI features) suggesting better explanation but not formally allowing other diagnosis (MS-plus patients).

The aims of this study were

- To identify an accurate PVL-f threshold that discriminates MS from other conditions, generated with a ROC (receiver operating characteristic curve) analysis including true positive cases (typical MS) and true negative cases (patients with other definite CNS diseases).
- To evaluate McDonald MS criteria accuracy in MS-plus patients categorized as true or false MS cases according to the PVL-f threshold previously identified with ROC analysis.
- To evaluate differences among MS-plus patients subgroups stratified according to the PVL-f threshold previously identified with ROC analysis.

Methods:

Typical relapsing remitting (RR)MS (n= 28), RRMS-plus (n=59) fulfilling the DIS and DIT based diagnostic criteria and Not-MS neurological syndromes with MS-like brain white matter lesions (WML) (n=32), received one brain MRI scan including conventional and FLAIR* sequences. PVL-f and conventional brain MRI characteristics were evaluated in each MS patient. The PVL-f threshold that best discriminates MS from other neurological conditions was obtained with ROC analysis including true MS (typical MS cases) and true negative cases (patients with not-MS definite neurological conditions). MS-plus patients fulfilling or not the PVL-f threshold generated by ROC analysis were categorized in two groups for evaluating the accuracy of the MS

diagnostic criteria for detecting fulfilment of this threshold. Data concerning clinical, demographic, conventional MRI, OCT and OCT-angiography were also collected and analyzed to find potential differences among patients' groups.

Results:

The threshold-value of PVL-f identified with ROC analysis to discriminate true-MS cases resulted ≥51%.

Typical MS patients had a median PVL-f = 91% (range 67–100%), the MS-plus = 55% (range 8–100%; p=0.001) and the non-MS = 23% (range 0-89%, p< 0.00001). The 51% PVL-f threshold - selected by ROC analysis - was fulfilled by 100% (28/28) of the Typical MS and by 3% (1/33) of non-MS (p< 0.00001) indicating 0.98 accuracy of the MS diagnostic criteria in this population. However only 52.5% (31/60) of the MS-plus patients fulfilled this threshold (p= 0.001), indicating in this patient population 0.68 accuracy of the MS diagnostic criteria. Conventional MRI measure did not contribute to the accuracy of the MS diagnostic criteria, but MS-atypical lesions resulted more frequent in MS-plus, representing the most reliable red flag for its identification. Presence of cerebrovascular comorbidities, high frequency of small lesions and in non-typical MS locations, segregated with the MS-plus patients who did not reach the ≥51% PVL-f threshold suggesting that in MS-plus these diseases may represent an explanation better than MS.

Interpretation:

In MS-plus patients categorized by PVL-f≥51% (the MRI *in vivo* hallmark of MS pathology), the DIS/DIT based MS diagnostic criteria have low performances, indicating that atypical MS cases (MS-plus) represent a group of patients at high risk of misdiagnosis and therefore need PVL-f evaluation.

Summary

CHAPTER 1

MULTIPLE SCLEROSIS

1.1 INTRODUCTION AND DEFINITION

Multiple Sclerosis (MS) is a chronic inflammatory, demyelinating, and neurodegenerative disease of Central Nervous System (CNS). It represents the main cause of non-traumatic disability in young adults, with a typical onset between the second and third life decades. MS has a multifactorial and complex genesis, in which environmental and genetic factors result in a dysregulation of immune system leading to a chronic autoimmune process. The most characteristic sign of the disease is the onset and accumulation, during acute inflammatory phases, of demyelinating lesions in both white and grey matter of brain, spinal cord and in the optic nerve, with a variable degree of concomitant axonal loss and neurodegeneration. Resulting clinical manifestations are highly heterogeneous. In the first phases of disease, when generally predominates an acute inflammatory activity, focal neurological signs prevail, with a good or complete recovery (relapsing remitting MS -RRMS). In later and often progressive phases, subtle signs and symptoms (as cognitive deficits or progressive motor disability worsening) appear, resulting from cumulative inflammatory and neurodegenerative load in the CNS (secondary progressive MS- SPMS)(Goldman et al., 2022; Thompson, Baranzini, et al., 2018). A minority of MS patients show a progressive course from the onset of disease, with a prevailing underlying degenerative process (primary progressive MS - PPMS)(Goldman & Amezcua, 2022).

MS diagnosis is based on clinical criteria, supported by paraclinical findings (laboratory, neurophysiological and MRI investigations), necessary to demonstrate the presence of demyelinating lesions in the CNS, with spatial and temporal dissemination,

in a patient presenting with a typical demyelinating clinical syndrome. Considering that MS pathognomonic markers are currently lacking, for the diagnosis is mandatory the exclusion of any possible alternative disease.

The universally recognized MS diagnostic criteria are the McDonald criteria, with the last revision published in 2017(Thompson, Banwell, et al., 2018b).

These criteria should be applied only in patients with typical demyelinating syndrome and with an accurate differential diagnosis workout: an incorrect application of McDonald criteria, in fact, could lead to MS misdiagnosis, that is currently estimated about 5-20% of all MS definite diagnoses(Solomon, 2019).

MS is a chronic disease without a definitive therapy. However, in the last decades, the prognosis of the disease showed a great improvement, thanks to the development of many disease modifying therapies (DMTs) and, for selected case, the use of Autologous Hematopoietic Stem Cells Transplant (aHSCT). To date, more than 15 DMT, with immunomodulant or immunosuppressive action, are used in MS, resulting in a great reduction of relapse occurrence and of disability progression. Specific DMTs are chosen according to the specific MS phenotype and the patient characteristics, prognostic factors, and lifestyl (Giovannoni et al., 2012) e.

1.2 PATHOGENESIS

The pathognomonic neuropathological marker of MS is represented by the formation of focal plaques (also referred to as 'lesions'), which are areas of demyelination typically located around the post-capillary venules and in which a break in the blood-brain barrier (BEE) is recognisable. The mechanism leading to the breakdown of the BEE is not fully understood, however, it seems to involve proinflammatory cytokines including IL-6, TNF, IL-1β, produced by resident cells and endothelial cells on the one hand, and a mechanism of direct chemokine-mediated or non-chemokine-mediated damage of activated leukocytes on the other(Minagar & Alexander, 2003). BEE damage promotes the trans-endothelial migration of activated leukocytes (including macrophages, T- and B-lymphocytes) within the CNS, from which the proinflammatory cascade and thus demyelination, and subsequently loss of oligodendrocytes, reactive gliosis and neuro-axonal degeneration ensue. Plaques form in both the white and grey matter of the entire CNS, including the optic nerve, brain and spinal cord. Although in some cases the location of the plaques may lead to a specific and obvious clinical manifestation, the

total volume of lesions correlates partially with overall disability and cognitive deficits, suggesting that there are other physio-pathological mechanisms, such as more extensive involvement of the grey matter or the presence of functional damage of apparently healthy brain tissue involving both white and grey matter (**Figure 1.1**) (Filippi, Preziosa, et al., 2016; Goldman et al., 2022)).

1.2.1 White Matter Lesions (WML)

In earliest stages of the diseases active demyelinating phenomena prevail, with concomitant progressive infiltration of immune cells such as T CD8, B CD20+ and, to a less extent, T CD4+ lymphocytes. Moreover, activated microglia, mainly at the edges of lesions, and abundant myelin degradation products, macrophages and hypertrophic reactive astrocytes appear.

The inflammatory activity leading to plaque formation is typical of active forms of MS and is significantly lower in primary or secondary progressive forms. In the latter, inactive lesions, or lesions with a tendency to slowly expand (smoldering lesions) prevail, which show a hypocellular pattern, perilesional reactive astrogliosis, variable microglial activation and a reduced lymphocyte infiltrate compared to that of active plaques(Kuhlmann et al., 2017).

PATHOPHYSIOLOGICAL ASPECTS OF DEMYELINATION

To understand the characteristic phenomenology of MS, some pathophysiological premises are necessary. The alteration of the myelin structure leads to impaired transmission of the impulse along the axon, and in particular the impairment of normal saltatory nerve conduction, which is responsible for the transmission of the impulse up to 120 m/s, which is impaired to the level of amyelinic fibres (< 5 m/s). This results in an increased latency, up to even a true conduction block with consequent limitation or loss of the neurological function involved. The conduction disfunction leads to specific MS symptoms. On the one hand, the alteration of the myelin structure leads to ectopic conduction of certain action potentials, e.g. in ephaptic mode between contiguous axons, from which irritative-type symptoms (e.g. paresthesias) and other symptoms, sometimes paroxysmal in character, are generated. In addition, the damaged myelin structure is more sensitive to an increase in body temperature, which is itself associated with a worse conductivity of the nerve stimulus through the myelin (Uhthoff phenomenon). Finally, the Lhermitte phenomenon has a similar genesis, i.e. the

sensation of an electric shock to the trunk and limbs caused by abrupt bending or extension of the neck, and which finds its explanation in the increased susceptibility to compression of the partially or totally demyelinated fibres located in the cervical cord(Brück, 2005; Lassmann, 2018).

REMYELINATION AND DEGENERATION

In MS, there is a variable degree of remyelination estimated at around 50% in white matter (WM) plaques and up to 90% in cortical plaques, constituting one of the most desirable future therapeutic targets. Several factors influence this process, for example a young age and an early stage of the disease are prognostically favourable factors. Remyelination results in so-called 'shadow plaques', areas in which there is complete or partial remyelination with unclear demarcation from the surrounding normal appearing white matter (NAWM) and axons with thinner myelin sheaths and shorter internodes(Albert et al., 2007; Patrikios et al., 2007; Strijbis et al., 2017).
Neuroaxonal degeneration constitutes the anatomopathological finding responsible for neurodegeneration in MS. It is present from the early stages of the disease and appears to influence the degree of overall disability(Mahad et al., 2015).

NORMAL APPEARING WHITE MATTER (NAWM)

It has been observed that NAWM in MS frequently shows signs of functional alteration and diffuse inflammation or/and neuroaxonal damage. NAWM appears to occur independently of previous mechanisms proposed to explain its genesis, such as axonal damage within focal lesions (Kutzelnigg et al., 2005).

1.2.2 GREY MATTER LESIONS

Although grey matter (GM) lesions are more characteristic of the progressive phases of the disease (with involvement in extreme cases of more than 60% of the cortex), they have been observed from the earliest stages (e.g. CIS and RIS (Filippi, Preziosa, et al., 2018; Giorgio et al., 2011). Such lesions are present at the level of the cerebral and cerebellar hemispheres, but also at the level of the basal and spinal cord nuclei (Gilmore et al., 2009; Petrova et al., 2018). At the hemispheric level, cortical lesions occur mainly in deep grooves and invaginations of the encephalic surface, topographically associated with the presence of ectopic leptomeningeal inflammatory infiltrates (Choi et al., 2012;

Howell et al., 2011). Their formation appears to be attributable to the presence of inflammatory mediators released from the meninges or present in the cerebrospinal fluid (CSF). Cortical lesions show less damage to the BBB, less oedema, less inflammation and more efficient remyelination after the acute phase, suggesting a different mechanism underlying lesion formation in the grey substance than in the white substance (Albert et al., 2007).

Four types of cortical lesions have been identifie (Filippi, Bar-Or, et al., 2018) d:

- type 1: at the cortico-subcortical junction
- type 2: small intracortical perivenular lesions that do not involve the WM or the subpial surface (most frequent in MS)
- type 3: lesions projecting inwards from the subpial surface.
- type 4: lesions extending through the entire thickness of the GM without involving the WM.

Type 3 lesions are the most frequent in MS and are mostly associated with a focus of lymphocytic infiltrative inflammation in the meninges (Choi et al., 2012). Figure 1.1 summarises the main features of MS lesions.

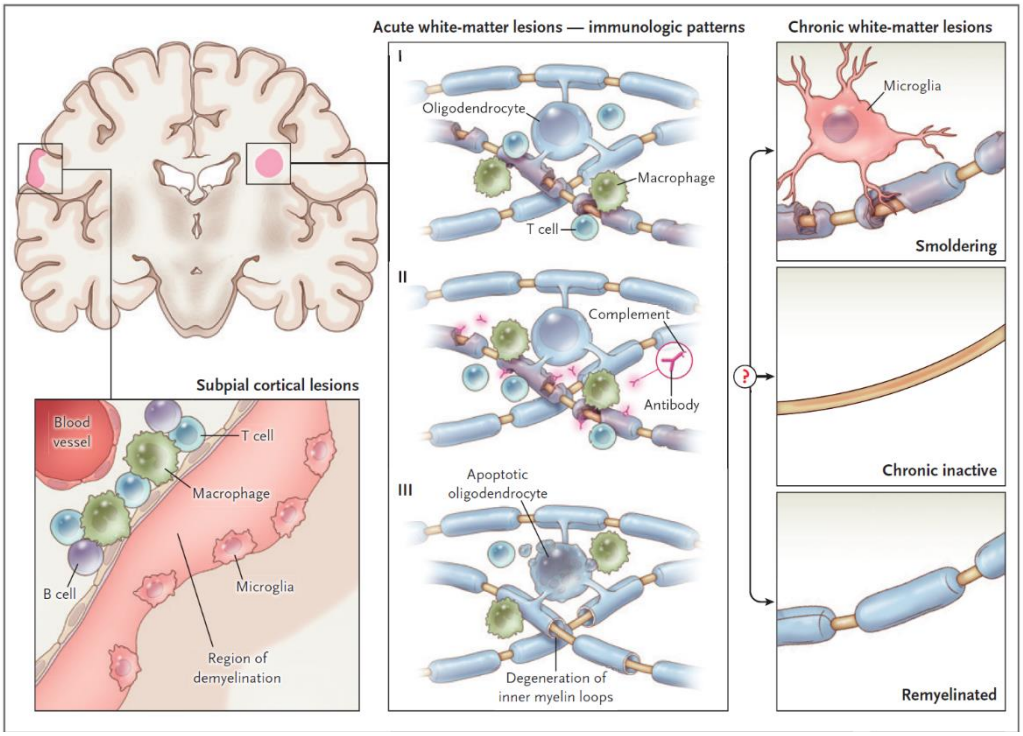
1.3 IMMUNOPATHOPHYSIOLOGY

Scientific progress in recent years has made it possible to overcome the classic model of immunopathogenesis of MS exclusively attributed to T cell activity: current knowledge suggests that there is an interpenetration between the two immunopathogenic mechanisms originally proposed as mutually exclusive, that is, the extrinsic model and the extrinsic one.

Briefly, the first model, derived from observations of Experimental Autoimmune Encephalomyelitis (EAE), predicts that the disease, mediated by a Th1 and Th17 response, is triggered following the migration through the BBB of autoreactive T cells from the periphery. The intrinsic model, instead, predicts that MS is triggered by events with an internal origin in the CNS and with only subsequent recall of inflammatory infiltrate from the periphery. To date, it is known that the basis of the disease is a complex interaction between T and B lymphocytes but also peripheral myeloid cells, and cells resident in the CNS (microglia, astrocytes) (Goldman et al., 2022). Together, these cellular actors are responsible for the initiation and maintenance of the demyelination and inflammation process, also mediated by the secretion of pro-inflammatory factors that recruit additional immune cells by self-sustaining the process

(Filippi, Bar-Or, et al., 2018). This context includes a phenomenon known as "epitope spreading", i.e. the exposure and continuous release of myelin antigens due to cellular damage which further increases the activation of autoreactive T lymphocytes (Croxford et al., 2002). Imputed antigens are components of myelin, including myelin basic protein (MBP), proteolipid protein (PLP), myelin oligodendrocyte glycoprotein (MOG), myelin associated protein (MAG), 2',3 ' cyclic nucleotide 3'phosphodiesterase (CNPase). Non-myelin antigens presented by MHC II cells including S100β protein, crystalline αB have also been associated.

Figure 1.1: **Lesions of white and grey matter**. Early active white matter demyelination falls into three main categories. The most common types (I and II) show an aggregate of mononuclear



phagocytes with perivascular infiltration and T cell parenchymal. Pattern II is further distinguished by the deposition of immunoglobulins and complement. In about 25% of active biopsy lesions (model III), oligodendrocyte apoptosis is accompanied by an oligodendrogliopathy "with retrograde degeneration", starting from the portion of myelin closest to the axon. These lesions resemble viral, toxic and ischemic processes and can be destructive. After the acute phase, yet largely unknown factors determine whether surviving axons will become coated with a thinner myelin sheath (remyelinated), whether inflammation will resolve without remyelination (chronic inactive), or whether inflammation and slow myelin degeneration (smouldering) will persist. Smouldering lesions are more common in progressive multiple sclerosis. Subpial cortical lesion, which is also more common in progressive multiple sclerosis, is characterized by demyelination of the superficial cortex, possibly in association with inflammation of the overlying leptomeninges and scattered macrophages and microglia at the boundary between demyelinated and myelinated neuropil. (Reich et al., 2018).

The presence of resident cells such as microglia and astrocytes is responsible for the compartmentalized inflammatory mechanisms in the CNS, and cause persistent

homeostatic disturbances independent of the presence of exogenous inflammatory infiltrates, and cause neurotoxic activity (production of cytokines and chemokines, hydroxyl radicals, synaptic disfunction) from which the damage neuroaxonal and thus neurodegeneration. Unfortunately, to date, the DMTs available have little or no impact on the inflammatory targets within the CNS, which predominantly contribute to the neurodegenerative component and therefore to progressive MS (Sallusto et al., 2012).

The modalities of alteration of the BBB, found in MS, are not yet clarified. Similarly, the role of the so-called cerebral glymphatic system, recently discovered, is not yet well defined. The CNS is considered an immune-privileged organ, in which immunosurveillance is carried out by T lymphocytes that migrate transiently from the periphery to the CNS; to this is added the continuous monitoring of microglial cells for any changes in the microenvironment (Sallusto et al., 2012). However, the pathophysiological chain seems to start from the moment in which an exogenous antigen is recognized at the peripheral level, showing molecular mimicry with an endogenous antigen expressed by CNS cells, causing activation and expansion of autoreactive T cell clones. Through the increase in membrane expression of α4-integrin, these T lymphocytes are conveyed towards the endothelium of the BBB through the binding of α4-integrin with the corresponding receptor (VCAM1) on the endotheliocytes. From that moment the migration of T lymphocytes into the CNS starts, tighter with secretion of inflammatory cytokines with consequent activation of microglia, in turn responsible for the secretion of chemokines capable of recruiting macrophages and dendritic cells.

There is also an increase in the permeability of the BBB due to the upregulation of the adhesion molecules and interleukins, among which the most important seems to be IL-22 (Wu & Alvarez, 2011). Figure 1.2 summarizes the main immunopathological mechanisms of MS.

1.3.1 T cells involvement

Classically in MS relapses were thought to be attributable to the inflammatory activity of aberrant T cells (CD4+ and CD8+) with specific activity against myelin, also in relation to animal models of EAE. Namely, the loss of tolerance towards myelin self-antigens and other components of the CNS has been hypothesized, resulting in a persistent peripheral T cell autoinflammatory activity.

CD4+ cells expressing IL-17 (Th17 cells), cause damage by secretion of proinflammatory cytokines such as IL-1, IL-22, IL-21, IL-9, TNFα, IL-17A, IL-17F which stimulate the activity of macrophages and other cells. CD8+ T cells are present

in increased concentrations both peripherally and centrally in MS patients and cause direct damage to oligodendroglia and neurons, constituting the preeminent component of the inflammatory infiltrate present in active plaques, playing a key role in the damage that occurs both during relapses and during the progressive phases (McFarland & Martin, 2007). Furthermore, CD4+ cells of the TH1 type (secreting INFγ, TNFβ, TNFα and IL-2) and T lymphocytes expressing GM-CSF are also involved. At the basis of this aberrant T cell activity, it is plausible that a deficit in the function of regulatory T cells (Treg) acts, in particular the CD4+ CD25+ FOXP3+ Treg and IL-10 producing T reg. Tregs normally perform an anti-inflammatory function through the secretion of IL-10 and other cytokines such as TGF-β1. In support of this hypothesis there is the finding of alterations in the circulating Tregs of patients with MS including the reduced expression of FOXP3 (Astier et al., 2006).

1.3.2 B cells involvement

In recent years, the decisive role of B lymphocytes in the pathogenesis of MS has clearly emerged, supported not least by the efficacy data of antiCD-20 therapies(Hauser et al., 2008, 2017). There is still speculation regarding the possible role of a small population of recently identified CD20-positive T cells (Palanichamy et al., 2014). B lymphocytes, on the other hand, play numerous roles, which can turn both in an anti-inflammatory and pro-inflammatory sense; healthy individuals display very low levels of antibodies in CNS, in contrast to what can be detected in patients with MS, in which this level is much higher and often constituted by monoclonal Ig (which accounts for the CSF oligoclonal bands). However, the role of these antibodies does not seem decisive in the disease, since in patients with a good response to anti-CD20 there is no decrease in CSF antibody titre; these observations led to the postulation of the existence of an antibody-independent B cell activity in MS. The latter probably involve functions of cellular interaction at the peripheral level, of chemoattraction towards the CNS of T cells and macrophages. Indeed, B lymphocytes of MS patients have a marked tendency to express proinflammatory cytokines such as IL-6, GM-CSF, TNF and are deficient in regulatory cytokines such as IL-10 (Barr et al., 2012; Duddy et al., 2007; R. Li et al., 2017). Among these, memory CD20CD27+ B cells are particularly high. The cytokine pattern of these cells can induce aberrant Th1 and Th17 responses, leading to activation of proinflammatory myeloid cells. Thus, B cell depletion (in particular of memory cells) may explain the reduction in inflammatory responses, and it has also been noted that B

cells (mostly naïve) that re-emerge after the end of anti-CD20 treatment show a profile more anti-inflammatory with higher expression of IL-10. It remains to be clarified whether their identification in each patient suggests a possible long duration of therapeutic response.

1.3.3 Role of cytokines

Cytokines are key players in MS, as they exert recruitment, survival, expansion and effector functions in autoreactive cells. High levels of Th1 cytokines were shown during EAE/MS relapses, whereas high levels of Th2 cytokines during remissions phases in MS patients. However, mechanisms underlying MS pathogenesis are more complex than a simple unbalance between Th1/Th2 response: EAE models demonstrated that INF-γ receptor KO mice are not spared from EAE; moreover they show an increased susceptibility to disease onset (Krakowski & Owens, 1996). As previously mentioned, Th17 response has a preminent role in MS (Aggarwal et al., 2003). Peripheral levels of IL-17 correlate, indeed, with inflammatory activity in MS patients (Hedegaard et al., 2008). Moreover, all Th17 cytokines and IL-22 are more represented in chronic white matter lesions. IL-23 appears as the main responsible for the switch towards Th17 phenotype, although this cytokine is not able alone to product Th17 cells *de novo* from T naïve cells. However, it has been shown that IL-23 and not IL-12 (involved in several autoimmune diseases such as arthritis, psoriasis, inflammatory bowel diseases) is essential to promote EAE (Cua et al., 2003).

In summary, this data suggest that MS pathogenesis is driven by a complex interaction between cytokines produced by T cells with a mixed phenotype (Th1, Th2, Th17). IL-1α and IL-1β are the main members of IL-1 superfamily, and are known for a high pro-inflammatory activity, and together with IL-6 are considered key players of systemic inflammation and several data confirm their relevance also in MS pathogenesis (Musella et al., 2020; Stampanoni Bassi et al., 2020). As previously mentioned in MS a disfunction of T-reg cells was shown. Moreover, GM-CSF (*granulocyte macrophage colony-stimulating factor)* plays a major role in the persistence of chronic inflammatory activity in MS, as it controls the migration and proliferation of leukocytes within CNS (McQualter et al., 2001) and interacting in the complex axis of adaptative and innate immunity. T, NK and NKT cells are all GM-CSF producers (Constantinescu et al., 2015).

Chemokines are proteins able to produce chemotactic responses; namely, to induce immune cells migration in response to chemical signalling. They are also involved in CNS development, as they can facilitate cellular migration, proliferation and survival.
New concepts concerning the pathogenesis of MS revealed that chemokines are not limited to the immunological functions of chemoattraction, but also play a role in the increase of neurotoxicity and neurodegeneration. Several studies have already demonstrated an increase in serum and CSF values of CCL11 (exotaxin 1, a potent eosinophil chemoattractant) in patients with neuroinflammatory disorders, including MS (Matsushita et al., 2013; Tanaka et al., 2008). CCL11, secreted by activated astrocytes, significantly promotes microglia migration and its production of reactive oxygen species by upregulating NOX1 (nicotinamide adenine dinucleotide phosphate oxidase 1), resulting in neuronal death from excitotoxicity (glutamatergic). For these reasons, CCL11 may be a potential marker associated with early neurodegeneration and unfavourable prognosis from the early stages of MS. A further B cell chemokine, CXCL13, implicated in the inflammatory process and associated with the intrathecal B cell response, has also been identified, a potential prognostic marker to predict conversion to clinically defined MS, especially if associated with a typical MRI picture (Brettschneider et al., 2010). The evolution of knowledge relating to these cytokine and chemokine biomarkers is precious since it can allow the stratification of prognostic risk in individual with MS and for this reason it can guide the selection of those patients at greater risk of disease evolution or aggressive phenotype and, consequently, it could also facilitate the choice of more targeted and adequate therapeutic treatments.

1.3.4 Role of innate immunity

A potential role of innate immunity has been supposed, in particular an excessive activation of peripheral neutrophils, able to induce autoreactive clonal T lymphocytes, as well as invariant NK cells, and mucosal-associated invariant T cells (MAIT) (Naegele et al., 2012; Sospedra & Martin, 2016).

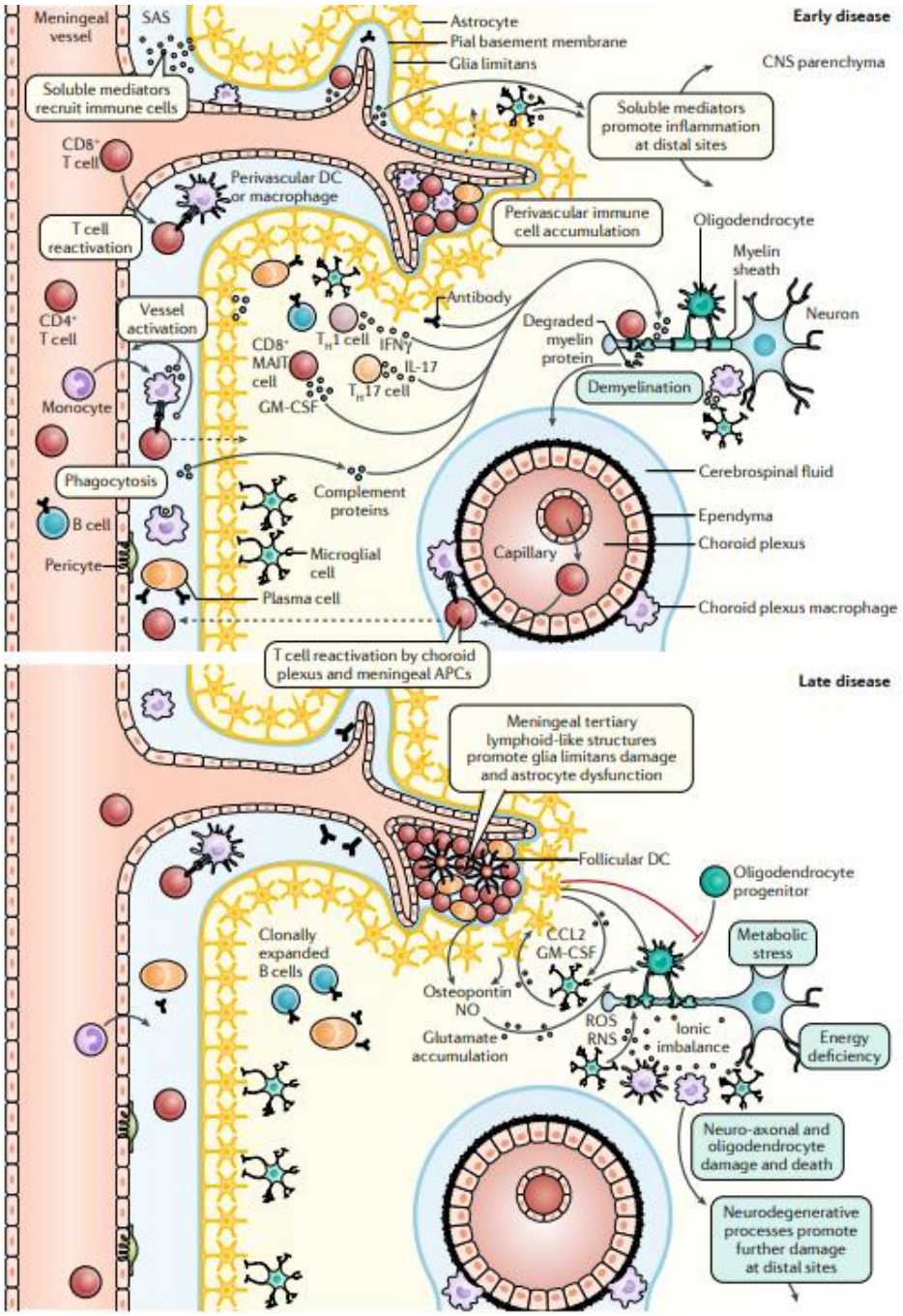


figure1.2 Dysregulation of the immune system (IS) within the CNS in the early and late stages of MS. IS cells enter the CNS via the BBB, subarachnoid space, and choroid plexuses. During relapses, cells of adaptive and innate immunity infiltrate the CNS with a perivascular distribution around the post-capillary venules of the BBB. These cells and resident cells (astrocytes and activated microglia) contribute to myelin, oligodendroglial, and axonal damage by contact mechanisms and by release of soluble factors. In the later stages, neurodegeneration prevails, leading to chronic oxidative stress promoted by chronic activation of IS cells, mitochondrial dysfunction, accumulation of extracellular iron, etc. Chronic inflammation is potentially mediated by compartmentalized inflammation within the CNS (meningeal infiltrates of follicular-like B lymphocytes).
APC, antigen presenting cell; **DC**, dendritic cell; **MAIT**, mucosal-associated invariant T; **NO**, nitric oxide; **RNS**, reactive nitrogen species; **ROS**, reactive oxygen species; **TH1**, T helper 1; **TH17**, T helper 17(Filippi, Bar-Or, et al., 2018)

1.4 PATHOLOGICAL ASPECTS

Although CNS involvement by the disease is broad and multifaceted, anatomically the most characteristic lesion of MS is the demyelination plaque, a specific macroscopic pathological marker. This plaque constitutes an area of focal demyelination predominantly of WM within the CNS, typically oval in shape, varying in size but generally 6 mm or more, and characterised by perivenular development. Plaques have a peculiar topography, with favoured sites such as the corpus callosum (Dawson's finger), optic nerve, iuxtacortical regions, periventricular regions, cervical spinal cord, cerebellum, lateral region of the brainstem, although they may be located in the most disparate areas. The number of plaques varies considerably from individual to individual and can obviously be influenced, in the same patient, by the duration of the disease. Spinal cord localisation can be observed in about 90% of MS patients, mostly cervical, and with extension less than two segments and, in the axial plane, less than the spinal cord hemisection (Charil et al., 2006; Fazekas et al., 1999; Filippi, Rocca, et al., 2016). Plaques can be distinguished on a histopathological level into active and inactive. The former are lesions in the acute phase, characterised by an impressive inflammatory infiltrate with edema and resulting tissue damage, the presence of a perivenular cuff of mononuclear cells (mainly CD4+ T lymphocytes, macrophages and to a lesser extent B lymphocytes and plasma cells) and resulting areas of mostly peri-axial demyelination (with relative sparing of the axon). Astrocytosis and reactive hypercellularity also co-exist. Subsequently, remyelination phenomena supported by oligodendrocytes may superimpose.

Active lesions are classically divided into four patterns (Lucchinetti et al., 2000):

- Pattern I: typical of MS in acute phase with clearly demarcated lesions, perivascular T cell infiltrates and active demyelination (with activated microglia and macrophages filled with myelin debris)
- Pattern II: lesions with preminent T cell and macrophage infiltration, with evident immunoglobulin deposition at the demyelination sites
- Pattern III: lesions with poorly demarcated margins, heavily damaged oligodendrocytes and vascular inflammation, bordered by an outer ring of spared myelin
- Pattern IV: lesions found exclusively in primary progressive forms, rich in T-cell infiltrate and activated microglia/macrophages. There is extensive oligodendrocyte degeneration of a non-apoptotic type.

In the course of the disease these active patterns converge towards a common histopathological phenotype, which is that of the inactive plaque: a clearly demarcated, demyelinated lesion, characterised by hypocellularity, loss of axons and minimal amounts of macrophages peripherally (they tend to disappear concentrically), with a central area of gliosis. Some plaques, however, undergo partial remyelination, generating so-called shadow plaques, characterised by thin myelin sheaths and enlarged internodal spaces (Patrikios et al., 2007).

Patients in the secondarily progressive phase typically show lesions with an activated gliotic centre and microglia/macrophages and with active demyelination at the lesion margins (smoldering lesion)(Mahad et al., 2015).

1.5 CLINICAL FEATURES AND DISEASE COURSE

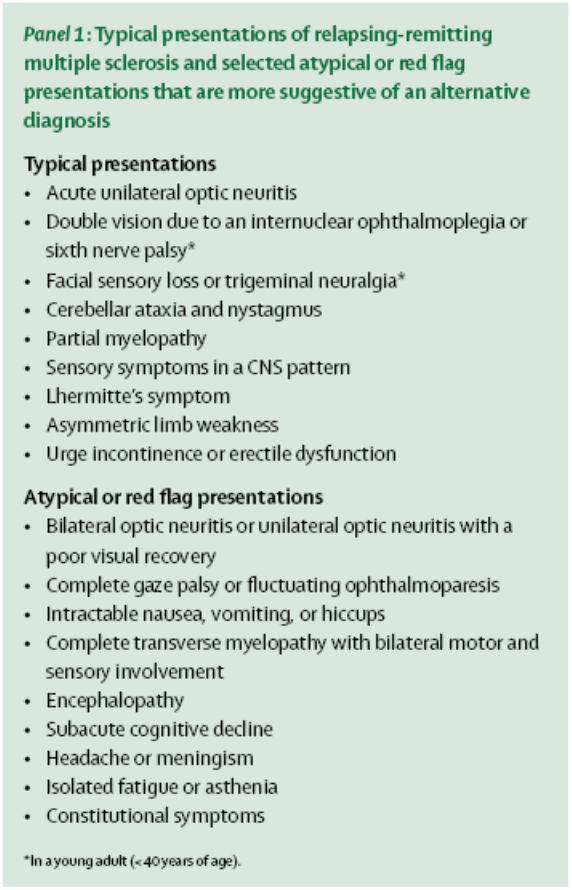
1.5.1 Typical demyelinating syndromes

The clinical signs and symptoms of MS presentation are highly variable in relation to the possible involvement of numerous CNS loci, however certain demyelinating syndromes are recognisable and occur more frequently.

The disease generally begins with a monophasic neurological symptom with acute or subacute onset, which reaches its acme within 2-3 weeks and constitutes the so-called CIS (clinically isolated syndrome). The most common disorders include:

- **Retrobulbar optic neuritis:** it constitutes the onset symptom in 25% of cases and 70% of patients are affected during the disease. Typically unilateral, with blurred vision, and pain evoked by eye movements. There is reduction of visual acuity with central scotoma, dyschromatopsia, deficit of the afferent component of the pupillary reflex. In the acute phase, papillary edema is found on fundus examination and on MRI it is possible to identify edema and gadolinium uptake in the optic nerve. Visual evoked potentials (VEPs) show increased P100 and OCT (optical coherence tomography) is impaired with reduced thickness of the retinal nerve fiber layer. Good recovery is seen in approximately 90% of patients (Toosy et al., 2014).
- **Myelitis,** generally partial: the most frequent form of onset, characterized by predominantly sensory symptoms (alteration of superficial tactile and pallesthesic sensitivity, paresthesias, dysesthesias) at the level of a limb and which then tend to spread above the trunk (typically the so-called "MS hug" with

constricting sensation) or to the contralateral limbs. Depending on the topography of the lesion, motor and/or sphincteric symptoms and Lhermitte's sign may be associated. MRI shows T2 hyperintense lesions with possible T1 gadolinium gain typically extending over 1-2 medullary segments.



***Panel 1*: Typical presentations of relapsing-remitting multiple sclerosis and selected atypical or red flag presentations that are more suggestive of an alternative diagnosis**

Typical presentations

- Acute unilateral optic neuritis
- Double vision due to an internuclear ophthalmoplegia or sixth nerve palsy*
- Facial sensory loss or trigeminal neuralgia*
- Cerebellar ataxia and nystagmus
- Partial myelopathy
- Sensory symptoms in a CNS pattern
- Lhermitte's symptom
- Asymmetric limb weakness
- Urge incontinence or erectile dysfunction

Atypical or red flag presentations

- Bilateral optic neuritis or unilateral optic neuritis with a poor visual recovery
- Complete gaze palsy or fluctuating ophthalmoparesis
- Intractable nausea, vomiting, or hiccups
- Complete transverse myelopathy with bilateral motor and sensory involvement
- Encephalopathy
- Subacute cognitive decline
- Headache or meningism
- Isolated fatigue or asthenia
- Constitutional symptoms

*In a young adult (<40 years of age).

Figure 1.3 Typical and atypical features for demyelinating syndrome from Brownlee et al, Lancet Neurol 2017

- **Brainstem and cerebellar syndrome**: onset symptoms in 25% of cases, they could have a very heterogeneous clinical presentation. Frequent symptoms are diplopia due to internuclear ophthalmoplegia from mono- or bilateral lesion of the medial longitudinal fasciculus, sixth cranial nerve palsy, trigeminal hypoesthesia, or ataxic syndromes, vertigo or tremor due to involvement of the cerebellar component.
- **Hemiparesis or hemi-hypoesthesia** from hemispheric lesions, less frequent manifestations together with isolated sphincteric symptoms such as bladder and/or bowel urge-incontinence.

All these presentations are typical for an inflammatory demyelinating event, while more atypical manifestations are summarized in **figure 1.3**.

In this phase, the main prognostic factor predicting the risk of conversion to defined MS is the presence and number of demyelinating lesions at the encephalic and/or spinal level; to this is added the presence at baseline of CSF oligoclonal bands, a sign of a compartmentalization of the inflammatory activity at the intrathecal level (Awad et al., 2010).

Several studies have tried to estimate the risk of conversion of clinical demyelinating syndrome to definite MS in relation to the type of onset: this estimate varies between 10 and 85% in the case of optic neuritis, between 40 and 60% in the case of myelitis and 53 -60% for brainstem onset (Miller et al., 2012). A study with a large number of patients (1058) also identified prognostic factors stratified by severity: low risk for socio-demographic factors, medium for the presence of OCB and high for >10 brain lesions (Tintore et al., 2015). In some cases, in the absence of clinically definite onset of the disease, MRI WMLs highly suggestive of m MS are found and satisfy the criterion of spatial dissemination (radiologically isolated syndrome, RIS). In this case the absence of anamnestic and objective clinical correlate (clinically silent disease) makes it impossible to formulate a diagnosis, but these patients remain at high risk of developing the disease (Lublin, 2014). Several studies have been concerned with identifying any negative prognostic factors. The conversion to a clinically manifest form 5 years after the detection of RIS occurs in 30% of patients, a progression of the radiological findings in 2/3 of them (Siva, 2013). A clinical conversion was associated with young age, male sex and the presence of spinal cord lesions (Labiano-Fontcuberta & Benito-León, 2016). Finally, approximately 10% show progressive course from the onset (PPMS); these patients had an older mean age, were male, and all presented asymptomatic spinal lesions in the preclinical phase (Okuda et al., 2009).

In addition to the aforementioned symptoms typically seen at onset, patients experience many other neurological symptoms during the course of the disease:

- **Sensitivity disorders**. Present from the onset in 1/3 of patients and seen globally in 90% of them during the disease. “Positive” manifestations such as dysesthesia and allodynia as well as neuropathic pain or the already mentioned Lhermitte's sign can be accompanied by “negative” symptoms such as tactile, pallesthesia or pain-relieving hypoesthesia.

- **Motor impairment**. Present in more than 90% of patients. Signs of pyramidal damage (Babinski sign, Hoffman sign, hyperreflexia, clonus) are often present with it. Sometimes spasticity, cramps or impaired ambulation are detected even in the absence of frank hyposthenia (Gelfand, 2014).
- **Fatigue**. Present in 50-80% of cases and prominent in the progressive forms, it constitutes one of the symptoms with the greatest impact on the patient's global level of functionality, reflecting negatively on the emotional and mood pattern, on the sleep-wake rhythm, on the level of productivity and quality of life. It can be defined as a reduction in both physical and mental energy levels with frequent need for rest. It can arise spontaneously or be induced by psycho-physical activity or by heat, infections and humidity. Although it can be found at any time, it generally reaches its peak in the afternoon (Mills & Young, 2008). The pathophysiology of this symptom has not been fully clarified but it is possible it is connected to the presence of a proinflammatory cytokine (IL-6, TNFα, INFγ) and to the presence of atrophy of structures such as the corpus callosum, cortex, dysfunction of the hypothalamus-pituitary-adrenal axis, axonal dysfunction, and the need for increased activation of axonal neuronal networks to perform a single task.
- **Sphincteric and autonomic alterations**: an important cause of disability and limitation in daily life, often present even in the early stages. The neurological bladder is present in about 2/3 of the patients, mostly caused by a detrusor-sphincteric dyssynergia, generally manifests itself with urge-incontinence, interrupted urination and reduced voiding, consequently there is a greater susceptibility to urinary infections. Bowel alteration is less frequent, and generally manifests itself with constipation, although faecal incontinence is possible in the case of severe marrow damage. Sexual disturbances (erectile dysfunction in men and decreased libido with hypo-anorgasmia in women) are present in 30-80% of patients. Less frequently, other autonomic deficits such as orthostatic hypotension, sudomotor and gastrointestinal dysfunction are found (Pintér et al., 2015).
- **Cognitive disorders**. Present in about 40-65% of patients and show prevalent involvement of the attentional-executive domains, in particular a reduction in the speed of information processing, and long-term memory which determine in most cases mild cognitive impairment, while definite dementia is rare. Cognitive deficit can also occur in the early stages (up to 24% of CIS) but is generally

found with decreasing frequency in the SP, PP, RR and CIS forms. Lesion load, degree of brain atrophy, and specific lesion or atrophy sites (hippocampus, thalamus, basal nuclei and cortex, third ventricular dilatation) show a specific correlation with the development of intellectual disability (Achiron & Barak, 2003; Korakas & Tsolaki, 2016).

- **Major depression** is present in up to 30-40% of cases and has a mixed pathogenesis: organic and reactive to the disease (Feinstein et al., 2014).
- **Headache**: reported in about 2/3 of patients and not infrequently has migraine characteristics.
- **Pseudorelapse**: Transient worsening of an old symptom in conjunction with heat, stress, intercurrent infections.

1.5.2 Clinical courses and phenotypes of MS

In 1996, the clinical course of MS was characterized as relapsing-remitting (RR), primary progressive (PP), secondary progressive (SP), or progressive relapsing (PR). Since then, a greater understanding of MS and its pathology prompted a re-examination of these clinical phenotypes which led to a 2013 review by Lublin and colleagues based mainly on the redefinition of the two main clinical courses of MS (relapsing and progressive) in relation to the presence of disease activity and progression as shown in **figure 1.4** (Lublin et al., 2014).

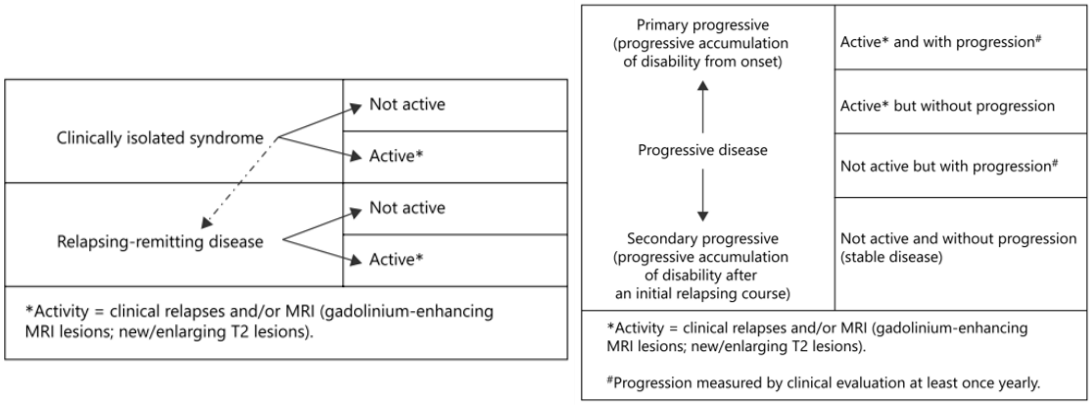


Figure 1.4: MS clinical phenotypes, Lublin revision 2013. On the left side are shown relapsing-remitting phenotypes of MS, on the right progressive ones (primary or secondary progressive) with the 4 possible sub-classifications (Lublin et al, 2014).

Based on this classification, the RR phenotype is divided into active or inactive forms, in relation to the presence of clinical and/or radiological relapses (new or enlarging lesions in T2 and/or gadolinium-enhancing in 2 consecutive MRI). There are no

standardized radiological markers to ascertain a progressive course of the disease, however the increase in lesion load in T1 and the progressive reduction of brain volume are being evaluated. The progressive course can be classified into 4 subgroups as shown in **figure 1.4**.

While CIS has been included as an entity belonging to the phenotypic spectrum of MS, RIS is excluded from this classification because the findings that characterize it are not sufficient to formulate a definite diagnosis of MS.

Therefore, 4 main phenotypes with specific course characteristics are currently identified (**figures 1.5, 1.6**):

1. Clinically Isolated Syndrome (CIS): is the first clinical presentation of a disease showing inflammatory demyelinating features, that could be multiple sclerosis, but has yet to meet the criteria for spreading over time (Lublin, 2014). A CIS is, by definition, always isolated in time (monophasic). Clinically, it is also usually spatially isolated (monofocal) with findings indicating lesion of the optic nerve (a common presentation in many CIS studies), spinal cord, brainstem or cerebellum, or (rarely) cerebral hemisphere (Miller et al., 2012).

2. Relapsing-Remitting Multiple Sclerosis (RR-MS): Accounts for 85% of MS onset cases and is characterized by unpredictable attacks (relapses). A relapse is defined as an episode of acute or subacute neurological dysfunction lasting a minimum of 24 hours, in the absence of fever or infection (Winsen et al., 2010a), usually evolving over days or weeks and then recovering to a variable extent, from a minimum full resolution recovery. A variable period (weeks, months, or even years) may pass before another relapse occurs, followed by a variable period of no symptoms. Recurrences are believed to be the clinical manifestation of recurrent episodes of inflammation and demyelination often accompanied by axonal damage usually destined to subside spontaneously within a few weeks. The fate of RRMS patients is variable, but most of them evolve, after a variable period of years, into the secondary progressive form of the disease (SPMS).

3. Secondary progressive multiple sclerosis (SPMS): is characterized by a change towards progressive worsening, with accumulation of irreversible neurological deficit and disability after an initial course of relapsing disease. Relapses can still occur but tend to decrease in frequency over time. Among patients with RRMS, about 50% developed SPMS after 10 years, about 80% after 20 years, about 95% after 30 years.

4. Primary Progressive Multiple Sclerosis (PPMS): Makes up approximately 15% of all MS cases. Patients with PPMS experience no attacks but a continuous gradual

deterioration of neurological function from the onset of the disease. Compared to RRMS, the gender distinction is less strong and the onset is later (average age 40 years). Clinically, the disability develops faster and there is a lack of response to any/most form of immunotherapy. Even if some evidence suggests that PPMS constitutes a distinct pathological entity, with a milder inflammatory component than RRMS, innumerable evidence document that the PP form is part of the spectrum of progressive phenotypes of MS (Lublin et al. 2014). Observational studies based on large cohorts of patients with natural history of the disease have shown that the accumulation of long-term disability in patients with RR onset is related to several clinical factors, including the relapse rate in the first years of the disease and the between the first two attacks (Scalfari et al., 2010). The term relapsing-progressive MS (RPMS) (Lublin 2014) previously used to characterize patients with a progressive course from onset and coexistence of clear relapses, is now obsolete. An acute attack in a patient with early progressive disease is now considered an expression of a "PPMS with disease activity".

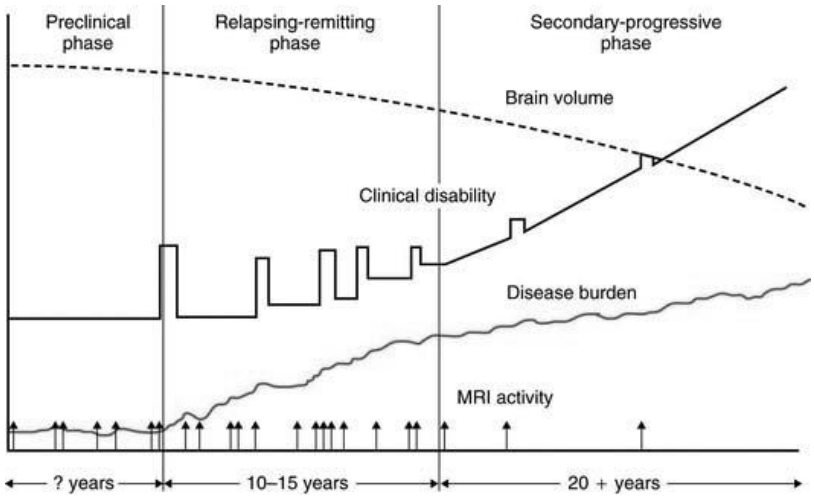


Figure 1.5 MS Phases. Clinical evident phase is preceded by a variable period of clinically silent inflammatory activity. Then, the first relapse occurs, followed by (in case of relapsing remitting course) several acute inflammatory MRI lesions and sometimes also clinical episodes. Generally, a good recovery is seen. After a variable period of time the disease shifts towards a progressive course, with a reduction of acute inflammatory relapse and a progressive disability accumulation. To date, current knowledge of the disease allows to affirm that neurodegeneration exists since the very early stages of MS, although with a variable extent from individual to individual and varying according to disease phenotype and stage (Fox RJ, Cohen JA. Cleve Clin J Med 2001)

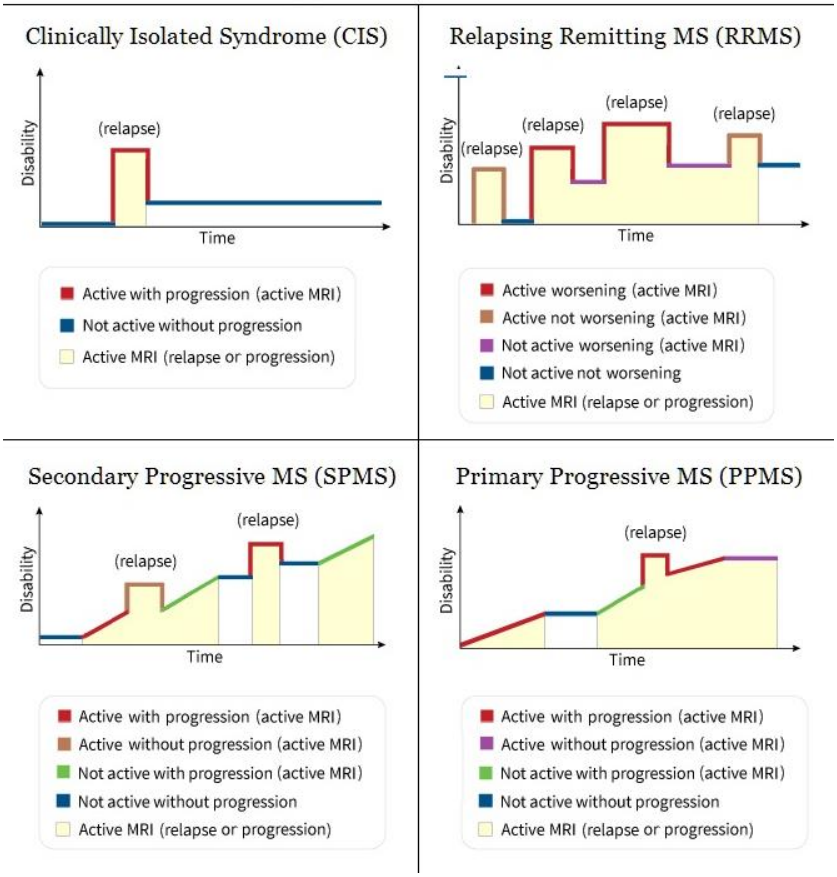


Figure 1.6 : the 4 main **MS phenotypes according to Lublin 2013** are represented (CIS, RRMS, SPMS, PPMS) together with the disability accumulation in time, showing the differente disease phases (active with or without progression, inactive with or without progression, MRI activity with or without progression

1.6 DIAGNOSIS OF MULTIPLE SCLEROSIS

1.6.1 Evolution of MS diagnostic criteria

The first clinical definition of Multiple Sclerosis can be attributed to Charcot who in 1868 had identified the presence of nystagmus, intentional tremor and marked voice as the characteristic triad of the disease. Understandably, Charcot was faced with a cerebellar onset variant that does not fully summarize the typical pleomorphism of MS. In 1906, Marburg also attempted to develop diagnostic criteria. He had postulated that the presence of Uhthoff's phenomenon, pyramidal signs and absence of the cutaneous-plantar reflex were sufficient to formulate the diagnosis of MS. Obviously, both Charcot's triad and Marburg's criteria had low specificity. In 1954, a first clinical classification of MS appeared made by Allison and Milliar: the appearance of clinical symptoms at different points in time and in different sites of the CNS was recognized as a typical feature of the disease, laying the foundations for the identification of two

fundamental concepts such as dissemination in time (DIT) and in space (DIS). Patients were also divided into "initial", "possible" and "probable" MS groups. This division of patients into groups was later used by Schumacher, who developed the first modern version of the diagnostic criteria for MS in 1965 (Milo & Miller, 2014). The concept of relapse was introduced together with a fundamental axiom, namely the need to exclude any other potential cause responsible for the clinical picture (the so-called "better explanation"). Diagnosis assumed the presence of all diagnostic criteria items (see **table 1.1**)

Table 1.1- Schumacher Criteria for the diagnosis of clinical definite MS (required 6/6)

1. 1. Clinical signs of a problem in the CNS
2. Dissemination in space, shown by clinical evidence of damage in two or more areas of CNS at examination or previous neurological history.
3. Evidence of white matter involvement
4. Dissemination in time shown by one of these: Two or more relapses (each lasting ≥ 24 hr and separated by at least 1 month) or disability progression (slow or stepwise)
5. Patient should be between 10 and 50 yr. old at time of examination.
6. No better explanation for patient's symptoms and signs should exist

Over the years it became clear that Schumacher's criteria were too restrictive and, apart from a few unsuccessful attempts at modifications, it was not until 1983 that a new, officially accepted version of the diagnostic criteria was drafted. The review by Poser et al was partly based on the previous one by Schumacher and identified 5 possible diagnostic categories: 1) clinically defined MS; 2) clinically probable MS; 3) defined MS supported by laboratory elements; 4) probable MS supported by laboratory elements; 5) non-MS.

For the first time, therefore, the diagnosis was made possible, in the event of insufficient clinical criteria, with the aid of paraclinical tests, in this case visual evoked potentials (VEP) and the detection of oligoclonal bands (at least 2 in the absence of correspondents in the serum) or high IgG index (high level of intrathecal IgG compared with serum). Relapse was described as an acute or subacute onset of 'typical' neurological symptoms for MS which must have been present for at least 24 hours and not attributable to an infection. It was therefore suggested not to take into consideration atypical symptoms for demyelinating syndrome (e.g., headache, psychiatric or consciousness disorders, etc.) and also to observe caution in classifying as relapse symptoms reported exclusively by the patient, and not documented by a clinical examination. Furthermore, to define a relapse it was specified that at least 30 days must have elapsed since the beginning of recovery from a previous exacerbation of the disease. The development of

neuroimaging, with particular reference to MRI, as well as the introduction of the first disease-modifying drug (interferon-beta) on the European market in 1996, led to a further revision of the diagnostic criteria of MS. Researchers aimed for a more rapid and accurate diagnosis of MS in its early stage, when therapeutic effects were thought to have broader action potential.

In 2001, the International Group on MS Diagnosis chaired by Ian McDonald therefore developed new criteria for MS diagnosis, now known as the 'McDonald's criteria', which led to several substantial changes from Poser's criteria (McDonald et al., 2001).

- Elimination of clinical or laboratory supportive classifications, and reduction of diagnostic categories to three: definite, possible, and non-MS.
- MS diagnosis was possible when the disease had a typical clinical course and met all the required criteria.
- Possible MS: a situation in which the patient's symptoms indicated multiple sclerosis, but did not meet McDonald's criteria; therefore, further observation of the patient was required (Nielsen et al., 2008).
- The diagnosis of "non-MS" rules out the disease.
- McDonald et al. also modified the definition of recurrence, as regards the minimum lapse of time necessary to consider two distinct relapses: it was established that a time of 30 days was sufficient between the onset of the previous relapse (and not the beginning of clinical recovery as postulated by Poser) and the onset of neurological symptoms of the following one.
- The neuroimaging must undoubtedly be counted as a diagnostic element in all respects. In Poser's criteria, MRI played only a supportive role, considered a tool to find a second subclinical focus; with McDonald's criteria, MRI has assumed a priority role since it can provide DIT or DIS criteria in place of clinical findings. Radiological DIS was demonstrated when there were enough lesions indicative of demyelination (with clear borders, greater than 3 mm in diameter) and when these lesions met the radiological criteria for MS (developed by Barkhoff, modified by Tintore through studies of conversion of CIS to SMCD) (Josey et al., 2012; Tur et al., 2008).

Specifically, it was necessary to satisfy at least 3 of the following 4 criteria (Tintoré et al., 2000):

- > 1 Gd-enhancing lesion or > 9 hyperintense lesions on T2-weighted sequences.
- >1 subtentorial lesion
- > 1 juxtacortical lesion
- >3 periventricular lesions

These neuroradiological criteria significantly increased the specificity of the diagnosis, while not significantly changing the sensitivity; thus, they have been incorporated into the McDonald's criteria. DIS, as well as through neuroradiological criteria, could be demonstrated in the presence of 2 asymptomatic T2 hyperintense lesions associated with the presence of CSF OCB or high IgG index.

DIT could be demonstrated by evidence of increased lesion load or by the presence of a contrast enhancing WML on a MRI performed $\geq$3 months after the previous one. Overall, the 2001 McDonald's criteria possessed high specificity (83%) and sensitivity (83%), positive predictive value of 75% and negative of 83%, accuracy of 83% in predicting the conversion of a CIS to SMCD (Dalton et al., 2002). Subsequent changes to the McDonald's criteria then occurred in 2005 and 2010. The changes were intended to facilitate and speed up the diagnostic process. In the introduction, the Panel emphasized that McDonald's criteria should serve as an aid, not a basis, in the diagnostic process. The revisions in question reduced the weight attributed to CSF diagnostics (diagnostic support role maintained only for RRMS in the 2005 edition but removed in the 2010 edition) and modified the radiological criteria for DIT and DIS as can be seen in **Table 1.2**.

Table 1.2: DIS and DIT criteria (Przybek et al., 2015)

Table 1 – Radiological criteria of 'dissemination in space' (DIS): a comparison.

Original McDonald criteria (2000)	Modified McDonald criteria (first revision, 2005)	Modified McDonald criteria (second revision, 2010)
Three conditions out of four must be met: 1. At least one gadolinium-enhancing lesion must be present or at least 9 T2 hiperintensive lesions must be present if gadolinium-enhancing lesions are absent. 2. At least one infratentorial lesion must be present. 3. At least 1 subcortical lesion must be present. 4. At least 3 periventricular lesions must be present.	Three conditions out of four must be met: 1. At least one gadolinium-enhancing lesion must be present or at least 9 T2 hiperintensive lesions must be present if gadolinium-enhancing lesions are absent. 2. At least one infratentorial lesion must be present. 3. At least 1 subcortical lesion must be present. 4. At least 3 periventricular lesions must be present.	'Dissemination in space' may be demonstrated by a presence of at least 1 T2 hiperintensive lesion[a] in 2 out of 4 typical locations: • periventricular • subcortical • infratentorial • in the spinal cord[b].

Important: The lesion in the spinal cord is equivalent to the infratentorial lesion. The gadolinium-enhancing lesion in the spinal cord is equivalent to the gadolinium-enhancing lesion in the brain. The lesions in the spinal cord can be counted together with the brain lesions to meet the required number of 9 lesions.

[a] Gadolinium-enhancing lesions are not required for DIS.

[b] If the patient has a relapse with spinal or truncal symptoms, the lesion responsible for these symptoms should not be counted.

Table 2 – Radiological criteria of 'dissemination in time' (DIT): a comparison.

Original McDonald criteria (2000)	Modified McDonald criteria (first revision, 2005)	Modified McDonald criteria (second revision, 2010)
DIT can be proven by: a) By a presence of at last one gadolinium-enhancing lesion in the brain in the MRI scan performed at least 3 months after the onset of first clinical symptoms*. This lesion should not be the one responsible for the clinical symptoms. b) By a presence of a new T2 hiperintensive lesion or a gadolinium-enhancing lesion in the second MRI scan performed not sooner than 3 months after the first.	DIT can be proven in two ways: a) By a presence of gadolinium-enhancing lesion in a MRI scan performed at least 3 months after the onset of first clinical symptoms. This lesion should not be the one responsible for the clinical symptoms. b) By a presence of a new T2 hiperintensive lesion in the next MRI scan if the previous one was done at least 30 days after the onset of first clinical symptoms.	DIT can be proven in two ways: a) By a presence of a new T2 hiperintensive lesion or a gadolinium-enhancing lesion in the next MRI scan. The time of the previous MRI scan is not important. b) By a presence of both gadolinium-enhancing and non-enhancing lesions in the first MRI scan. The time of the MRI scan is not important.

* If the first MRI scan is done too early, it cannot be used for diagnosing DIT. Performing a second MRI scan no sooner than 3 months after the onset of clinical symptoms is recommended.

In 2016, the MAGNIMS group (Magnetic Resonance Imaging in Multiple Sclerosis) proposed further changes in the neuroradiological criteria also in the light of the technological progress acquire (Filippi, Rocca, et al., 2016) d. In this case it was proposed:

- to include the optic nerve and cortical regions among the areas of interest within the CNS in the assessment of DIS
- the restoration of the Barkhof criteria entry which required the presence of at least 3 periventricular lesions.
- the abolition of the distinction between symptomatic and asymptomatic lesions for the criteria of DIT and DIS
- the adoption of the same DIS criteria for the diagnosis of RR and PP forms.
- reintroduce the support of CSF analysis for uncertain forms of PPMS.
- to use imaging of the whole spinal cord to define DIS (especially in patients who do not meet the DIS at the brain level), while there is a limited role of spinal cord imaging to document DIT.
- Consider making a diagnosis of Clinical definite MS in a RIS as soon as a compatible clinical event occurs as DIT is also satisfied (DIS is by definition present in a RIS)
- To exercise caution in applying McDonald's criteria in patients <11 years of age even if an ADEM-like form is excluded.

Figure 1.7: Comparison of McDonald's criteria 2010 and 2017 (Van Der Vuurst De Vries et al., 2018)

McDonald 2010
Dissemination in Space
1. Objective clinical evidence of at least 2 lesions or objective clinical evidence of 1 lesion with reasonable historical evidence of a prior attack involving a different CNS site
2. At least 1 T2 lesion in at least 2 of 4 MS-typical regions of the CNS: Periventricular Juxtacortical Infratentorial Spinal cord (Symptomatic lesions in patients with brainstem or spinal cord syndrome are excluded)
Dissemination in Time
1. At least 2 attacks separated by a period of at least 1 month
2. Simultaneous presence of asymptomatic gadolinium-enhancing and nonenhancing lesions at any time
3. A new T2 and/or gadolinium-enhancing lesion on follow-up MRI, irrespective of its timing with reference to a baseline scan
McDonald 2017
Dissemination in Space
1. Objective clinical evidence of at least 2 lesions or objective clinical evidence of 1 lesion with reasonable historical evidence of a prior attack involving a different CNS site
2. At least 1 T2 lesion in at least 2 of 4 MS-typical regions of the CNS: Periventricular (Juxta)cortical Infratentorial Spinal cord
Dissemination in Time
1. At least 2 attacks separated by a period of at least 1 month
2. Simultaneous presence of gadolinium-enhancing and nonenhancing lesions at any time
3. A new T2 and/or gadolinium-enhancing lesion on follow-up MRI, irrespective of its timing with reference to a baseline scan
4. Demonstration of CSF-specific OCBs (as substitute for demonstration of DIT)

Abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; MRI, magnetic resonance imaging; OCB, oligoclonal bands.

Furthermore, it was suggested that the presence of the central venule within the white matter lesions visualized through T2* sequences with high-field devices (7T) could constitute a distinctive feature of MS, although for obvious reasons not yet usable in clinical practice (Filippi, Rocca, et al., 2016).

1.6.2 CURRENT CRITERIA: MCDONALD 2017

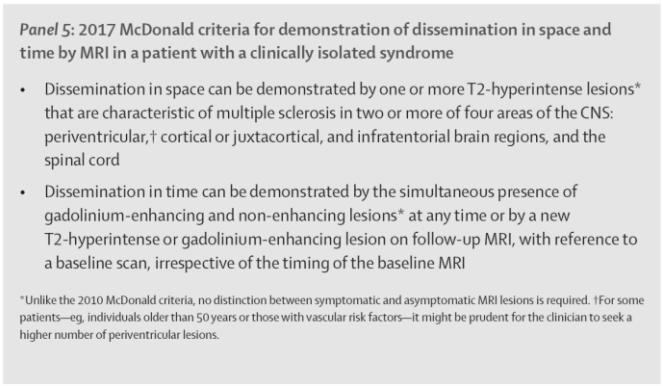
The current version of the diagnostic criteria was drafted in 2017 (Thompson et al., 2018b). The main changes, compared to the 2010 version (**figures 1.7, 1.8, 1.9**) are:

	Number of lesions with objective clinical evidence	Additional data needed for a diagnosis of multiple sclerosis
≥2 clinical attacks	≥2	None*
≥2 clinical attacks	1 (as well as clear-cut historical evidence of a previous attack involving a lesion in a distinct anatomical location†)	None*
≥2 clinical attacks	1	Dissemination in space demonstrated by an additional clinical attack implicating a different CNS site or by MRI‡
1 clinical attack	≥2	Dissemination in time demonstrated by an additional clinical attack or by MRI§ OR demonstration of CSF-specific oligoclonal bands¶
1 clinical attack	1	Dissemination in space demonstrated by an additional clinical attack implicating a different CNS site or by MRI‡ AND Dissemination in time demonstrated by an additional clinical attack or by MRI§ OR demonstration of CSF-specific oligoclonal bands¶

If the 2017 McDonald Criteria are fulfilled and there is no better explanation for the clinical presentation, the diagnosis is multiple sclerosis. If multiple sclerosis is suspected by virtue of a clinically isolated syndrome but the 2017 McDonald Criteria are not completely met, the diagnosis is possible multiple sclerosis. If another diagnosis arises during the evaluation that better explains the clinical presentation, the diagnosis is not multiple sclerosis. An attack is defined in panel 1. *No additional tests are required to demonstrate dissemination in space and time. However, unless MRI is not possible, brain MRI should be obtained in all patients in whom the diagnosis of multiple sclerosis is being considered. In addition, spinal cord MRI or CSF examination should be considered in patients with insufficient clinical and MRI evidence supporting multiple sclerosis, with a presentation other than a typical clinically isolated syndrome, or with atypical features. If imaging or other tests (eg, CSF) are undertaken and are negative, caution needs to be taken before making a diagnosis of multiple sclerosis, and alternative diagnoses should be considered. †Clinical diagnosis based on objective clinical findings for two attacks is most secure. Reasonable historical evidence for one past attack, in the absence of documented objective neurological findings, can include historical events with symptoms and evolution characteristic for a previous inflammatory demyelinating attack; at least one attack, however, must be supported by objective findings. In the absence of residual objective evidence, caution is needed. ‡The MRI criteria for dissemination in space are described in panel 5. §The MRI criteria for dissemination in time are described in panel 5. ¶The presence of CSF-specific oligoclonal bands does not demonstrate dissemination in time per se but can substitute for the requirement for demonstration of this measure.

Figure 1.8: McDonald 2017 criteria for MS diagnosis in patients with a typical demyelinating attack at onset (Thompson et al, 2018)

- The ability to also use symptomatic lesions to demonstrate DIS and DIT (there is an increase in sensitivity without a decrease in specificity).
- The inclusion of cortical lesions among the site-specific lesions of MS, albeit including them in the ambit of juxtacortical lesions, also in consideration of the technical difficulty in visualizing pure cortical lesions which would require specific and scarcely widespread neuroradiological techniques (e.g., DIR, double inversion recovery).
- The re-evaluation of CSF diagnostics: the presence of OCB can confirm the diagnosis in case of insufficient clinical-neuroradiological criteria to formulate the diagnosis and above all if there is the intent to undertake a DMT, thus acting as an element capable of demonstrating DIT.
- Furthermore, CSF analysis is recommended in all those cases of diagnostic doubt between MS and another condition in which therefore the value of a typical CSF test can serve as a support to the diagnosis of MS while an atypical test can reinforce the idea of being faced to an alternative condition (better explanation). The expert consensus did not consider it appropriate to include the optic nerve among the typical lesion sites (due to the slight increase in sensitivity counterbalanced by a significant decrease in specificity) nor to modify the



Panel 5: 2017 McDonald criteria for demonstration of dissemination in space and time by MRI in a patient with a clinically isolated syndrome

- Dissemination in space can be demonstrated by one or more T2-hyperintense lesions* that are characteristic of multiple sclerosis in two or more of four areas of the CNS: periventricular,† cortical or juxtacortical, and infratentorial brain regions, and the spinal cord
- Dissemination in time can be demonstrated by the simultaneous presence of gadolinium-enhancing and non-enhancing lesions* at any time or by a new T2-hyperintense or gadolinium-enhancing lesion on follow-up MRI, with reference to a baseline scan, irrespective of the timing of the baseline MRI

*Unlike the 2010 McDonald criteria, no distinction between symptomatic and asymptomatic MRI lesions is required. †For some patients—eg, individuals older than 50 years or those with vascular risk factors—it might be prudent for the clinician to seek a higher number of periventricular lesions.

Figure 1.9: Above the criteria for demonstrating dissemination in space and time in a patient with clinically isolated syndrome, below the 2017 McDonald criteria for diagnosing MS in a patient with a course characterized by progression from onset (primary progressive MS)

number of periventricular lesions required from 1 to 3 (which would determine a negligible increased specificity with decreased sensitivity).

1.6.3 Paraclinical markers to support the diagnosis

MRI

The development of MRI from the 80s onwards led, as previously mentioned, to the progressive identification of neuroradiological key features of MS up to the definition of the first neuroradiological disease criteria (Barkhof et al., 1997).

The main neuroradiological features of demyelinating lesions in MRI are represented by:

- Presence of "enhancement" after administration of Gadolinium during phases of inflammatory activity, the most typical contrastographic pattern is that of an external ring. The enhancement results from the passage and accumulation of contrast medium in the CNS due to the breakdown of the blood-brain barrier,
- T2 hyperintensity and T1 iso-hypointensity. T1 hypointense lesions are called black holes and are markers of severe demyelination and axonal loss.
- WML shape: typically oval flame shape in the brain. Spinal cord lesions are cigar-shaped, extending at least 3 mm but less than two vertebral segments in length and less than half the diameter of the spinal cord.
- WML location: typically juxtacortical and periventricular localization at the brain level, with frequent perpendicular arrangement with respect to the ventricles, involvement of the corpus callosum (which gives rise to the characteristic Dawson fingers, **figure 1.10**) and of the U fibers. There is an asymmetric and random distribution of the WML (at least in the earliest stages); lateral localization at the level of the brainstem or at the level of the cerebellar hemispheres; at the medullary

level they are generally eccentric, rarely show a mass effect and prefer the cervical cord and posterior columns (Polman et al., 2011).

- WML size: generally greater than 3 mm and less than 1 cm. Occasionally, in rarer forms of MS, it is possible to find larger lesions (>2cm), which can also show mass effect and surrounding edema, atypical contrast patterns (e.g.: open ring, concentric) leading to problems of differential diagnosis with other conditions such as cancer and infections.

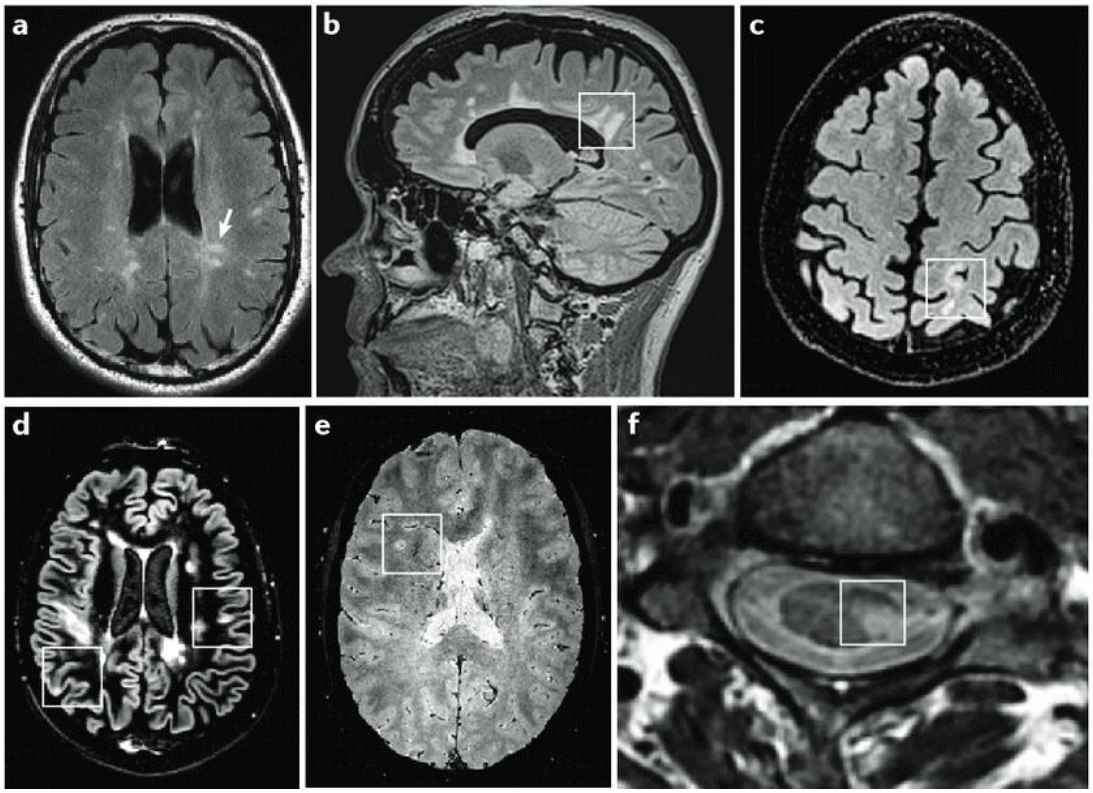


Figure 1.10: MRI features typical of MS a) Dawson finger (arrow), visible as ovoid, hyperintense, periventricular lesions perpendicular to the body of the lateral ventricle and/or the callosal junction, in a FLAIR image. b) Dawson finger (inset) in a sagittal FLAIR image. c) An S-shaped juxtacortical lesion (inset) in an axial FLAIR image. d) Cortical lesions (inset) shown on a double inversion recovery (DIR) image e) The central vein sign (inset), visible on magnetic susceptibility weighted imaging (T2* axial image). f) Eccentric spinal cord lesions (inset) typical of multiple sclerosis, shown in a T2-weighted axial image. (Geraldes et al., 2018a).

LABORATORY BIOMARKERS

Considerable efforts have been made over the years to identify MS-specific biomarkers. Despite this, a pathognomonic marker is not yet available, and the laboratory support for the diagnosis mainly focuses on the confirmation of the presence of CSF oligoclonal bands (a non-specific but very frequent finding in MS), in fact the only biomarker of importance for the diagnosis of MS. Moreover, are currently used blood chemistry panel

for differential diagnostics, and therefore simply to exclude other pathologies. More than 95% of Caucasian MS patients have undetectable IgG-class CSF OCBs in serum (Link & Huang, 2006). The IgG index (or Link index) considers the serological levels of albumin and Ig according to this relationship:

$$\frac{\textit{CSF IgG/serum IgG}}{\textit{CSF albumin/serum albumin}} \quad \textit{(normal<0,7)}$$

(Link, 1987)

70 to 90% of MS patients have an elevated IgG index (Awad et al., 2010; Link & Huang, 2006). However, disorders other than MS can cause an increase in OCB and IgG index. Furthermore, some studies have documented that in Asian populations there is a significantly higher rate of CSF OCB negative patients (B. Li et al., 2007; Lu et al., 2019; Nakashima et al., 2002). Other CSF findings include the presence of mononuclear cells (generally <50 cells/mm3) and a slight increase in total protein count, which is an indication of BBB damage. Other biomarkers are under investigation, including CSF IgM oligoclonal bands, CSF kappa free light chains but their use is limited to research contexts as further confirmation is needed.

NEUROPHYSIOPATHOLOGY

Multimodal evoked potentials (visual, auditory, motor or sensory) in MS typically show a delayed evoked response with preserved waveform, revealing the presence of areas of demyelination. Any relief of an altered waveform indicates the presence of areas of axonal loss (Comi et al., 1999).

1.7 INTRODUCTION TO THERAPY

MS therapeutic approach is unfortunately not decisive to date to arrest the disease, but it allows to considerably reduce the number, duration and severity of relapses as well as to slow down the progression of the disability. There are three main categories of intervention: treatments for clinical exacerbations, symptomatic treatments, and disease-modifying treatments (DMTs).

1.7.1 Drugs for clinical relapse

Corticosteroids are first choice treatment in case of clinical relapse. They are administered in high doses for short periods of time (steroid *bolus*), classically intravenous methylprednisolone, at a dosage of 500mg - 1g/day for 3-5 days (extendable

up to 7-10 days in case of no response), followed or less by progressive tapering. Alternatively, adrenocorticotropic hormone (ACTH) may be used subcutaneously or intramuscularly. The beneficial effect of steroids is attributable to their powerful anti-inflammatory and anti-edema activity, capable of accelerating clinical recovery, however about 15% of patients do not benefit from steroid therapy. In this case, plasmapheresis can be used, in 5-10 sessions administered in consecutive days, which have demonstrated high efficacy (up to 72%). Although some centers use intravenous immunoglobulin (IVIG), there is no evidence to support the efficacy of this treatment (Noseworthy et al., 2000).

1.7.2 Symptomatic drugs

The therapeutic management MS patients also includes several symptomatic drugs, with the aim of reducing the impact of disease symptoms on patient's quality of life. The integrated approach with pharmacological and rehabilitative strategies is essential, with a significant impact not only on physical disability but also on cognitive impairment. Table 1.3, taken from Thompson A, 2018 summarizes the main pharmacological and non-pharmacological options employed in this setting.

1.7.3 Disease modifying therapies (DMTs)

In the last 15-20 years there has been a profound renewal of the therapeutic panorama for MS treatment, with the approval to date of over 15 DMTs and many other drugs under final development (phase III trials). The available drugs act both peripherally and centrally, mainly through the control of the inflammatory activity of the disease, with a potential indirect effect also on the neurodegenerative mechanisms (**Fig. 1.11**).
In relapsing forms of MS, the use of DMTs produces a reduction in inflammatory activity detectable both clinically (relapses) and radiologically (new/enlarging T2 lesions and Gd-enhancing lesions), with a moderate effect on the worsening of disability. The efficacy of the approved DMTs is instead marginal in the progressive forms, probably due to partly different pathogenetic mechanisms underlying the latter.
In accordance with the European Medicines Agency (EMA), DMTs are conventionally divided into first line and second-line treatments, in relation to the safety profile of the individual drugs and the resulting risk-benefit ratio for the patient. Tendentially more effective than first-line drugs, second line DMTs are characterized by a greater risk of adverse events and therefore reserved for patients who have previously failed

treatment with first line DMTs or who present more aggressive forms since debut.

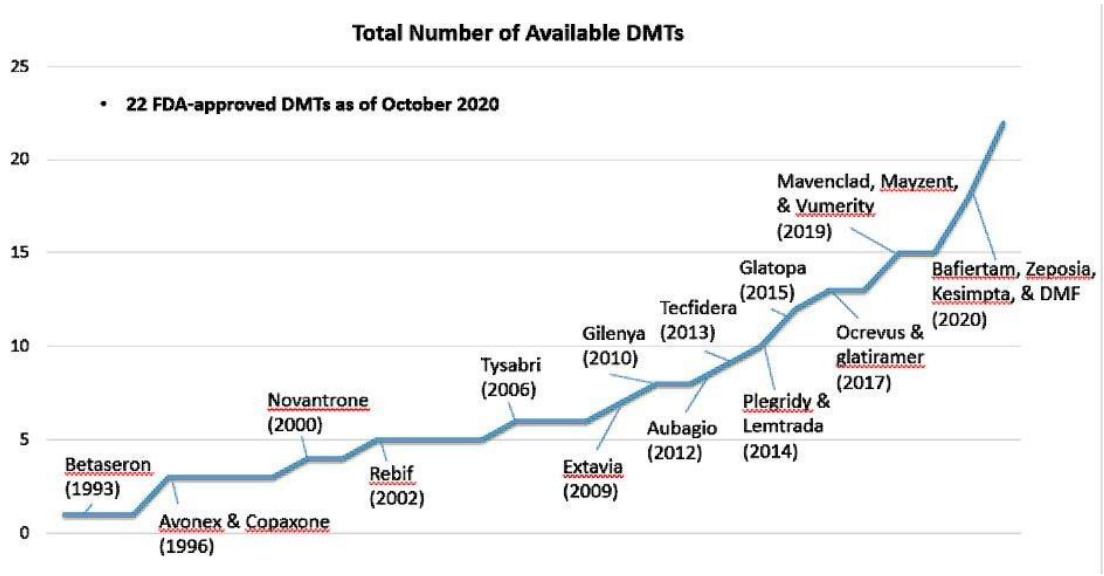


Figure 1.11: disease-modifying drugs (DMTs) for MS and their year of discovery or approval. RMS: relapsing multiple sclerosis, RRMS relapsing remitting multiple sclerosis, PPMS primary progressive multiple sclerosis, SPMS secondary progressive multiple sclerosis. Daclizumab was withdrawn from the market in March 2018 due to the detection of serious adverse events including encephalitis and meningoencephalitis.

SCALAR AND INDUCTION THERAPY

At the basis of MS treatment there are two opposing strategies:

- on the one hand the so-called "escalating therapy", i.e., the first instance use of DMTs with a moderate effect with the possible subsequent sequential use of increasingly effective (and equally less safe) drugs in case of therapeutic failure;
- on the other "induction therapy", based on the principle of inducing a disease remission using highly effective treatments (and greater risk of adverse events), followed by first-line treatments or by no therapy.

The appropriateness of one or the other strategy is obviously evaluated in relation to the clinical and demographic characteristics of the patient, and the risk-benefit ratio of the treatment in each case. With the introduction of increasingly effective therapies, a new surrogate marker of therapeutic response has emerged in recent years, defined NEDA (No Evidence of Disease Activity) and consisting of the combined absence of clinical and radiological disease activity, represented by the following: clinic, disability progression (assessed by EDSS scale) and radiological activity (increase in lesion load detected in T2 and/or as the presence of Gd-enhancing lesions). Over the years, the NEDA concept has expanded to include additional domains, such as brain atrophy

(NEDA-4) and neurofilament level (NEDA-5). Further therapeutic targets have recently emerged, such as NEPAD (No Evidence of Progression or Active Disease) intended for use in progressive forms of the disease, and MEDA (Minimal Evidence of Disease Activity), the latter aimed at indicating an acceptable level of residual disease activity as a therapeutic target (e.g., Rio score) (Giovannoni et al., 2018). Data relating to the mechanism of action and efficacy of the individual treatments goes beyond this discussion, a table taken from McGinley et al (McGinley et al., 2021) is shown for the schematization of the treatments currently available (**figure 1.12**).
Finally, it should be remembered that the availability of the various DMTs is subject to considerable differences in the various regions of the world and often even within the same country, mainly in relation to the high costs of the treatments (Thompson et al. 2018).

1.7.4 MS treatment guidelines

Given the growing complexity of the therapeutic landscape, the European Commission for Treatment and Research in Multiple Sclerosis (ECTRIMS) together with European Academy of Neurology (EAN) has recently published guidelines based on levels of evidence defined in accordance with the GRADE system, capable of support the clinical neurologist in therapeutic decisions within an increasingly complex scenario. The guidelines provide 23 recommendations aimed at 10 clinical questions that encompass the main therapeutic aspects of the disease, from the timing of the start of treatment, to problems related to pregnancy, to the suspension of therapy in the event of clinical and instrumental stability (Montalban et al., 2018). Some months later, also the American Academy of Neurology (AAN) published MS treatment guidelines (Bittner & Zipp, 2018). Finally, in 2019, followed the revisions of the Consensus Recommendations for the Diagnosis and Treatment of Multiple Sclerosis by the MENACTRIMS group (Middle East North Africa Committee for Treatment and Research in Multiple Sclerosis) (B. Yamout et al., 2020).

Table 3. Disease-Modifying Therapies for Multiple Sclerosis

Medication category, medication	Reduction of annualized relapse rate, % (sample size)	Absolute relapse rate reduction difference[a]	Route and frequency of administration	Required baseline testing	Required monitoring	Recommended additional testing	Common adverse effects	Rare serious adverse effects
Interferons								
Interferon beta[26-29b]	34% (n = 372)[26] 32% (n = 172)[30] 28% (n = 1516)[28]; 32%	0.41 0.15 0.141 0.83	Subcutaneously every other day or ×3/wk; intramuscularly once a wk or every 2 wk[c]	CBC with differential and liver function	CBC with differential and liver function every 6 mo	None	Headache (44%-65%) Flu-like symptoms (47%-57%) Injection site reaction (8%-89%) Leukopenia (7%-28%)	Liver toxicity
Amino acid copolymer								
Glatiramer acetate[31]	29% (n = 251)	0.25	Subcutaneously daily or ×3/d	None	None	CBC with differential and liver function	Injection site reaction (8%) Immediate postinjection reaction (2%-16%)	Skin necrosis
S1P receptor modulators								
Fingolimod[32]	54% (n = 1272)[32] 52%[d] (n = 1153)[33]	0.22 0.17	Oral once daily	CBC with differential, liver function, VZV antibodies, fundus examination, ECG, FDO[e]	Fundus examination 3-4 mo after initiation, CBC with differential and liver function every 6 mo	Skin examinations[f]	Headache (25%) Liver enzyme elevation (15%) Back pain (10%) Hypertension (8%)	Infections, PML, macular edema, liver toxicity, PRES, hypertension, bradyarrhythmia, heart block, respiratory effects
Siponimod[34]	55% (n = 1651)	0.09	Oral once daily[g]	*CYP2C9* genotype, CBC with differential, liver function, VZV antibodies, fundus examination, ECG, FDO[h]	None	CBC with differential and liver function every 6 mo	Headache (15%) Hypertension (13%) Liver enzyme elevation (11%)	Bradycardia, heart block, liver toxicity, macular edema, respiratory effects
Ozanimod[35]	31%[d] (n = 1346)	0.17	Oral	CBC with differential, liver function, VZV antibodies, ECG, fundus examination[i]	None	CBC with differential and liver function every 6 mo	Upper respiratory tract infection (26%) Liver enzyme elevation (10%) Orthostatic hypotension (4%) UTI (4%) Back pain (4%) Hypertension (4%)	Bradycardia, heart block, liver toxicity, macular edema, respiratory effects
Fumarates								
Dimethyl fumarate[36,37] Diroximel fumarate[38]	44%[d] (n = 1417)[36] 53% (n = 1234)[37]	0.18 0.19	Oral twice daily	CBC with differential, liver function	CBC with differential every 6 mo	Liver function annually	Skin flushing (40%) Diarrhea (14%) Nausea (12%) Abdominal pain (18%) Vomiting (9%)	Infections, liver toxicity, lymphopenia, PML

(continued)

Figure 1.12: DMT for Multiple Sclerosis (McGinley, JAMA 2021)

Table 3. Disease-Modifying Therapies for Multiple Sclerosis (continued)

Medication category, medication	Reduction of annualized relapse rate, % (sample size)	Absolute relapse rate reduction difference[a]	Route and frequency of administration	Required baseline testing	Required monitoring	Recommended additional testing	Common adverse effects	Rare serious adverse effects
Pyrimidine synthesis inhibitor								
Teriflunomide[39,40]	31% (n = 1088)[39]	0.17	Oral once daily	CBC with differential, liver function, PPD or QFT, blood pressure	Liver function monthly for first 6 mo then every 6 mo	CBC with differential every 6 mo	Headache (16%) Increased liver enzymes (15%) Diarrhea (14%) Nausea (11%) Alopecia (13%)	Hepatotoxicity, teratogenicity
Purine analogue								
Cladribine[41]	58% (n = 1326)	0.19	Two oral treatment courses 12 mo apart	CBC with differential, liver function, VZV antibodies, HIV, PPD and QFT, hepatitis panel, age-appropriate cancer screening	Age-appropriate cancer screenings, CBC with differential 2 and 6 mo after starting each course; if lymphocyte count <200/μL, monitor monthly until month 6	None	Upper respiratory tract infection (38%) Headache (25%) Lymphopenia (24%) Nausea (10%) Back pain (8%)	Malignancy, teratogenicity, pulmonary tuberculosis, herpes infections, PML
Anti-α_4 integrin receptor monoclonal antibody								
Natalizumab[42]	68% (n = 942)[42]	0.5	Intravenously once every 4 wk	CBC with differential, liver function, JCV serology, brain MRI	JCV serology every 3-6 mo, brain MRI every 6-12 mo for JCV-seronegative patients	CBC with differential and liver function every 6 mo; antinatalizumab neutralizing antibodies at 6 mo	Headache (38%) Fatigue (27%) Arthralgia (19%) Abdominal discomfort (11%) UTI (21%) Lower respiratory tract infection (17%)	PML, hepatotoxicity, herpes infections, hypersensitivity reactions
Anti-CD20 monoclonal antibodies								
Ocrelizumab[43]	46% (n = 821) 47%[d] (n = 835)	0.13 0.13	Intravenously once every 6 mo	CBC with differential, liver function, hepatitis panel	None	PPD or QFT at baseline, CBC with differential and liver function annually	Infusion reactions (34%) Upper respiratory tract infections (40%) Herpes infections (6%)	Hepatitis B reactivation, PML, malignancy risk potential
Ofatumumab[44]	51% (n = 927) 59% (n = 955)	0.11 0.15	Subcutaneously monthly after initial load	Hepatitis panel, serum immunoglobulins	None	PPD or QFT at baseline, CBC with differential and liver function annually	Infections (51.6%) Injection reaction (20.2%) Headache (13.3%)	Hepatitis B reactivation, PML, reduction in immunoglobulins

(continued)

erapies for Multiple Sclerosis (continued)

ed, ize)	Absolute relapse rate reduction difference[a]	Route and frequency of administration	Required baseline testing	Required monitoring	Recommended additional testing	Common adverse effects	Rare serious adverse effects
.63)[45]	0.21	Intravenously, 2 courses 12 mo apart	CBC with differential, creatinine, thyrotropin, ALT, AST, hepatitis panel, VZV antibodies, PPD or QFT, urinalysis	CBC with differential, creatinine, and urinalysis every mo until 48 mo after last drug dose; thyrotropin every 3 mo until 48 mo after last drug dose, skin examination annually	HIV at baseline, liver function, gynecologic examination/HPV screening annually	Rash (53%) Headache (52%) Infusion reactions (92%) Thyroid disorder (34%) Infection (71%) Herpes infection (16%)	Autoimmune conditions (ITP, antiglomerular basement membrane disease, hepatitis), HPV infection, stroke, TB, PML, malignancy risk potential
28)[46]	0.26						

otransferase; AST, aspartate aminotransferase; CBC, complete blood cell count; t-dose observation; HPV, human papillomavirus; ITP, immune nningham virus; MRI, magnetic resonance imaging; PML, progressive multifocal ed protein derivative; PRES, posterior reversible encephalopathy syndrome; sphingosine 1-phosphate; TB, tuberculosis; UTI, urinary tract infection; VZV,

e, along with a number-needed-to-treat analysis, should be interpreted with of relapses.

rferon beta-1b, peginterferon beta-1a.

tration dependent on interferon.

[d] Trial had an active comparator.

[e] All patients must have an FDO that includes observation after taking the first dose for at least 6 hours, with pulse and blood pressure assessment hourly and ECG prior to dosing and at end of observational period.

[f] Increased risk of basal cell carcinoma and melanoma.

[g] Target dose and titration depends on *CYP2C9* genotype.

[h] FDO only required in patients with a cardiac history.

[i] Fundus examination only required for patients with a history of diabetes mellitus and uveitis that increase the risk of macular edema.

CHAPTER 2

CONTROVERSIES IN THE DIAGNOSIS OF MULTIPLE SCLEROSIS

2.1 McDONALD CRITERIA LIMITATIONS

McDonald's criteria, conceptually based on the two paradigms of the spatial and temporal diffusion of findings (DIS and DIT), have a strong clinical connotation, supported by the use of paraclinical instruments (mainly MRI, but also CSF laboratory and neurophysiological investigations) in which, however, the lack of a pathognomonic marker emerges as the main shortcoming.

In fact, despite the innumerable progress in the pathophysiological understanding of the disease, and despite the undoubted evolution of neuroimaging and laboratory diagnostic methods, despite constant efforts aimed at identifying MS-specific biomarkers, a pathognomonic marker has not yet been identified unequivocally. Precisely because of the absence of a pathognomonic marker, as well as of a univocal panel of exclusion criteria, since the 1965 Schumacher criteria, MS has remained a diagnosis of exclusion, determinable only if any alternative underlying cause has been reasonably ruled out of the neurological picture ("better explanation") (Solomon, 2019; Solomon, Naismith, et al., 2019; Solomon & Corboy, 2017). Although the disease is characterized by a rather characteristic phenomenological pattern, it must in fact be remembered that the clinical presentation and the course itself of MS unfortunately can be mimicked by numerous diseases with profoundly different etiopathogenesis from MS. Added to this is a further limitation, inherent in the neuroradiological criteria: they too, in fact, were mainly conceived for prognostic purposes (to predict the risk of conversion of CIS into MS and

therefore promote early treatment) (Beesley et al., 2018; Solomon & Corboy, 2017). For this reason, the MRI criteria have a suboptimal specificity and can be met by numerous different pathologies (MS mimics) with the frequent finding of white matter lesions (WML) that can resemble the demyelinating lesions typical of MS in terms of location, shape and site. An inaccurate interpretation of the neuroradiological criteria is in fact one of the main causes of MS misdiagnosis. One study found that of all patients referred to an MS centre for MRI "matching" lesions, only 11% were ultimately diagnosed with MS (Carmosino et al., 2005). Furthermore, situations often arise such that it is precisely the finding of a neuroradiological picture that inappropriately raises a diagnostic doubt. For example, in the case of patients performing brain MRI for non-demyelinating neurological symptoms (e.g., headache) in which a RIS picture is found, or, sometimes, patients in which aspecific WML are erroneously interpreted as demyelinating lesions. Although the criteria specify that only WML that touch the cortex and the ventricles, respectively, must be considered as juxtcortical and periventricular, this specification is not always taken into consideration in the evaluation of the lesions of the WM. A further issue in the interpretation of neuroradiological findings is due to the fact that periventricular lesions are found with a decidedly high frequency (> 60%) in patients aged > 60 years and subjects with small vessel disease (SVD). Further neuroradiological (and clinical) characteristics of MS-mimics will be described in more detail later.

Overall, it is therefore easy to understand how the absence of a pathognomonic marker, combined with the lack of an absolute specificity for MS of the neuroradiological criteria, cause a profound limitation of the McDonald criteria. On the other hand, as the authors themselves also point out in the latest 2017 version, the criteria "were not developed to differentiate MS from other conditions" (Thompson, Banwell, et al., 2018a), but rather to facilitate a diagnosis of MS and early treatment in patients with typical demyelinating syndromes and who are therefore at high risk of developing clinically defined disease. Conceptually it follows that these criteria, tending to favour sensitivity, can partially damage specificity with a consequent greater risk of erroneous diagnoses, especially if they are applied to atypical clinical pictures due to demographic, clinical and/or neuroradiological characteristics of the patient (Gelfand, 2014; Solomon & Corboy, 2017).

In this regard, in a recent study (Gobbin et al., 2019) the 2010 and 2017 versions of the diagnostic criteria were compared in terms of specificity, sensitivity, positive and negative predictive values (Table 2.1). The study found that the 2017 criteria,

Table 2.1: Sensitivity and specificity of 2010 and 2017 McDonald Criteria for clinical definite MS (*Gobbin et al, 2019*)

Diagnostic criteria	Fulfillment of criteria before CDMS conversion	**Final diagnosis at last follow-up visit at study center**			Predictive values
		CDMS (N)	**Not CDMS** (N)		
2010 McDonald criteria	Fulfilled (N)	21	16	37	**PPV=56.8%** (47.3%-65.7%)
	Not fulfilled (N)	5	13	18	**NPV=72.2%** (51.8%-86.3%)
	Totals	26	29	55	
		SENS=80.8% (60.7%-93.4%)	**SPEC=44.8%** (26.5%-64.3%)		
2017 McDonald criteria	Fulfilled (N)	**26**	**25**	51	**PPV=50.9%** (47.3%-54.6%)
	Not fulfilled (N)	**0**	**4**	4	**NPV=100%**
	Totals	26	29	55	
		SENS=100% (86.8 %-100 %)	**SPEC=13.8%** (3.9 %- 31.7 %)		

Sensitivity, specificity, PPV, and NPV were calculated with 95% confidence intervals (in parentheses) according to Clopper–Pearson and Mercaldo.
Abbreviations: CDMS = clinically definite multiple sclerosis; NPV = negative predictive value; PPV = positive predictive value; SENS = sensitivity; SPEC = specificity.

compared to the 2010 version, show a substantial increase in sensitivity for the diagnosis of SMCD in cases of an initial demyelinating event. Furthermore, the 2017 revision allows for a significant reduction in the diagnostic delay after the clinical onset of symptoms. These findings, in line with those of other similar studies, seem to be largely justified by the introduction of the positivity of OCB as an element of confirmation of DIT (Beesley et al., 2018; Mantero et al., 2018; McNicholas et al., 2018). However, the authors found a significant decrease in specificity in the assessment of a second attack after demyelinating onset in the 2017 criteria compared to the 2010 criteria.

While this was partially attributed to the inclusion of patients who had started DMTs early, it was hypothesized that this finding could be justified by the fact that the 2017 McDonald's criteria can collect a subset of MS patients with low activity of disease, and this could have relevant implications on the therapeutic approach in newly diagnosed cases. The authors concluded by emphasizing the value of the high sensitivity of the criteria but asking a question about the risk that an early diagnosis could expose patients belonging to a group with low risk of experiencing a second clinical event to the risks of DMTs.

Finally, in evaluating the reliability of the McDonald's criteria, it must always be taken into account that they have been tested for a Western adult population and are not applicable to children and must be used with caution in subjects of other ethnic groups (Solomon, Naismith, et al., 2019).

2.2 MAIN MS-MIMICS

As stated, many diseases can enter the differential diagnosis with MS, mostly those that show a relapsing course, but there is also a more limited number of conditions that can emulate a MS with a primarily progressive course. Generally, all these diseases are characterized by a concomitant MS-like neuroradiological picture, especially by the presence WMLs, which may resemble or, in some cases, be indistinguishable from that of MS. In clinical practice, the situations that give rise to differential diagnostic problems are usually limited, due to the rarity of some SM-mimics, or, in other cases, due to the possibility of correctly reaching the diagnosis for the presence of specific systemic or neurological clinical signs, thus as highly suggestive paraclinical tests. However, it is not possible to overlook the weight of some of these pathologies and how some of them significantly affect the MS misdiagnosis rate, due to inexperience and/or incorrect application of McDonald's diagnostic criteria by clinicians. Here are some of the main MS-mimics.

CEREBROVASCULAR DISEASES AND BRAIN AGING

WMLs are found in many diseases with an ischemic basis. The most frequent is small vessel disease (SVD), which actually encompasses a diverse spectrum of conditions.

Small vessels (< 500 μm) are difficult to study due to spatial resolution limitations, and for this reason a series of surrogate markers can be used in MRI: microbleeds, lacunae, enlargement of Virchow Robin's perivascular spaces, leukoaraiosis. Furthermore, although less frequent in the early stages, confluent and periventricular lesions can be found which can therefore mimic MS. SVD is a frequent condition in subjects with cardiovascular risk factors but can also be found in subjects aged >50 years in the absence of specific comorbidities.

Differences with MS: the lesions in SVD depend on ischemic damage to the arterioles, and therefore do not show contrastographic enhancement, do not involve the U-shaped fibers (which have a double arterial supply, from cortical and medullary arteries), do not cause lesions of the medulla, generally do not involve the corpus callosum, at the brainstem level they are centrally located and involve the cerebellum only in very advanced stages. Moreover, they are generally < 3mm in size and rounded in shape, they can be associated, as stated, with microbleeds and lacunae, in the case of amyloid angiopathy cortical hemosiderosis can also coexist (Charil et al., 2006; Geraldes et al., 2018b).

MIGRAINE

In several studies, migraine emerges as one of the most frequent causes of MS misdiagnosis. Especially in forms with aura it can be associated with the presence of WMLs, which can sometimes have periventricular localization and meet the neuroradiological criteria of MS. In addition, silent infarctions, especially involving the cerebellum and deep gray matter, may frequently occur.

Differences with MS: lesions are generally small (<3mm), with poor tendency to confluence, involving deep WM and not the typical sites of MS. In particular, spinal or cortical lesions are never found, they are generally more stable over time (Geraldes et al. 2018; Gelfand 2014).

NMOSD

NMOSD is one of the conditions that can show clinical overlap with the more evident RRMS, both in the forms linked to the presence of anti AQP4 and anti MOG antibodies, as well as in the double-seronegative variants. Over time, the concept that NMOSD was not associated with brain lesions was undermined, and it is now known that approximately 40% of patients with NMOSD will meet the Barkhoff criteria for

MS over time (Kim et al., 2015). WMLs in NMOSD can be pleomorphic (**figure 2.1**), they can be localized at the level of the corpus callosum, periventricular, brainstem or even be short extending transverse myelitis (mostly in the MOG+ forms).

As already mentioned, in some cases there is positivity to CSF OCBs (especially in the MOG + forms).

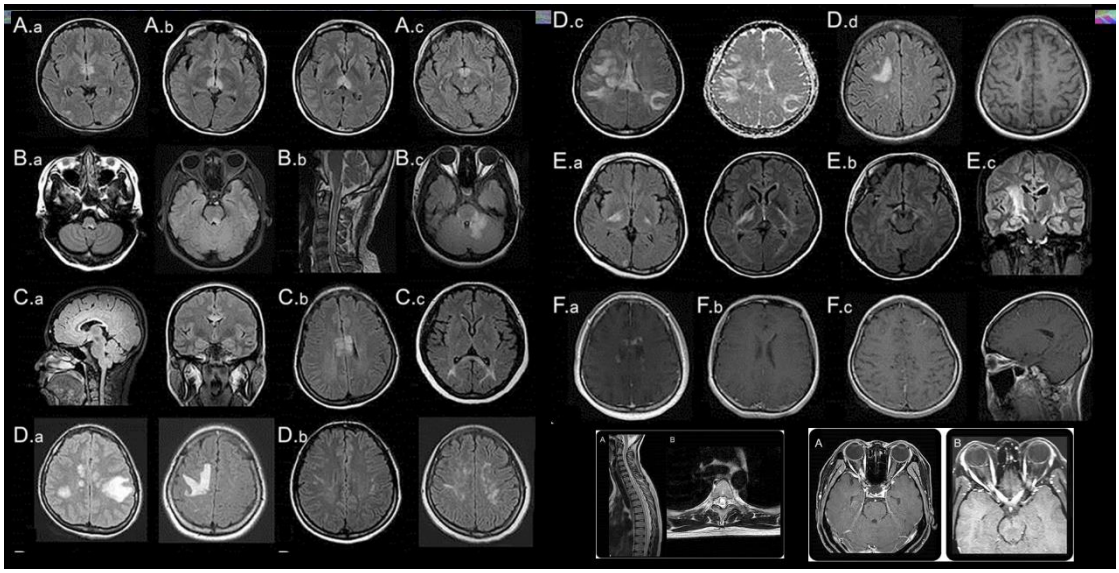


Figure 2.1 MRI in NMOSD Diencephalic lesions surrounding (A.a) the third ventricle and cerebral aqueduct (A.b) and including the thalamus, hypothalamus, and (A.c) anterior border of the midbrain (B.a) Dorsal brainstem lesion adjacent to the fourth ventricle (B.b) bulbar lesion linear contiguous with a cervical cord lesion (B.c) extensive edematous dorsal brainstem lesion involving the cerebellar peduncle. (C.a) Callosal lesion immediately adjacent to the lateral ventricle, following the ependymal border (C.b) Callosal lesion with marbled pattern (C.c) Callosal lesion with arch bridge pattern (D.a) Tumefactive hemispherical WML (D.b) Lance-shaped lesions or radial following WM tracts (D.c) Extensive and confluent hemispherical lesions with increased diffusivity in DWI maps suggesting the presence of vasogenic edema (D.d) Hemispherical lesions showing pseudocystic cavity changes in the chronic phase (E.a) Corticospinal tract lesions involving the posterior arm of the internal capsule and (E.b) the cerebral peduncle (E.c) longitudinal lesion of the pyramidal tract (F.a) Cloud-like enhancement, (F.b) linear enhancement of the ependymal surface of the lateral ventricles (F.c) meningeal enhancement Adapted from Ho Jin Kim , Neurology 2015

Differences with MS: The most classic cases of NMOSD are easily classifiable due to their typical prominent involvement of the optic nerve, with retrobulbar optic neuritis (RBON) of a more severe entity, and with less recovery (mostly in the forms linked to anti-AQP4), often bilateral and synchronous, due to the presence of longitudinally extended transverse myelitis (LETM, classically > 3 medullary segments). Furthermore, the AQP4+ forms show a characteristic lesional distribution, linked to the involvement of the aquaporin-rich areas and therefore the peripendymal areas around the lateral ventricles, the 3rd and 4th ventricles including diencephalic structures and typically the area postrema with consequent characteristic symptoms (nausea, vomiting, uncontrollable hiccups, etc.).

Furthermore, a characteristic involvement of the conus medullaris is typical of anti-MOG related forms. In NMOSD there is no cortical, juxtacortical involvement, there are no Dawson fingers (Jarius et al., 2016). A summary comparison between MS, NMOSD AQP4+ and NMOSD MOG+ is shown in table 2.2, Nowadays, the advent and diffusion of sensitive laboratory methods such as cell-based assay kits allow the diagnostic doubt to be resolved quite easily in these cases, thanks to research of autoantibody positivity, thus leaving room for doubt only for the potentially double serum negative forms of NMOSD.

Table 2.2 Main differences between MS, anti AQP4 + and anti MOG+ NMOSD

	SM	**NMOSD anti AQP +**	**NMOSD anti MOG+**
Demographic factors	Caucasian, F:M=7:1, onset 20-40 ys	Asian, F:M=9:1, onset 30-50 ys	Caucasian, F:M=2:1 onset 13-40 ys
Previous infection/vaccination	Not relevant	Not relevant	Frequent
CSF	< 50 cells, lymphocytes, OBC+> 85-90%	>50 cells, granulocytes, OCB + < 20%	generally OCB -
Bilat. optic neuritis	Rare	+	+++
Brain	Several WMLs, Periventricular, juxtacortical, dorsal and pial surface of brainstem structures, lateral pontine lesions, trigeminal involvement, rarely in diencephalon	less frequent, rare at onset, few lesions, area postrema syndrome and symptomatic narcolepsy. Periependymal periventricular lesions, Arch bridge pattern, around 4th ventricle, dorsal surface of brainstem. ++ involvement of diencephalic structures, rare cortical lesions.	Supratentorial (30-45%), infratentorial 15-30% Possible ADEM-like presentations
		Sometimes voluminous, tumefactive and blurred edges lesions	
Spinal cord	Usually <3 segments, less than 1/2 spinal diameter, ++ cervical; Eccentric (++ posterolateral) lesions, homogeneous/nodular enhancement. Over time they become less noticeable	++ LETM (>3 segments), Entire spinal diameter, central GM involvement. Variable Enhancement, sometimes patchy. Very frequent edema. Malacic/syringomyelic evolution	mainly<3seg but LETM is possible. Conus medullaris frequently involved. Central medullary lesions. Frequent enhancement with variable pattern
Early sphincteric/sexual disfunction	Rare	+	+++
Antibodies	No	AQP4	MOG
Recovery	Full/variable	Generally incomplete	Generally full
Course	RR, P	Generally relapsing	50% monophasic

Abbreviations: OCB: oligoclonal bands, ADEM acute disseminated encephalomyelitis, LETM longitudinally extended transverse myelitis, AQP4 aquaporin 4, MOG myelinic oligodendrocytic glycoprotein, RR relapsing remitting, P progressive

ADEM (ACUTE DISSEMINATED ENCEPHALOMYELITIS)

ADEM traditionally constitutes a monophasic and paediatric disease. However, cases with onset in adulthood and exceptionally with a relapsing course are possible. The presence of demyelinating lesions can in some cases therefore pose a problem of differential diagnosis with MS(Dale & Branson, 2005). **Table 2.3** below shows the main differences between the two pathologies.

Table 2.3 Main differences between ADEM and MS

	ADEM	MS
Age and sex	Often <10 ys. No sex diff.	> 10 ys F >> M
Previous infections/vaccinations	Really frequent	Variable
Encephalopathy	Constant	Rare
Attacks	Fluctuations in 3 months, ++ monophasic	Separate by at least 1 month, relapsing
WMLs	Large, often symmetrical and confluent, synchronous, sometimes tumefactive, undefined, cottony, Gd+ Tends to resolve within 6 months	Ovoidal, 3-6 mm thick, confluent over time, sharp margins, asymmetrical, black holes
Deep WM involvement	Frequent (++ parieto-occipital areas). Relative periventricular sparing	Rare
Deep GM involvement	Yes, basal nuclei involvement	No/very rare
Spinal cord	Often LETM, mainly thoracic segments involvement, often edema	<3 segments
CSF Leukocytes	Often>50 cells/mm^3	<50 cells/mm^3
OCB, pattern II	Variable, sometimes pattern IV	Frequent (90%)
Antibodies	Possible anti-MOG positivity	no
Gd	Often coexistence of Gd+ and Gd- WMLs	Variable

Abbreviations: MS multiple sclerosis, **ADEM** acute disseminated encephalomyelitis, **Gd** gadolinium, **WM** white matter, **GM** grey matter, **LETM** longitudinally extended transverse myelitis

SUSAC SYNDROME

This retinocochlear vasculitis is an immune-mediated endotheliopathy that affects precapillary arterioles present above all at the retinal, cochlear and callosal level, causing microinfarctions visible on MRI as small hyperintense lesions on T2. The result is a characteristic clinical triad consisting of sensorineural deafness, blindness/visual impairment (due to retinal branch occlusion) and encephalopathy. However, there is often an onset with focal neurological symptoms that can mimic MS and, added to the finding of T2 hyperintense lesions, pose problems of differential diagnosis with MS.

Differences with MS: in addition to the aforementioned pathognomonic clinical triad, at a neuroradiological level snowball lesions of the corpus callosum (with central involvement and spared periphery) are characteristic, which appear hypointense in T1 in the chronic phase. Hemispheric lesions are much less frequent. Furthermore, in the acute phase, perilesional and/or leptomeningeal contrast enhancement is possible in some cases, furthermore restriction in DWI of the lesions is associated (supporting the ischemic genesis)(Chen et al., 2016; Siva, 2018a; Susac et al., 2003) (figure 2.2).

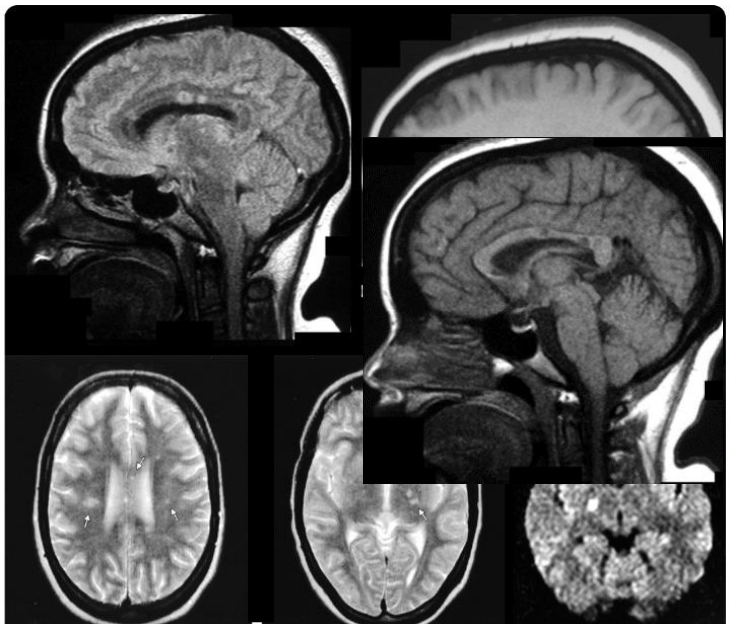


Figure 2.2: MRI in Susac Syndrome

Above: Snow ball lesions of corpus callosum, hyperintense in T2 and hypointense in T1 (in chronic stages) sequences.

Below, on the left: MS like WML. On the center: deep grey matter lesions. On the right: DWI restriction in one snow ball lesion

(Susac JO, Neurology 2003)

CADASIL (CEREBRAL AUTOSOMAL DOMINANT ARTERIOPATHY WITH SUBCORTICAL INFARCTS AND LEUKOENCEPHALOPATHY)

CADASIL is a rare systemic vasculopathy of monogenic genesis (in > 95% of patients NOTCH3 mutation) determining a cerebral microangiopathy with predominant involvement of the long perforating and leptomeningeal arteries of the brain. Although CADASIL has a rather characteristic sequence of symptoms, in some cases (especially during the early or preclinical phases of the disease), neuroradiological findings (T2 hyperintense brain WMLs) can enter the differential diagnosis with MS, also considering the relatively young age in at which this pathology tends to begin.

Differences with MS: symptoms onset can be ictal, since it is linked to the presence of transient ischemic attacks or real stroke; moreover, the disease is typically accompanied by a positive family history, migraine, psychiatric disorders, and cognitive deficits. At the neuroradiological level, WMLs distribution follows a vascular pattern (deep subcortical lesions with a preference for the anterior temporal pole, external capsule,

frontal lobe) and is mostly symmetrical, with sparing of the corpus callosum and infratentorial regions. Deep grey matter involvement, lacunae, and widening of perivascular spaces may also coexist (O'Riordan et al., 2002; Phillips et al., 2010).

PRIMARY CENTRAL NERVOUS SYSTEM VASCULITIS (PACNS)

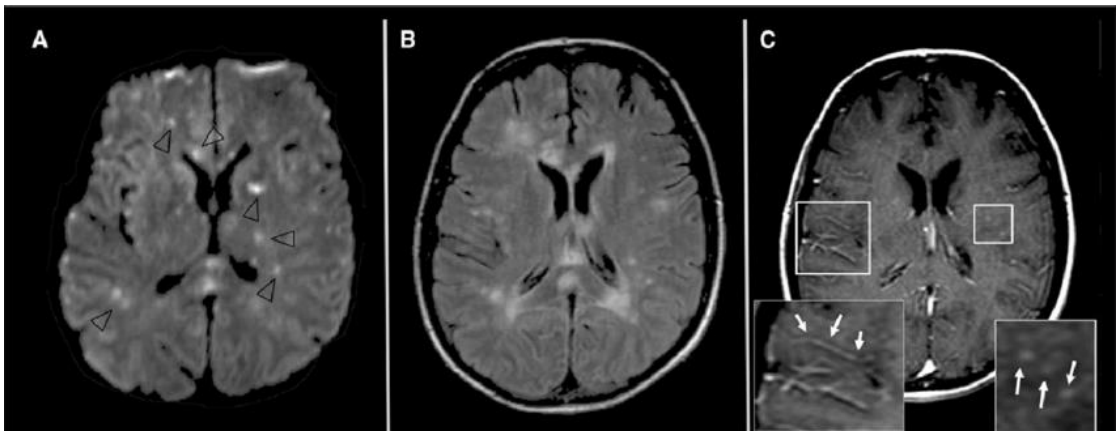


Figure 2.3 Some MRI features of primary angitis of CNS (PACNS): A. Several punctiform DWI hyperintense areas signaling ischemic damage (cytotoxic edema) **B.** MS-*like* pattern of WML in a patient with PACNS **C.** Leptomeningeal Enhancement (*Boulois et al, Stroke 2017)*

This condition, defined as a vasculitis with exclusive involvement of the cerebro-afferent vessels and not associated with other systemic manifestations, can manifest itself with a very variable number of signs and symptoms, considering the casual involvement of different brain structures and vessels of different calibre and site (Salvarani et al., 2016). In certain situations, i.e., where the involvement of small-calibre vessels afferent to WM predominates, PACNS can cause differential diagnostic problems with MS, also considering the possible relapsing course and the difficulty of analysing the morphological characteristics of vessels with a diameter < 500 µm with angiographic or CT angiography/ MRI angiography methods.

Differences with MS: PACNS is an ischemic pathology and for this reason lesions show an arteriolar vascular distribution. In MRI, WMLs often show acute signal restriction in DWI sequences; moreover, when PACNS is determined by alteration of small calibre vessel is associated with acute/hyperacute onset clinical presentation, with frequent headache, cognitive deficits, epilepsy and encephalopathy, partially linked to the frequent concomitant leptomeningeal inflammation (visible on neuroimaging with contrastographic enhancement) (**figure 2.3**). Moreover, evident cortical lesions, deep gray matter involvement, microbleeds often coexist in PACNS; the contrastographic pattern of WMLs is typically radial or linear, while in MS it is typically ring-shaped.

Spinal cord involvement in PACNS is possible but has only been described anecdotally in case reports or case series (Chen et al., 2016).

NEUROSARCOIDOSIS

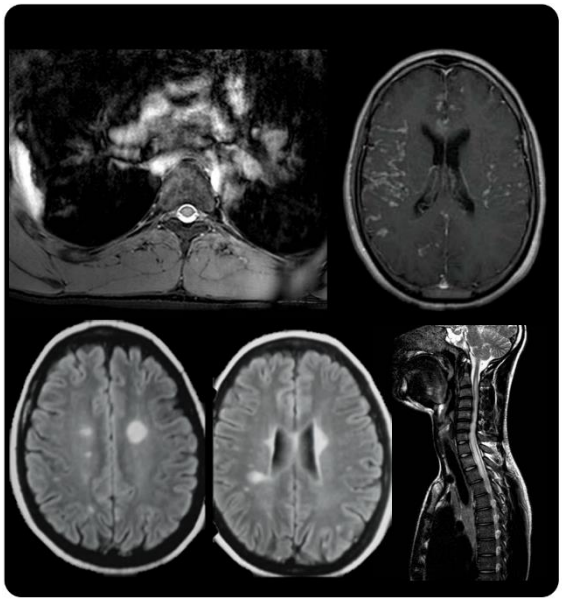


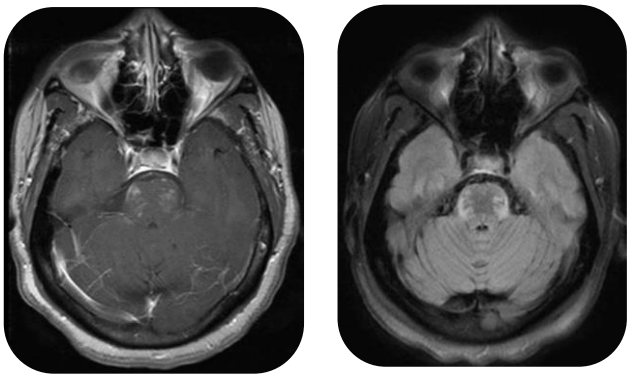
Figure 2.4 MRI lesion patterns of neurosarcoidosis. Above, a dorsal mid-medullary lesion is observed on the left, evident leptomeningeal enhancement on the right. Bottom left SM-like pattern of lesions, right a characteristic LETM (longitudinally extended transverse myelitis) (Dutra et al, 2012).

This non-caseous granulomatosis can involve the CNS in a clinically manifest way in about 10% of cases and less frequently (about 2% of the total) it presents as isolated neurosarcoidosis. It frequently involves cranial nerves (in order of frequency VII, II and not infrequently presentation with multineuropathies). Concerning MS differential diagnosis, it must be considered that in neurosarcoidosis retrobulbar ON occurs in approximately 1/3 of patients, that CSF OCB is appreciated in more than 50% of patients, and that in 10% of cases spinal cord involvement is present (Krumholz & Stern, 2014).

Differences with MS: is often observed an involvement of multiple cranial nerves, which is infrequent for MS; in the same way the retro-bulbar ON is not infrequently bilateral and severe. Furthermore, atypical localizations for MS may coexist such as pituitary-hypothalamic lesions, leptomeningitis and/or pachymeningitis especially in posterior cranial fossa, hydrocephalus. On the other hand, hemispheric and brainstem involvement is less present. Myelitis is often presents as a longitudinally extended form, with a predilection of the dorsal segments, and sometimes there is a subpial linear contrast enhancement that persists for months (**figure 2.4**). Radiculopathy may also be present. CSF may document severe protein elevation and higher white blood cell counts than in MS. In sarcoidosis, uveitis is also frequently found, and at the laboratory level increased levels of angiotensin converting enzyme - ACE (which, however, can also be present in a non-specific way in MS) and

chitotriosidase (which is instead a more specific marker of sarcoidosis) (MacLean & Abdoli, 2015).

CLIPPERS (CHRONIC LYMPHOCYTIC INFLAMMATION WITH PONTINE PERIVASCULAR ENHANCEMENT RESPONSIVE TO STEROIDS)



Dudesek et al, Journal of transl immunol 2014

Table 3. Core features of CLIPPERS (chronic lymphocytic inflammation with pontine perivascular enhancement responsive to steroids) (adapted from Simon *et al.* [7]).

I. Clinical

- Subacute progressive ataxia and diplopia
- A range of other clinical features referable to brainstem pathology plus cognitive and spinal features occur in some patients

II. Radiological

- Numerous punctate or nodular enhancing lesions bilaterally within at least two of the three following anatomical locations: pons, brachium pontis, cerebellum
- Individual radiological lesions are small but may coalesce to form larger lesions (mass effect may suggest an alternative diagnosis)
- Lesions may occur in the spinal cord, basal ganglia or cerebral white matter but should be decreasing density with increasing distance from the pons/hindbrain
- Absence of the following radiological features:
  - Restricted diffusion on diffusion weighted imaging
  - Marked hyperintensity on T2-weighted images
  - Abnormal cerebral angiography

Figure 2.5 MRI in CLIPPERS: The characteristic punctiform pontine salt-and-pepper enhancement is noted on the contrast-enhanced T1 sequences and the associated appreciable lesions on the T1 sequence.
Down are reported the **diagnostic criteria** (Tobin et al, 2017)

CLIPPERS, a clinical entity first described by Pittock et al in 2010 (Dudesek et al., 2014), is an inflammatory disease of the CNS with immune-mediated genesis and unknown etiology. It is characterized by the detection of a long T cell infiltrate the perivascular spaces of WM, with pathological features suggestive of vasculitis. MS is the most important differential diagnosis. There are T2 hyperintense lesions and frequently CSF restricted OCB. Specific diagnostic criteria have been elaborated (Tobin et al., 2017) (see figure 2.5).

Differences with MS: neuroimaging in most cases can be decisive in the diagnosis of CLIPPERS. The most characteristic element is the presence of multiple punctiform salt-and-pepper pontine lesions with curvilinear contrast enhancement reflecting the ongoing inflammation (Ferreira et al., 2013).

NEUROBEHÇET

CNS involvement in Behçet's disease can manifest itself in two main patterns:

- on the one hand a vascular inflammatory form,
- on the other a picture in which thrombosis of the cerebral venous sinuses and hydrocephalus predominate (Siva & Saip, 2014).

The forms that enter differential diagnosis with MS fall into the first pattern. In particular, some Behcet's disease patients show MS-like WMLs on MRI, especially if the site of the lesions is perivenular (typical of MS). Perivenular lesions are a relatively frequent finding in thromboembolic forms of Behçet, as the disease typically involves both the venular and arterial side of vessels. The differential diagnosis can be difficult if the neurological presentation precedes the systemic one, and in case of absence of the more typical neuroradiological findings of neuroBehçet. In addition, a presentation with spinal cord syndrome is rare but possible.

Differences with MS: in most cases neuroBehçet presents with typical neuroradiological elements (**figure 2.6**). Among these, a prominent involvement of the brainstem stands out, with voluminous, often edematous and confluent lesions with frequent involvement of the basal nuclei. At follow-up, these lesions may show considerable reduction up to complete regression. However, a characteristic focal brainstem atrophy may remain, in the absence of cortical atrophy. Any spinal cord involvement differs from that present in MS due to the more frequent LETM pattern and the presence of a recently described marker, the bagel sign(Uygunoglu et al., 2017), i.e., the visualization in axial MRI sequences of a roundish lesion with hypointense core and hyperintense contour on T2, with or without contrast enhancement on T1 (Siva 2018).

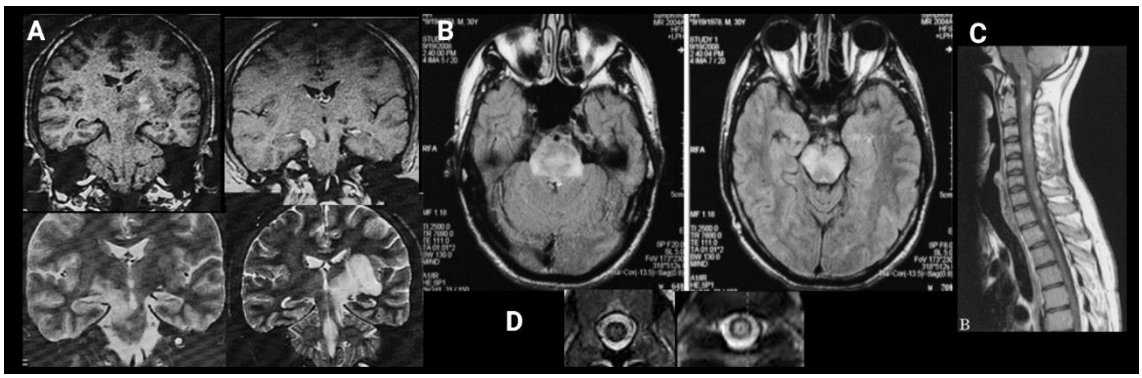


Figure 2.6: MRI in NeuroBehçet: A. brainstem lesions with variable extension, down are visible voluminous lesions, with mass effect and basal nuclei involvement **B.** bilateral extensive involvement of pons and midbrain **C.** Several short extended cervicodorsal spinal lesions **D.** Spinal cord Bagel sign (Siva, 2018)

NEUROSLE AND ANTIPHOSPHOLIPID SYNDROME

In the context of neuropsychiatric systemic erythematous lupus (NPSLE) the finding of WMLs is extremely frequent (50-75% of patients), but in most cases they are non-specific and asymptomatic. The main cause of a MS-like clinical picture is instead determined by the presence of an antiphospholipid antibody syndrome (in recent years the concept of lupus demyelinating syndrome has instead been progressively overcome). Clinically, it can present with episodes of focal neurological deficit with a relapsing course, determined by transient ischemic attack or stroke and which can therefore account for a dissemination in time and space of the lesions.

Furthermore, in some cases, CSF findings can be superimposable to those of MS with possible CSF restricted OCB, mild leucocytosis, and mild elevation in CSF proteins. Although very rare, a further presentation of NPSLE is made up of spinal cord syndrome (Govoni et al., 2016).

Differences with MS: generally, WMLs have a deep subcortical distribution, sparing the juxtacortical regions and, moreover, due to their intrinsic ischemic nature, they can involve the deep gray matter. Sometimes WMLs show punctiform enhancement and calcifications. Lupus myelitis most frequently presents as LETM, is often associated with regular leptomeningeal enhancement, from a clinical point of view it shows an acute onset with severe neurological deficit subtended in most cases by an ischemic etiopathogenesis. Furthermore, as far as CSF findings are concerned, in the NPSLE the detection of a small number of OCBs (<4) is very characteristic (Bortoluzzi et al., 2018). In NPSLE, a fundamental support in the differential diagnosis is given by the autoantibody findings which are present in almost all cases in active phases of the disease, with reference not only to any antiphospholipid antibodies but obviously to the presence of high titre of antinucleus antibodies (ANA) with possible specificities such as the presence of anti-Ribosomal P (a good marker of NPSLE) (Briani et al., 2009).

INFECTIOUS MS-MIMICS

Lyme Diseases with CNS involvement (Neuroborreliosis)

Neuroborreliosis is typically described by the triad of meningitis, cranial nerve impairment (particularly bilateral facial nerve palsy) and radiculoneuritis. It can manifest itself in variable ways, and sometimes with T2 hyperintense brain WMLs (particularly in frontal lobes). In addition, unilateral facial palsy or, very rarely, myelitis may present onset.

Differences with MS: in addition to the possible presence of the other components of the neurological triad (and systemic symptoms), differential elements with respect to MS are the more frequent: cranial nerves multineuropathy and the more often dorsal medullary site or the involvement of the cauda (Siva 2018b; Gelfand 2014b).

Other infectious diseases: they only exceptionally enter the differential diagnosis with MS; among these it is possible to include, for example, PML (progressive multifocal leukoencephalopathy), toxoplasmosis, HIV and HTLV-1 infections (the latter sometimes mimicking progressive MS and more frequently found in populations living in the tropics).

LEUKODYSTROPHIES AND OTHER HEREDO-METABOLIC DISEASES

Although in most cases diseases with hereditary or metabolic etiology are clinically manifest from childhood, and often have a systemic clinical correlate, some differential diagnosis problems can arise with MS if there is an onset in adulthood, and mostly in case of incidental MRI WMLs finding (Renaud, 2016).

Differences with MS: generally, WMLs pattern in these conditions differs from that of MS for symmetry, for the presence of a much more widespread involvement of WM (without a preference for the typical areas of demyelination), for the saving of U-shaped fibers (with some exceptions such as Kearn Sayre syndrome), due to the absence of contrast enhancement (except for Alexander disease). There may also be characteristic multisystem involvement (e.g., mitochondrial diseases, Fabry disease, etc.), neuroradiological characteristics peculiar to each specific condition and moreover there is often a positive family history (Luo et al. 2015; Siva 2018b).

A separate mention deserves **LOHN** (Leber's hereditary optic neuropathy), a mitochondrial pathology that enters the differential diagnosis with MS in its earliest stages because it can mimic a picture of RBON, with centro-cecal scotoma, dyschromatopsia, in some cases optic nerve contrastographic enhancement, and sometimes recovery phases (mostly transient). Moreover, it can be associated with the presence of brain demyelinating lesions. Involvement of the contralateral eye at the same time or over a period of weeks (average 8 weeks) as well as the severity of the disorder, the absence of pain on eye movements and little/no response to steroids distinguish LHON from MS. In some cases, systemic symptoms typical of mitochondrial diseases (LOHN plus) may also be associated (Finsterer, 2006; Matthews et al., 2015).

OTHER CONDITIONS

Among the other conditions that can rarely present as MS-mimics, it is possible to mention deficiency conditions, of which the best known is vitamin B12 deficiency, and other systemic dysimmune conditions, including Sjögren's disease (which can cause both WMLs and very rarely LETM), celiac disease (with frequent WMLs but coexistence of calcifications and frequent cerebellar atrophy); while a MS-like phenotype is decidedly more rare in the case of ANCA-related granulomatosis or in inflammatory bowel disease.

2.3 THE ISSUE OF MISDIAGNOSIS IN MS

For the reasons just listed, the frequency of MS misdiagnosis is surprisingly high, even today. Despite the methodological inhomogeneity and the variable definition of "misdiagnosis" in the various studies (e.g. patients with suspected diagnosis of MS versus patients with already confirmed diagnosis of MS), the frequency of erroneous diagnoses would still seem to be estimated in a range between 5 and 20% of all MS cases, particularly after the application of more recent versions of McDonald's criteria which allowed earlier diagnosis to first attack (Solomon, Naismith, et al., 2019). A recent study found that among new cases referred to MS centers for a diagnostic second opinion, MS was eventually ruled out in 30-67% of cases, despite several patients having already received a prior formal diagnosis of MS and some they had undergone treatment with DMTs (Kaisey et al., 2019a; B. I. Yamout et al., 2017). A recent American study (Solomon, Bourdette, et al., 2016) focused on the analysis of the characteristics of a cohort of 110 patients in whom MS diagnosis was rescinded after re-evaluation by expert MS neurologists from 4 major US universities. Globally, it emerged that:

- misdiagnosis also occurs in tertiary academic centers specialized in MS,
- the duration of misdiagnosis is high (on average at least 3 years) and that,
- in the series under consideration, 70% of patients were subjected to a DMTs in the absence of effective clinical justification of the risk/benefit ratio associated with these drugs.

Solomon et al have also shown that, surprisingly, in most cases the pathologies mistaken for MS were non-specific and mild conditions such as migraine, fibromyalgia, psychiatric disorders and non-specific WML on MRI. Moreover, the role of non-specific

WML at brain MRI had often had a determining weight in the diagnostic process, and in most cases the misdiagnosis was attributable to an erroneous application of the McDonald 2010 criteria (**figure 2.7**)

McDonald's 2017 criteria, regarding the presence of past neurological symptoms, postulate that it is possible to consider them for the purpose of establishing DIT also on the basis of the medical history, only if they are highly suggestive of a demyelinating event. This position is partly controversial since it opens up the possibility of erroneous interpretations of non-objectifiable events, and therefore is a potential tool for misdiagnosis. As suggested by some authors (Solomon, Naismith, et al., 2019) the need for objective feedback from the neurological examination or the support offered by paraclinical tools (MRI, PEV, OCT, etc.) consistent with the involvement of the anamnestically reported site could guarantee greater protection from misdiagnosis in the evaluation of the aforementioned past events.

In essence, the main limitation of the McDonald's diagnostic criteria is their clinical and to some extent subjective nature. Indeed, an accurate diagnosis relies on clinical judgment (e.g., was the reported episode a relapse?), experience (e.g., is a neurological sign really abnormal?), test interpretation (e.g., T2 hyperintensities suggest MS?), and on the reasoning and effort required to identify an alternative diagnosis (e.g., is genetic investigation needed for autosomal dominant cerebral arterial disease with subcortical infarcts and leukoencephalopathy- CADASIL?). Given the subjective nature of the judgments involved and the numerous conditions that can be confused with MS, it is not surprising that the diagnostic process is difficult in many cases (Deisenhammer et al., 2013).

The consequences of a misdiagnosis of MS are extremely relevant and can be simplified to three consequences:

1. Inability to guarantee the patients a specific treatment for their pathology with possible exacerbation of the underlying pathology and accumulation of neurological disability.

2. Treatment of the patient with MS-specific DMTs which, in addition to not being effective in some cases in pathologies other than MS, are burdened by an absolutely negatively unbalanced risk-benefit profile (just think of the risk of PML in a patient treated with natalizumab or the reduction in the quality of life associated with the side effects of some drugs, for example the interferon parainfluenza syndrome or working hours lost due to the administration of infusion drugs);

3. Economic impact: given the high cost and/or the need to administer many specific MS drugs in a hospital environment, a treatment based on an incorrect diagnosis places an unjustified burden on the NHS from an economic point of view.

2.4 RED FLAGS

The presence of a large number of MS mimics, associated with the limitations of the McDonald criteria (suboptimal specificity on the one hand, and the frequent erroneous application by clinicians on the other) has imposed the need over time to try to cope with the risk concrete of misdiagnosis. For this reason, the experts have tried to define as extensive a set as possible of atypical elements for a diagnosis of MS (red flags of better explanation), which could serve as a guide for the clinician at the moment of the differential diagnostic process, as well as a series of recommendations to guide clinicians in a diagnostic process free from methodological errors that could invalidate the correct diagnosis or exclusion of diagnoses in MS.

MAGNIMS in 2006 had published a first panel of purely radiological red flags and also the association with possible underlying alternative pathologies (**figure 2.8**) (Charil et al., 2006). Subsequently, in 2008, an international consensus on the differential diagnosis of MS led by Miller had drawn up an in-depth list of red flags classified according to a priority criterion (major, intermediate and minor) and which ranged from the clinical to the laboratory to that of MRI. Algorithms were also proposed to guide the differential diagnosis with suboptimal specificity in optic neuritis, myelitis, and brainstem syndrome (Miller et al., 2008a).

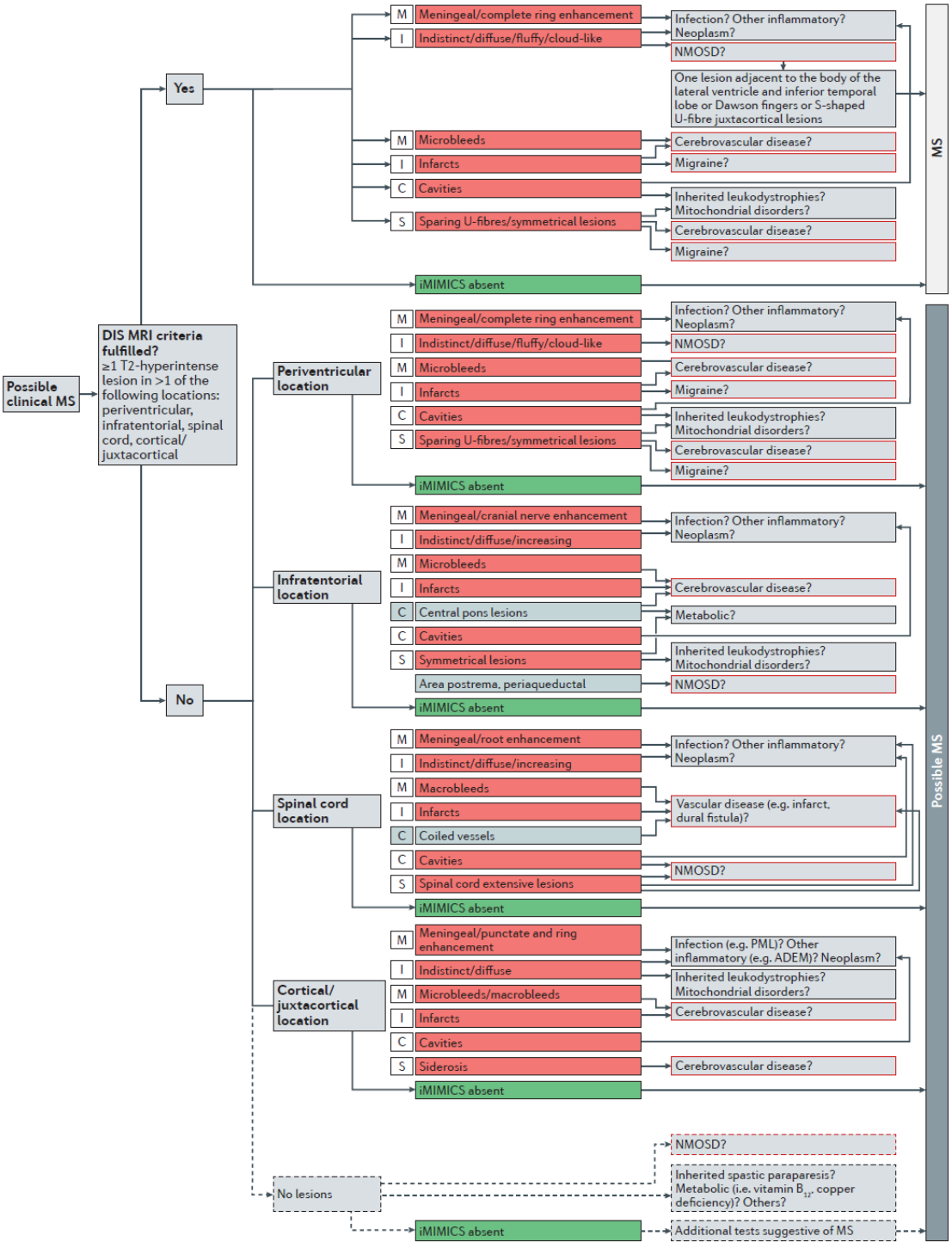
The most recent consensus on MS-mimics has been elaborated again by MAGNIMS, therefore focused on the role of MRI in the differential diagnosis of MS updated to 2018, and with two further focuses, the first on the role of new MRI techniques in MS differential diagnosis (including the central vein sign, discussed later, and the sequences to detect cortical lesions) and the second on the progress made in the identification of neuroimaging hallmarks capable of differentiating MS from its mimics. They developed an acronym, iMIMICs, which constitutes a mnemonic aid for the main MRI red flags (**Table 2.4**) to be considered in the differential diagnostic process. The authors have also developed a useful algorithm that showed how the application of the iMIMICs scheme is useful in situations where the DIS is respected, but also in those where it is not. In the latter case, they reported the scheme with some small site-specific modifications

depending on the site of lesion location among the 4 typical sites of MS (cortical/juxtacortical, periventricular, infratentorial, and spinal). In the absence of all the elements of iMIMICs the diagnosis of MS is possible while in case of detection of one or more red flags it is necessary to hypothesize alternative causes, which are suggested by the authors themselves in the algorithm (figure 2.9) (Geraldes et al., 2018a). The efforts of the international community to develop strategies for the detection of red flags in order to reduce misdiagnosis are of primary importance, however the most important unmet clinical need in the diagnostic field of MS remains the lack of a specific biomarker of the disease.

Table 2.4 Red flags features in mnemonic scheme iMIMICs.

Letter	Meaning	Minimum essential MRI sequences
M	• Meningeal enhancement	2D axial or 3D contrast-enhanced T1 weighted
I	• Indistinct lesions • Increasing lesions	Sagittal 2D or 3D T2-weighted FLAIR
M	• Macrobleeds • Microbleeds	2D axial T2*-weighted gradient echo
I	• Infarcts	2D axial, 3D T1 weighted and DWI
C	• Cavities • Complete ring enhancement	2D axial or 3D contrast-enhanced T1 weighted
S	• Symmetrical lesions • Sparing of U-fibres	2D axial or coronal or 3D FLAIR
	• Siderosis	2D axial T2*-weighted gradient echo or FLAIR
	• Spinal cord extensive lesions	Sagittal dual echo (proton-density and T2-weighted) and/or fast spin echo, contrast-enhanced T1-weighted spin echo and axial 2D and/or 3D T2 and contrast-enhanced T1 weighted fast spin echo

Figure 2.9: iMIMICS algorithm to evaluate the presence of MRI red flags in case of a possible picture of MS. In the first place it must be assessed whether the criterion of spatial dissemination (DIS) is respected, if so, only the first path of the flow chart can be considered. In case the DIS is not respected, the authors have developed algorithms for evaluating the specific site red flags, which can be observed in this figure (Geraldes et al, Nat Rev Neur. 2019)


Possible clinical MS
DIS MRI criteria fulfilled?
≥1 T2-hyperintense lesion in >1 of the following locations: periventricular, infratentorial, spinal cord, cortical/juxtacortical
Yes
No
Periventricular location
Infratentorial location
Spinal cord location
Cortical/juxtacortical location
No lesions
M Meningeal/complete ring enhancement
I Indistinct/diffuse/fluffy/cloud-like
M Microbleeds
I Infarcts
C Cavities
S Sparing U-fibres/symmetrical lesions
iMIMICS absent
Infection? Other inflammatory? Neoplasm?
NMOSD?
One lesion adjacent to the body of the lateral ventricle and inferior temporal lobe or Dawson fingers or S-shaped U-fibre juxtacortical lesions
Cerebrovascular disease?
Migraine?
Inherited leukodystrophies? Mitochondrial disorders?
MS
M Meningeal/cranial nerve enhancement
I Indistinct/diffuse/increasing
C Central pons lesions
Metabolic?
Area postrema, periaqueductal
M Meningeal/root enhancement
M Macrobleeds
Vascular disease (e.g. infarct, dural fistula)?
C Coiled vessels
Spinal cord extensive lesions
M Meningeal/punctate and ring enhancement
Infection (e.g. PML)? Other inflammatory (e.g. ADEM)? Neoplasm?
Siderosis
Inherited spastic paraparesis? Metabolic (i.e. vitamin B12, copper deficiency)? Others?
Additional tests suggestive of MS
Possible MS

CHAPTER 3

THE CENTRAL VEIN SIGN

The Central Vein Sign

The premises relating to misdiagnosis issue in MS, and therefore to the lack of a pathognomonic marker of the disease allow us to introduce this chapter.

The advancement of MRI analysis techniques in recent years has allowed the identification of new diagnostic markers associated with MS pathology. Among these, the central vein sign (CVS) is particularly promising, that is a brain MRI marker based on an anatomical-pathological assumption characteristic of MS, i.e., the perivenularity of classic white matter plaques. In fact, the post-mortem anatomopathological studies of Dawson date back to the early 1900s in which was documented the presence of a small vein sited approximately at the center of MS WML. Since then, a multitude of further studies have confirmed that veins and venules surrounded by a perivascular sheath of mononuclear cells were consistently recognizable centrally within MS lesions. The perivenular perivascular space is in fact a locus of extreme interest from a pathogenetic point of view for the disease, since the key immune responses responsible for initiating the inflammatory cascade at the base of MS are expressed at that level. It is here that the interaction, mediated by resident antigen-presenting cells (APCs), occurs between lymphocytes and various white matter antigens (Adams, 1975; Barnett & Prineas, 2004).

3.1 TECHNICAL BASIS OF CVS ANALYSIS

The CVS is based on this anatomopathological assumption and allows the observation of the perivenularity of MS lesions to be translated *in vivo*. WML are highlighted through T2FLAIR sequences (fluid attenuated inversion recovery), the venular structure can instead be visualized by exploiting the paramagnetic properties of venous deoxygenated haemoglobin using T2* sequences or gradient echo sequences.

The technique was initially devised by Tallantyre and colleagues in 2008 in a pilot study with 8 subjects affected by MS, in which the percentage of perivenular lesions (%PVL) out of the total was 83% (Mistry et al., 2012). In 2011 they then tested, on a 7T device, the method on two groups of patients with WMLs, respectively with and without MS. They had demonstrated that in the MS group the %PVL was 80% while in the non-MS group it was 19%, establishing that a threshold of 40% was associated with sufficient accuracy to MS (Tallantyre et al., 2011). Since then, a series of studies have compared the %PVL between MS and various distinct pathologies, also documenting how the method can also be performed on 3 and 1.5T devices.

- **MS vs NMOSD**: the %PVL was 80% and 32% respectively, the threshold able to distinguish MS was a %PVL > 54% (Cortese et al., 2018)
- **MS vs migraine**: %PVL was 84% and 22% respectively, the threshold identified to distinguish MS was a %PVL > 40% (Solomon, Schindler, et al., 2016)
- **MS vs SVD**: %PVL was 72% and 8% respectively, the indicative threshold of MS identified with %PVL>45% (Mistry et al., 2016)
- **MS vs systemic inflammatory vascular disease**: the %PVL was 88% and 14% respectively, the threshold identified to distinguish MS was a %PVL >50% (Maggi et al., 2018a) (**figure 3.1**).
- **MS vs Susac syndrome**: %PVL was 92% and 54%, respectively. Considering the small sample (6 patients with S. Susac) a threshold of %PVL indicative of MS has not been establishe (Wuerfel et al., 2012) d.

3.2 NAIMS CONSENSUS

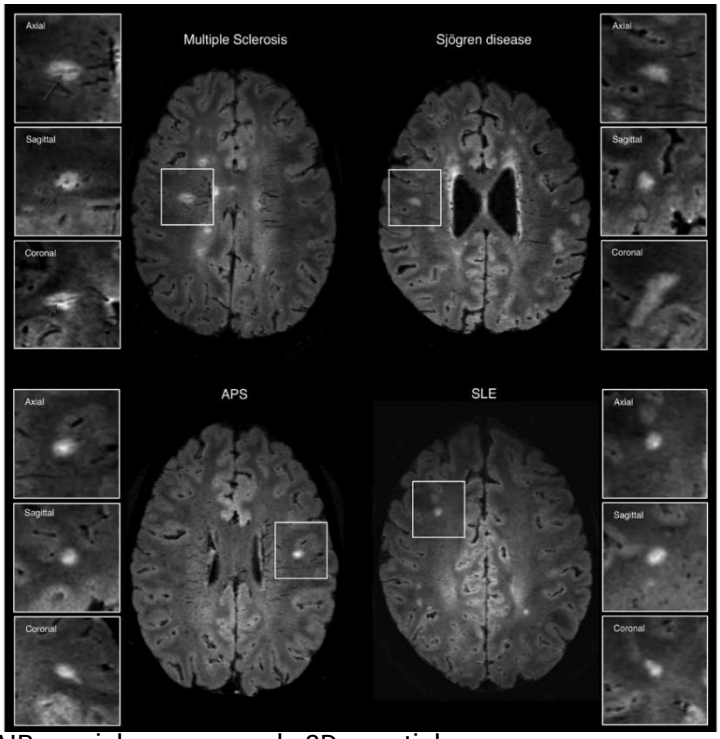


Figure 3.1: 3T FLAIR* axial scans and 3D spatial reconstruction for CVS analysis (inset). In the case of MS, a thin line or small hypointense point (CVS) can be seen within the lesions. On the other hand, CVS is not present in the lesions of patients with different pathologies (Sjogren's syndrome, anti-phospholipid antibody syndrome, neuroSLE). (Maggi et al, 2018)

In 2016 the NAIMS (North America Imaging for Multiple Sclerosis Cooperative) elaborated a consensus statement to define the recommendations, standardization and clinical evaluation of the central vein sign (CVS) in the diagnosis of MS of which the salient concepts are reported (Sati et al., 2016):

- the presence of a central vein within MS lesions is a recognized marker both in *ex vivo* pathological studies and in *in vivo* neuroimaging studies and exists in all clinical phenotypes of MS (RRMS, SPMS, PPMS).
- The location of the PVL should be considered during neuroimaging analysis. Current evidence suggests that PVL are predominant in the periventricular and deep WM areas, while the presence of cortical, infratentorial, and spinal PVL remains under determination, and further imaging studies are needed.
- the presence of comorbidities (e.g., vascular) cannot be ignored in considering the final percentage of PVL;
- Available evidence from MRI studies indicates that compared to MS patients, individuals with positive anti-AQP4 NMOSD, with systemic vascular diseases with CNS involvement (e.g., neuroSLE, neuroBehçet, etc.), with Susac syndrome, SVD and migraine have a significantly lower PVL%. Studies will be needed to describe CVS in other pathologies such as neurosarcoidosis and Sjögren's syndrome, but also ADEM.
- Regarding the technical standards of acquisition and analysis of neuroimaging, the consensus has not actually established definitive indications. Except for the

assertion that visualization of the venules is better with magnetic susceptibility sequences (T2*, SWI or FLAIR*, the latter based on the FLAIR-T2* co-registration) no categorical indication is given on the specific sequence to be used. However, the 3D gradient echo sequences with echo planar imaging (3D EPI) allow images to be acquired in a reduced time (< 7 minutes) and at the same time guarantee excellent coverage of the brain surface and an isotropic resolution of the voxels. The latter is particularly useful for reformatting images into any desired plane and allows for adequate visualization of venules regardless of their orientation. Furthermore, the use of small isotropic voxels increases the sensitivity in identifying small parenchymal veins within lesions, while simultaneously reducing artifacts due to inhomogeneity in the background field.

BOX1: Neuroradiological features of CVS.

A "central vein" shows the following features in T2 * sequences:

- Appears as a thin line or small hypointense dot;
- Can be visualized in at least two perpendicular MRI planes and appears as a thin line in at least one plane;
- Has a small apparent diameter (<2 mm)
- Runs partially or entirely through the lesion;
- Is located centrally in the WML (ie is approximately equidistant from lesion edges and passes across the edge in no more than two places), regardless of the shape of the WML

Exclusion Criteria. The WML:

• Has a diameter <3 mm in any plane;
• Merges with another lesion (confluent lesions);
• Has multiple distinct veins within it;
• Is poorly visible (due to motion or other MRI-related artifacts).

The NAIMS consensus also underlined how CVS analysis can be performed with excellent sensitivity (80%) even with 3 or 1.5T scanners through the use of optimized T2* protocols (Dixon et al., 2013; Maggi et al., 2018a), however recommending acquiring the images with the highest resolution level possible (submillimetre voxels). Another method to highlight the venules is the use of SWI sequences after the injection of gadolinium-based contrast medium (which is paramagnetic) and which is routinely used in MS diagnosis and follow-up protocols. Enhancement with post-contrast sequences is particularly recommended when low-field (1.5T) MRIs are used. Venular imaging at the medullary level is technically artificial and difficult (due to the small size of the medulla itself and the presence of numerous artefacts due to the surrounding tissues), and although the presence of PVL is theorized to date, no evidence has yet been reported at the medullary level. of literature.

3.3 METHODS FOR %PVL DEFINITION

While the definition of the central vein sign is unequivocal, there is no definitive agreement on how to globally interpret the CVS analysis at the radiological level in order to improve the diagnosis of MS. Most studies tend to consider the totality of analysable WMLs to perform the final PVL percent count. A proposed method is that of the "40% rule", introduced by Evangelou and colleagues, whereby the presence of at least 40% PVL among all evaluable WMLs is indicative of MS, and distinguishes it from non-MS pathologies. This approach had been successfully confirmed in a prospective study of the same group tested on CIS, with a positive and negative predictive value for MS diagnosis of 100% (Mistry et al., 2013) and also in a study of RRMS patients compared with patients with non-MS neurological syndromes (George et al., 2016). In other studies, a threshold of 50% has appeared to be more accurate in differentiating MS from other pathologies (Maggi et al., 2018a). Some groups have tried to overcome the limit derived from the need to analyse the totality of the lesions, due to the waste of time and the poor clinical applicability, evaluating the CVS analysis considering a reduced number of WMLs. The select3 and select3* method are based on the evaluation of only 3 lesions in FLAIR and FLAIR* sequences or exclusively FLAIR*. Three lesions, exclusively from the subcortical site or deep white matter, are arbitrarily selected and the amount of PVL is determined. The authors found good specificity and moderate sensitivity defining typical for MS a positivity of 3/3 lesions, or slightly lower sensitivity and specificity with a positivity of 2/3 lesions (Solomon, Watts, et al., 2019). A last algorithm instead provides for the random selection of 10 WMLs, defining the picture compatible with MS if at least 6/10 are PVL (Tallantyre et al., 2011).

A recent study prospectively evaluated the CVS in patients with CIS and MS with simultaneous red flags and compared the application of the 4 different criteria just mentioned (40% rule, 50% rule, 3 and 10 lesion algorithm) finding how not only did the CVS analysis prove to be an instrument with a high predictive value for the diagnosis of MS, but in comparing the various methods it highlighted how there is a slightly better result, in terms of diagnostic accuracy, for the method based on the 40% versus the 50% rule (100% diagnostic sensitivity, 92% specificity, 97% accuracy, positive and negative predictive values 96% and 100%, respectively), while between

the two methods with simplified counts the 3-lesion method was more reliable (diagnostic sensitivity of 93%, specificity of 92%, accuracy of 92%, positive and negative predictive values of 96% and 85% respectively (Sinnecker et al., 2019)). Similar results were found in a study conducted by MAGNIMS on a large multicenter series in 2018, in which a PVL threshold between 35-40% or the technique based on the 3 lesions was associated with the best results in terms of specificity and sensitivity (for the 35% rule 83% specificity and 61% sensitivity, for the 3-lesion rule 89% specificity and 62% sensitivity). In this study it also emerged that there was an increase in sensitivity when specific lesion sites of MS (periventricular and juxtacortical) were considered). A more recent study provided by MAGNIMS group (Cagol et al., 2023) evaluated the CVS in patients with a diagnosis of MS, clinically isolated syndrome (CIS), or non-MS conditions. The median (IQR) proportion of CVS-positive lesions per patients was 62.1% (44.4-79.2) in MS, 68.4% (32.9-90.2) in CIS, 10.7% (0-40.5) in AQP4-positive NMOSD, 20.0% (0.0-50.0) in seronegative-NMOSD, 33.3% (13.7-50.0) in MOGAD,0.95% (0.0-18.2) in migraine. Using the previously mentioned 40% threshold, the CVS provided sensitivity, specificity, and accuracy of 78.7% [95% CI, 75.5-82.0], 86.0% [95% CI, 82.1-89.5], and 81.5% [95% CI,78.9-83.7], respectively.

3.4 FIELDS OF APPLICATION OF CVS

In conclusion, the CVS analysis is emerging more and more clearly as a tool with great potential in the process of differential diagnosis of MS, even though today it needs further validation both in terms of standardization of acquisition and of analysis methodology, also in order to reliably define the real specificity and sensitivity.

CHAPTER 4

AIMS AND METHODOLOGY OF THE STUDY

4.1 STUDY RATIONALE

As previously mentioned, MS misdiagnosis is estimated at a frequency of 10–20% of all cases, (Kaisey et al., 2019b; Midaglia et al., 2021; Solomon & Corboy, 2017). A major reason is that the currently adopted MRI-based criteria are mainly nonspecific, and qualitative markers and reliable non-invasive biomarkers pathognomonic of MS are lacking (Filippi et al., 2019; Geraldes et al., 2018a; Siva, 2018a).

In the absence of reliable diagnostic biomarkers, indeed, when the accuracy of new iterations of the McDonald criteria was evaluated, the comparison has usually been made only vs the above-mentioned older criteria (Gobbin et al., 2019; Van Der Vuurst De Vries et al., 2018).

As stated, CVS is a promising non-invasive neuroradiological marker, capable of showing MS-specific lesions *in vivo*, accurately differentiating them from those of other pathologies that may mimic it. The validity of this marker has already been experimentally established in recent years in several studies, showing how a threshold between 40 and 50% of PVL was able to distinguish MS from its mimics. Consistently, a high PVL frequency per patient (PVL-f), usually above the order of 40–50% (the 40/ 50% rules), proves highly sensitive and specific for typical MS cases, with accuracy approaching 100% in many studies (Cortese et al., 2018; Maggi et al., 2018b; Mistry et al., 2016; Solomon, Schindler, et al., 2016; Tallantyre et al., 2011), but the optimal PVL-f is still controversial.

These studies compared populations of typical MS and several other well-defined pathologies (migraine, SVD, systemic inflammatory-dysimmune diseases with CNS involvement, NMOSD, Susac syndrome).

However, in clinical practice, diagnostic challenges derive from a specific group of patients: those with definite MS according to McDonald criteria that are at risk of misdiagnosis because of clinical, imaging, or laboratory red flags in which is not possible to reach a definite alternative diagnosis (henceforth denoted "**MS-plus**").

Starting from these considerations, the main aim of the present study was to identify a cut-off value of PVL-f that discriminates MS from other conditions, and then to use the identified cut-off value as gold standard to test the accuracy of current MS diagnostic criteria in different MS populations (typical patients and MS-plus patients).

For this purpose, PVL-f were analysed in typical MS and in MS-plus cases both fulfilling clinical DIS and DIT criteria according to the McDonald criteria and its iterations (McDonald et al., 2001; Polman et al., 2011; Poser et al., 1983; Thompson, Banwell, et al., 2018a), and compared with non-MS controls with MS-like brain lesions, finding that the PVL-f allowing the optimal accuracy is $\geq$52% and that when this threshold is applied in MS-plus patients, specificity and predictive value of the most accurate diagnostic criteria for MS is low. In the MS-like syndromes thus identified, risk factors for alternative disease were also explored.

4.2 DEFINITION OF MS-PLUS AND STUDY-SPECIFIC RED FLAGS

MS plus: MS diagnosed according to 2010 or 2017 McDonald Criteria with$\geq$1 clinical, laboratory and/or MRI red flag, suggesting but not sufficient to reach a definite alternative diagnosis to MS.

Red flag definition: the term "red flag" will be hereafter referred to a specific list of laboratory (CSF and serum), clinical and neuroradiological items supporting a possible alterative diagnosis to MS. The most relevant red flags were included in a study-specific form used for MS-plus patients' enrolment (figure 4.1). The list was elaborated after a revision and summary of the main available literature data (Aliaga & Barkhof, 2014; Brownlee et al., 2017; Chen et al., 2016; Geraldes et al., 2018a; Miller et al., 2008a; Siva, 2018b).

Figure 4.1: Study specific list of red flags adopted for MS-plus patients enrolment.

APPENDIX 1: Study specific form for detection of Red flags

CLINICAL RED FLAGS:
- ☐ Synchronous bilateral retrobulbar optic neuritis
- ☐ Positive anamnesis of Retinopathy (e.g. retinitis pigmentosa, retinal vasculitis, not diabetic or hypertensive) or Uveitis.
- ☐ Systemic symptoms suggestive of autoimmune/rheumatologic disease (i.e. diffuse arthritis, recurrent oral/genital ulcers, livedo reticularis, malar rash, xerostomia, xerophthalmia, myalgias, recurrent clinical relevant gastrointestinal disturbances, Raynaud phenomenon etc)
- ☐ Patent foramen oval with a documented medium-severe shunt entity
- ☐ Onset before 14 year or after 55 years
- ☐ Recurrent abortivity and/or arteriovenous thromboembolism
- ☐ Iperacute onset and/or fulminant course
- ☐ Systemic comorbidity that suggests a concurrent genetically determined syndromic state (e.g., various associations between early cataract, juvenile diabetes, hepatopathies or nephropathies, etc.)
- ☐ No clinical response to corticosteroids within 30 days from symptoms/relapse onset
- ☐ Concomitant myopathy and/or neuropathy
- ☐ Complete or fluctuating paralysis of the gaze
- ☐ Stereotyped and monomorphic attacks
- ☐ Postrema or diencephalic area syndrome

Within 12 months from "MS" symptoms onset:
- ☐ New onset relevant psychiatric symptoms as psychosis, behavioural and personality changes (depression excluded)+;
- ☐ New onset atypical, severe, persistent, drugs resistant headache
- ☐ Persistent (≥3months) flu of unknown origin or recurrent/periodic, generalized malaise, constitutional symptoms (flu-like)
- ☐ New onset epilepsy
- ☐ New onset neurosensory hypoacusia
- ☐ Encephalopathy (lethargy, mental confusion, slowing down, etc.)
- ☐ Concomitant cranial nerves multineuropathy (excluding II and VII) or polyradiculonevritis

MRI RED FLAGS

1.Atypical lesion distribution/features
- ☐ Predominance of small lesions (<3 mm)
- ☐ Sparing: corpus callosum, U-shaped iuxtacortical fibers, iuxtaventricular areas, i.e no "Dawson's fingers".
- ☐ Predominance of cortical lesions or deep grey matter involvement
- ☐ exclusive and widespread involvement of the anterior and inferior anterior temporal lobe pole
- ☐ Symmetric White Matter Lesions (WML)
- ☐ Medullary cone involvement
- ☐ Longitudinally extended transverse myelitis
- ☐ Predominant brainstem central lesions
- ☐ Brain WML necklace distribution

2.Enhancement
- ☐ Synchronous and widespread WML enhancement (e.g. ADEM like)
- ☐ Persistent enhancement of the same lesion;
- ☐ Atypical enhancement pattern;
- ☐ Punctate pontine (CLIPPERS like salt-and-pepper) or punctate supratentorial (e.g. sarcoidosis)
- ☐ Enhancement of multiple cranial nerves/spinal nerve roots
- ☐ Leptomeningeal or pachimeningeal enhancement

3. Atypical lesions
- ☐ Voluminous and/or with mass effect
- ☐ With poorly defined margins, marbled, cottoned
- ☐ Edematous, with mass effect at brainstem level
- ☐ T1 hyperintensity of thalamic pulvinar
- ☐ T2 hyperintensity of dentate nuclei
- ☐ Diffuse calcifications at CT/MRi scans

4. Small vessel disease and cerebrovascular disease markers
- ☐ Lacunae
- ☐ Status cribrosus
- ☐ Microbleeds
- ☐ Leukoaraiosis
- ☐ Cerebral infarcts, macrohaemorrhages
- ☐ Cortical hemosiderosis

LABORATORY RED FLAGS

1.Positivity to Persistently elevated systemic inflammatory/disimmune markers
- ☐ ANA (antinuclear antibodies) >1:160 or 1:160 persistent
- ☐ Anti ds-DNA antibodies
- ☐ ENA (Extractable Nuclear Antibodies)
- ☐ Antiphospholipid antibodies (anti-cardiolipin, anti-phosphatidylserine, anti β2GPI)
- ☐ Anti citrullinated cyclic peptide (anti-CCP)
- ☐ ANCA (Anti-Neutrophil Cytoplasmic Antibody;
- ☐ Anti endomysial, and/or anti deamidated gliadine, and/or anti transglutaminase antibodies
- ☐ C-reactive protein
- ☐ Erythrocyte sedimentation rate
- ☐ Sieroamyloid-A
- ☐ Rheumatoid factor IgG/IgM
- ☐ Circulating immune complexes
- ☐ C3 and/or C4 complement fractions reduction, hypogammaglobulinemia

4. ☐ Increased levels of ACE (angiotensin converting enzyme) or/and chitotriosidase

5. ☐ Thrombocytopenia, leukopenia, anaemia of possible autoimmune origin

6. Cerebrospinal fluid (CSF) analysis
- ☐ Absence or different from pattern II intrathecal IgG oligoclonal production
- ☐ CSF leukocytes > 50 cell/mm3
- ☐ CSF proteins > 0.9 g/L
- ☐ CSF Glucose alteration

The items considered most relevant and easily analysable were included, on the basis of a consensus established among the members of the MS center of the Neurology 2 Unit of the Careggi University Hospital.

4.3 AIMS OF THE STUDY

Primary endpoints:

- To identify a PVL-f threshold that discriminates true MS from not-MS neurological condition.
- To test the diagnostic accuracy of McDonald criteria applied in MS-plus patients using as gold standard for true-MS cases the PVL-f threshold previously identified

Secondary endpoints:

- To evaluate basal clinical-demographic differences among MS-plus subgroups stratified according to the PVL-f threshold previously identified.
- To evaluate disease course and conventional MRI differences among MS-plus subgroups stratified according to the PVL-f threshold previously identified.
- To test advanced neuroophthalmological techniques to find potential differences in MS-plus subgroups categorized according to the PVL-f threshold previously identified.
- To hypothesize possible alternative diagnosis in Ms-plus patients with PVL-f lower than the identified threshold.

Exploratory endpoints

- To evaluate a possible correlation between the number of WML<3mm and PVL-f lower than the identified threshold
- To evaluate if there is a significative change in patients' distribution in MS-plus subgroups using a CVS analysis method alternative to the NAIMS standardized one (namely, the inclusion of WML<3mm, considering it as non-PVL)

4.4 SUBJECTS/MATERIALS AND METHODS

4.4.1 Study design

PVL-f and T2 hyperintense lesions were analyzed in relapsing-remitting (RR) RRMS MS-plus (experimental group) or in typical MS patients (positive controls). Not-MS patients with MS-like neurological syndromes and brain WMLs were also analyzed (negative controls). The patients received one MRI scan, including conventional FLAIR sequences and a FLAIR* acquisition, allowing PVL-f analysis in each patient. A PVL-f threshold able to discriminate true MS from other CNS conditions was determined with ROC analysis, including the PVL-f of typical MS (true positive cases) and definite not-MS diseases (true negative cases). The accuracy of the MS Diagnostic Criteria was then evaluated in all patients using this threshold as the gold standard for true MS detection, in particular in MS-plus patients. The number and site location of WMLs were also evaluated in each case, as well as the baseline clinical characteristics and history. The clinical, MRI and demographic characteristics of patients fulfilling or not the PVL-f threshold were also compared. Moreover, a subset of MS and MS-plus patients, underwent an ophthalmological evaluation with high-resolution retinal imaging techniques to investigate possible differences among groups, especially in MS-plus with low PVL-f in which the

underling diseases could be potentially microvascular or a small vessel vasculitis. The term "red flag" refers to predefined clinical, laboratory, and/or MRI characteristics that, when present, suggest an alternative diagnosis rather than MS (Charil et al., 2006; Geraldes et al., 2018b; Miller et al., 2008b) (list in Figure 4.1).

4.4.2 Patients

Consecutive RRMS patients 18–65 years old, were prospectively included at the Tuscany Regional Referral MS Center (Careggi University Hospital, Florence, Italy) between 2017 and 2021 (n=59) according to the following inclusion criteria:

MS-plus:

Inclusion criteria:

- demyelinating syndrome with DIS and DIT according to the 2001 McDonald's criteria (the latter version of McDonald criteria was chosen according to its high diagnostic specificity and accuracy, and considering that all patients respect also 2017 version of criteria) without atypical signs according to Brownlee (Brownlee et al., 2017);
- CSF examination at diagnosis;
- "red flags" suggesting "better explanation"(see: **appendix 1**).

Exclusion criteria:

- "better explanation of the diagnosis", i.e. fulfillment of the diagnostic criteria of other neurological diseases;
- contraindication to MRI scanning or administration of gadolinium-based contrast material.

Typical MS cases fulfilling the same inclusion criteria as above apart from the presence of red flags and with disease duration long enough to allow certain exclusion of better explanation" ($\geq$ 5 years; Typical MS) were included as positive controls (n=28).

As, according to these criteria, in these first two groups different disease durations were expected, only the baseline annualized relapse rate (ARR) prior to inclusion related the first ten years after diagnosis were evaluated.

Non-MS neurological cases with MS-like brain WMLs (n=32), were also included as negative control cases for CVS analysis (data derived from a previous study conducted by our group). This group encompassed Systemic Autoimmune Diseases with neurological

involvement (Systemic Erythematous Lupus, n=5; antiphospholipid syndrome, n=5; Bechet disease, n= 6; Rheumatoid Arthritis, n= 2; systemic vasculitis ANCA+, n=1), isolated inflammatory vasculopathies of the CNS (Susac Syndrome n= 2; small vessel PACNS n= 9; Fabry disease, n= 1) and primary brain lymphoma (n=1).
The study received approval from the local independent Ethics Committee/Institutional Review Board. Clinical-demographical and disease course data from each patient were recorded on a predefined Case Report Form.

4.4.3 MRI acquisition protocol and analysis

Each patient underwent a single MRI exam, on a 1.5-tesla Philips Achieva scanner, with a protocol optimized for PVL-f analysis, as previously described and adapted to the scanner (Maggi et al., 2018b; Sati et al., 2016). The protocol included the following acquisitions: 3-dimensional (3D), 1-mm-isotropic, T2-FLAIR; 3D, 1x1-isotropic mm, T2*-weighted echo-planar imaging (EPI). Both scans were acquired during or after intravenous injection of a single dose (0.1 mmol/kg) of gadolinium-based contrast agent (Gadovist). Data were collected as DICOM images and analyzed with the Medical Image Processing, Analysis, and Visualization (MIPAV) software (https://mipav.cit.nih.gov/). Two neurologists with MRI research training and experience masked to the clinical data, performed the WMLs assessment. Interrater reliability score (R2) for the PVL frequency assignment resulted 0,94 ($p< 0.00001$). A sample of scans (n= 10) were also retested with the same conventional and SWI protocol in a 3T Philips device, observing optimal concordance between the two acquisitions ($R= 0.96$; $p< 0.00001$) and differing only as for evaluation time.

WMLs site/topography was classified as follows (Filippi et al., 2019):

- periventricular (abutting on the ventricule),
- cortical/juxtacortical (abutting on the cortex),
- infratentorial and
- subcortical/deep white matter.

Volume and number of the WMLs were also evaluated by semiautomatic contouring.
PVL identification was based on a previously described adaptation (Maggi et al., 2018a) of the consensus criteria of the North American Imaging in Multiple Sclerosis (NAIMS) Cooperative (Sati et al., 2016). Briefly, discrete WMLs $\geq$3-mm diameter in at least one plane were included in the primary analysis. Confluent or poorly visible lesions were excluded. WMLs were defined as PVL when containing a small (<2-mm) hypointense line or dot, positioned centrally and running partially or entirely through the lesion, which can be visualized in at least two perpendicular imaging planes.

Both total PVL number and PVL-f (defined as PVL number/ ≥3 mm WMLs number x100) were recorded.

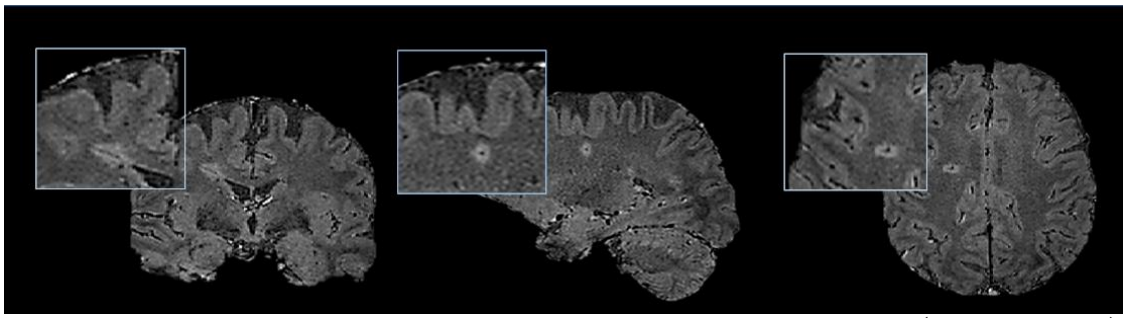


Figure 4.2: Triplanar view of a typical perivenular lesion in a MS-plus patient (SWI sequences), analysed with MIPAV software

4.4.4 Neuroophthalmological evaluation

Trained neuro-ophthalmologists evaluated MS and MS-plus patients. All patients underwent a baseline ophthalmic examination including medical and ocular history, family medical history, measurement of best corrected visual acuity (BCVA) using the early treatment retinopathy diabetic study (ETRDS) chart, slit lamp examination of the anterior and posterior segments, measurement of intraocular pressure, dilated fundus examination, B-scan OCT and OCT Angiography. The RS-3000 Advance 2 spectral domain OCT (NIDEK Co. Ltd., Gamagori, Japan) was used to acquire structural OCT, OCTA in all eyes.

All B-scans were performed 4 times and averaged for higher sensitivity. A real time SLO based active eye tracker was used to compensate for eye movement during image acquisition. In all cases the SLO image was captured prior to OCT-A analysis. Low-quality OCTA images, severe artifacts due to poor fixation or cases of failed automatic layer segmentation were excluded from analysis. Images were reviewed by two investigators (CL and DB) for segmentation accuracy.

The default RS-3000 Advance 2 AngioScan software has been used (%) to evaluate the vessel density, defined as the percentage of the total area occupied by vessels. In addition, FAZ area, perimeter and circularity (an index that is equal to 1 when the FAZ shape is a circle) were automatically calculated by the in-built software.

Structural B-scan OCT measurements included:

- Central foveal thickness (CFT) (μm) (figure 4.3)
- RNFL quadrant analyses (superior, inferior temporal, nasal) (μm) (figure 4.4)
- Ganglion Cell Complex superior sector (μm) (figure 4.3)

- Ganglion Cell Complex inferior sector (μm) (figure 4.3)

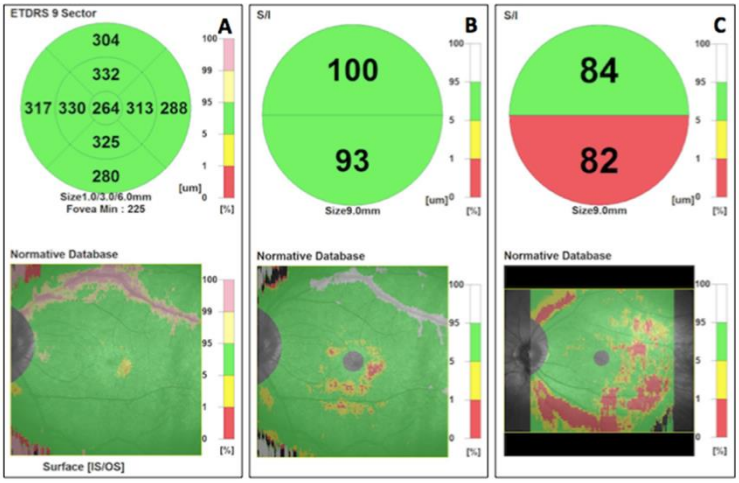


Figure 4.3. Automatic analysis of the macular region using structural OCT. A) Central foveal thickness (264 micron) detected automatically with the *thickness map* analysis. B) Ganglion cell complex (GCC) using the *thickness map* analysis in "glaucoma" section. The GCC were evaluated both for the superior and inferior sector and measured in μm, and differentiated in color scale from green (normal thickness) to red color (reduction of thickness). C) GCC analysis of a MS-plus patient, revealing a reduction of the mean thickness in the inferior sector, highlighted in red.

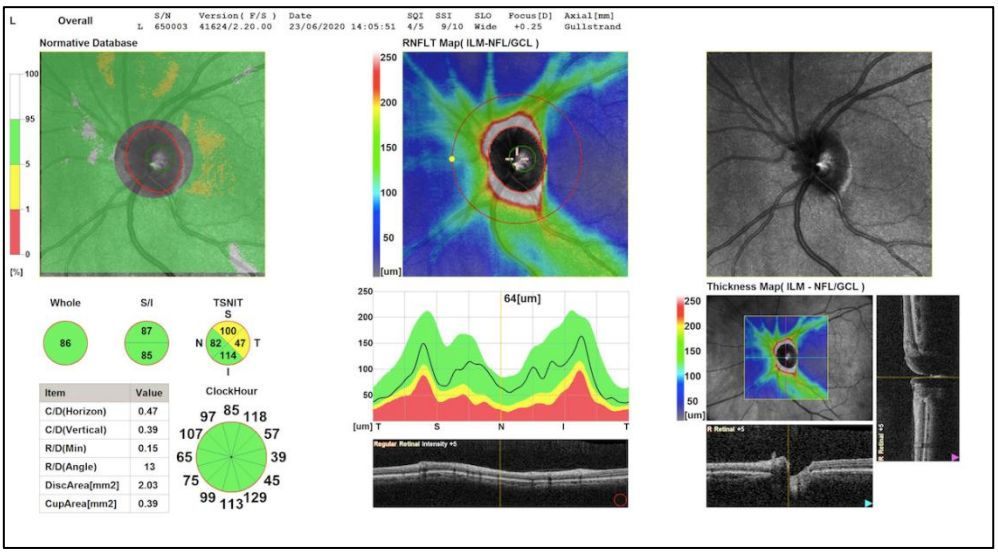


Figure 4.4. Evaluation of retinal nerve fiber layer (RNFL) using OCT. The four different optic disc sectors superior (S), inferior (I), temporal (T) and nasal (N) were automatically evaluated by the instrument and reported in numeric parameter (μm) and color scale (green to red).

Using OCT-Angiography, the following quantitative parameters were evaluated:

- Foveal avascular zone (FAZ) area (mm^2)
- FAZ perimeter (mm)
- FAZ circularity
- Optic disc whole vessel density
- Optic disc radial peripapillary capillary (RPC) network whole density

- Optic disc lamina cribrosa vessel density

Vessel densities of the superficial capillary plexus (SCP) and deep capillary plexus (DCP) were automatically calculated by the software on OCTA 3 x 3 mm volume scans in the whole foveal and the inner and outer retina

4.4.5 Analysis

Primary analysis.

PVL number and PVL frequency/patient (PVL-f) were analyzed in all patients as the number of PVLs/ total lesion number of each patient.

A PVL-f threshold able to discriminate MS from other CNS diseases was determined with a ROC analysis, including PVL-f of true positive cases (Typical MS patients) and true negative cases (other definite CNS diseases). The evaluation of the DIS/DIT-based MS diagnostic criteria accuracy was then calculated using the resulting PVL-f threshold as diagnostic gold standard (the proportion of patient in each group who had a PVL-f above that established by the ROC analysis was considered true MS).

Secondary Analysis

To evaluate possible differences among subset of MS-plus patients stratified with the identified PVL-f threshold, the following data were collected:

- Demopgraphic characteristics
- EDSS at onset, time to first confirmed EDSS worsening (CDW), EDSS al last follow up.
- Relapses
- DMTs (ongoing and previous, time to first DMT)
- Cardiovascular Risk Factors (diabetes, smoking, arterial hypertension, hypercholesterolemia, etc.)
- Any other relevant medical condition

To evaluate possible confounders that could impact the PVL-f assessment, two additional sensitivity analysis of the PVL-f were conducted:

- considering as non-PVL the WMLs <3 mm in diameter, to test the impact of the methodological limitation of excluding the small lesions.
- re-calculating the PVL-f after excluding MS-plus patients older than 50 years, to test possible confounding effects of nonspecific age-related WMLs. Moreover, to identify possible correlation between age at MRI and resulting PVL-f in patients subgroups.

T2 hyperintense lesion number/volume was evaluated in FLAIR sequences. Their distribution in the brain white matter was also analyzed and classified in MS typical/not-typical according to juxtaventricular/juxtacortical/infratentorial or to deep subcortical location.

4.5 Statistical analysis

Baseline demographic, clinical, and MRI differences between groups and subgroups were assessed using non-parametric tests (Mann–Whitney test for continuous variables; Chi square/Fisher exact test for frequencies). Significance levels were corrected for multiplicity of observations, when indicated and reported as *adjusted p value*, taking in account correlations among the variables involved, according to Hommel. A two-tailed p-value <0.05 was considered significant. The statistics software used was SPSS version 25 (Windows).

The PVL-f threshold associated with the best accuracy of the MS diagnostic criteria was established by Receiver-Operating Characteristics (ROC) analysis. To this purpose the PVL-f above the selected threshold was considered the marker of true MS and therefore the "gold standard" of MS diagnosis. Accordingly, the frequency of the patients fulfilling or not the selected PVL-f threshold was used for evaluating accuracy of the MS diagnostic criteria by two entries tables.

CHAPTER 5

RESULTS

A total of 87 MS patients were included, 28 typical MS and 59 MS-plus patients. The clinical-demographic features of the two groups of patients are reported in **Table 5.1**.

No differences emerged between the two groups concerning the age at MRI scan, at diseases onset and of basal EDSS. Median disease duration resulted significantly longer in typical MS group, as expected considering the inclusion only of patients with disease duration $\geq$ 5 years in this group ($p<0.0001$).

In MS-plus group the presence of cardiovascular risk factors (CVRF) was higher than in the MS group ($p=0.008$).

Table 5.1. Demographic and clinical characteristics of MS plus and MS patients

		MS plus N=59	**MS N=28**	**p values** (adjusted for multiplicity
Female	*n(%)*	51 (86%)	17 (61%)	p= 0,12 $^{\chi}$
Age at disease onset (ys)	*m(r)*[1]	38 (15-62)	31 (14-52)	p=0,42^{u}
Age at inclusion (ys)	*m(r)*	45 (20-65)	49 (23-65)	p=0,9^{u}
Disease duration at inclusion (ys)	*m(r)*	6 (0,2-28,4)	19 (7-43,3)	**p<0,0006^{u}**
Basal EDSS[2]	*m(r)*	0 (0-2,5)	0 (0-4)	p=0,84^{u}
Negative OCB[3]	*n(%)*	19 (32%)	0(0%)	**p<0.001** $^{\chi}$
Patients with CVRF[4]	*n(%)*	20 (34%)	2 (7%)	p<0,06 $^{\chi}$
Treated with DMTs[5]	*n(%)*	45 (76%)	27 (96%)	p=0,12 $^{\chi}$
ARR[6]	*m(r)* *Median (range)* *mean (±SE)*	 0,1 (0-0,5) 0,1(0)	 0,2 (0-0,5) 0,3 (0)	**p< 0,006^{u}**

$^{\chi}$ Fisher exact χ^2 test; ^{u} Mann Whitney U test

1 m(r): median (range) **2 EDSS** expanded disability status scale **3 OCB:** oligoclonal bands **4 CVRF**: cardiovascular risk factors, **5 DMT** disease modifying therapy **6 ARR** annualized relapse of the first 10 years from disease onset

5.2 PVL-f analysis and MS-plus subgroup stratification

Primary analysis.

WMLs PVL≥3 mm in largest diameter, n./patient, PVL number and frequency/ patients in the Typical MS cases (n= 28) in the MS-plus cases (n= 59) are reported in **Table 5.2**. In these groups the median PVL-f/patient was 91% (range 67–100%) and 55% respectively (range 8–100%; p=0.001; **Table 5.2, Fig. 5.1**). In the non-MS patients (n= 32) PVL-f/patient was 23% (range 0-89%; p< 0.00001; **Fig. 5.1**).

For estimating the best accuracy of the MS diagnostic criteria used in this study, a PVL-f threshold of 52% was first identified by ROC analysis (**Fig 5.2**).

Fulfilling this PVL-f threshold was met by 28/28 Typical MS cases (100%), but only by 31/59 MS-plus cases (52.5%; p< 0.0001, **Fig.5.1**). The 31 MS-plus who met the threshold had a median ≥3mm PVL number of 12 (range 2-76) corresponding to a median PVL-f 71% (range 54–100%), similar to that of the Typical MS cases (91%, p= NS; **Fig.5.1, Table 5.3**), whereas the 28/59 MS-plus patients who did not meet the threshold (47.5%), had a median PVL number of 5 (range 1-29) and a median PVL-f of 22% (range 8–43%; p< 0.001 as for the PVL number; $p<10^{-6}$, as for the PVL-f both between the MS-plus with a PVL-f > 52% and vs the Typical MS; **Fig. 5.1, Table 5.3**), similar to the non-MS cases (23%, p= NS).

According to the frequencies of patients fulfilling the PVL-f 52% threshold, accuracy of the MS diagnostic criteria used for patient inclusion in this study (Poser & Brinar, 2001; Thompson, Banwell, et al., 2018a) resulted 0.98 in Typical MS and and 0.68 in MS-plus. In typical MS, resulting in Typical MS from: positive predictive value (PPV) = 1.0, - sensitivity (Se) = 0.96; - specificity (Sp) 1.0. In the MS plus: PPV 0.52; Se 0.97; Sp: 0.52. Negative predictive value (NPV) was 0.97 as in both analysis it derived from the same non-MS cases (**fig. 5.3**).

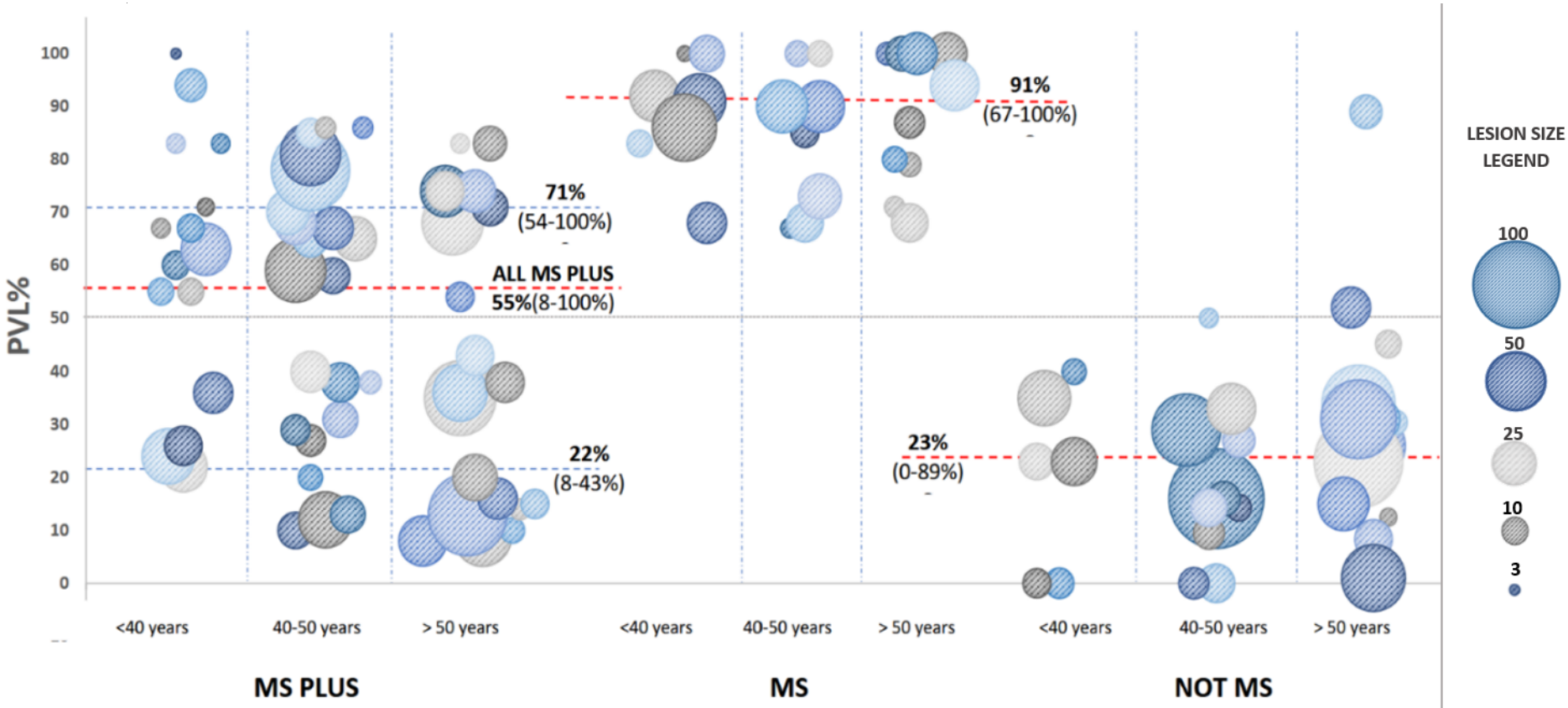


Figure 5.1. Frequency of perivenular lesions (PVL-f) in MS (n= 28) in MS plus (n=59) and in non-MS patients (n=33). The frequency is expressed as PVL number/ WML number x 100. Each circle represents one patient and its size is proportional to the total number of WMLs observed in each patient **(**see **lesion size legend)**. The red dotted lines represent the median PVL-f in each group (difference of the Typical MS vs MS-plus p= 0.001, vs non-MS p< 0.10^{-6}; Mann Whitney U test). Categorization for age did not show associations with this variable. Comparing Typical MS vs not-MS with MS-like brain lesions, ROC analysis indicated the 52% PVL-f threshold for optimal accuracy of the MS diagnostic criteria. The MS-plus patients that fulfilled this PVL-f threshold were n= 31/59 (52,5%) with a median PVL-f of 71% (range 54-100%) close to the PVL-f observed in the Typical MS, whereas those who did not meet this PVL threshold were n= 28/59 (47,5%) with a median PVL-f of 22% (range 8-43%; blue dotted lines), close to the non-MS patients. The low PVL-f observed in the MS-plus population with PVL≤52%, indicates that in this category, this pathological hallmark of MS is not present in a large proportion of patients thus indicating low specificity and low positive predictive value of the MS diagnostic criteria when applied to the MS-plus patients as the MS diagnostic criteria can be met by other diseases.

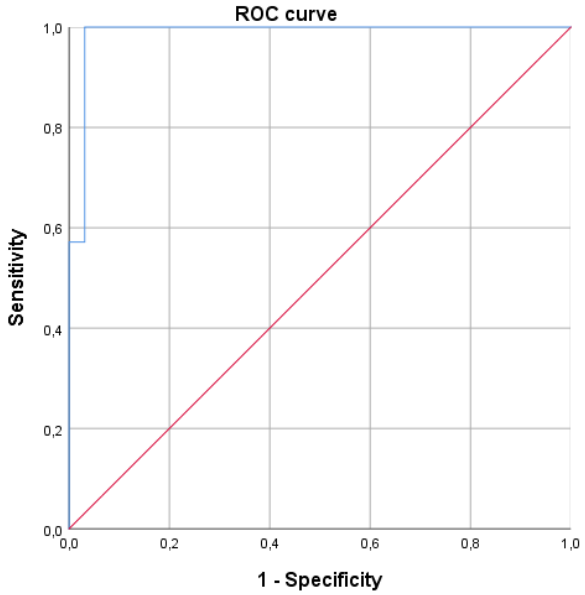


Figure 5.2. ROC analysis to evaluate the best PVL-f threshold discriminating MS from other conditions. A threshold of 52% was identified.

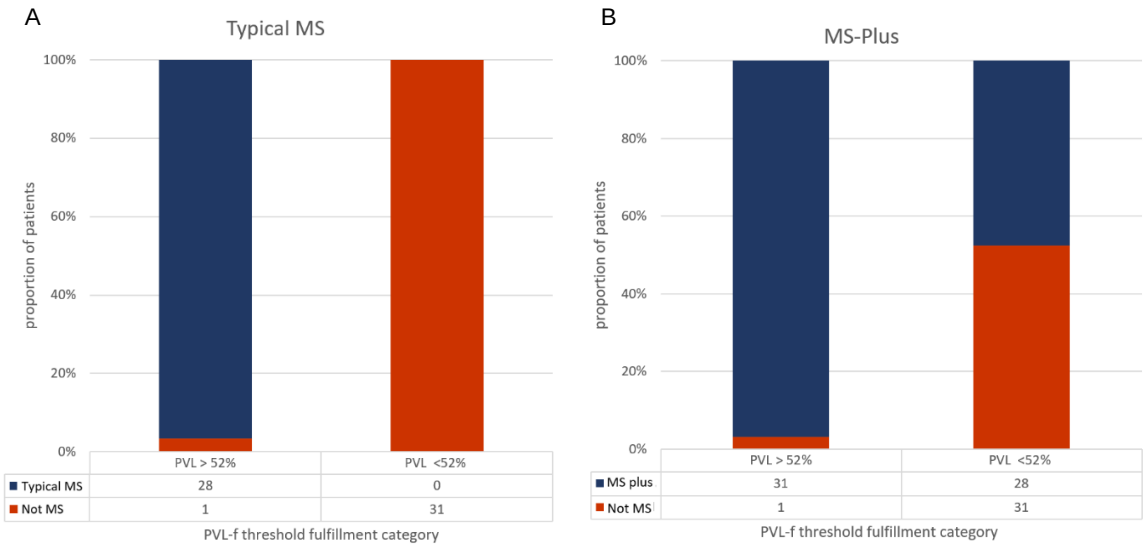


Figure 5.3. MS diagnostic criteria In Typical MS (**A**) and in MS-plus patients (**B**). Perfomance in detecting the >52% PVL-f threshold (the gold standard of true MS). In the optimal performace of the critera Typical MS, reaching positive predictive value and specificity = 1.0 (95% CI 1.0-1.0) and = 1.0 (95% CI 1.0 -1.0) respectively with sensitivity 0.96 (95% CI 0.89- 1.0). Unsatisfactory performance in the MS-plus patients, as positive predictive value and specificity were 0.52,5 (95% CI 0,40-0,66) and 0.52.5 (95% CI 0.39-0.65) repectively, with sensitivity 0.97 (95% CI 0.91-1.0). The negative predictive value detected in non-MS patients was 0.97 (95% CI 0.91-1.0). These data, confirm high accuracy of the MS diagnostic criteria in Typical demyelinating syndromes, but also indicates that in demyelinatiing syndromes carrying red flag of other diagnosis these criteria do not perform well.

TABLE 5.2. Conventional brain MRi characteristics in Typical MS and in MS-Plus			
MRI	**MS** N=28	**MS plus** N=59	**P values** (adjusted for multiplicity***)**
WMLs load/ patient, mm^3			
median *(range)* mean (*sd)*	4819(1118-18882) 6785 (5761)	2109(486-2678)1) 3851 (462	**p=0,004** [u]
WMLs volume/ lesion/ patient, mm3			
median *(range)* mean (sd)	302 (124-873) 318(171)	92 (20-477) 120 (92)	**$p<10^{-8}$** [u]
WMLs /patient			
All, n./patient median *(range)* *mean (sd)*	25 (6-66) 25 (15)	26 (3-122) 33 (26)	p=0,9 [u]
≥3mm, n./patient, median *(range)* n./patient, *mean(sd)* *frequency/patient, %, median (range)*	25 (6-60) 24 (14) 100% (72-100)	21 (3-100) 28 (22) 84% (58-100),	p=0.9 p= 0,2
<3mm, n. (% of tot WMLs) *n/patient, median(range)* *frequency/ patient, % median(range)*	26 (4%) 0 (0-7) 0 (0-28)	345(17%) 4 (0-41) 16 (0-42)	**$p=10^{-5}$** [u] **$p=10^{-5}$** [u]
PVL (>3 mm WMLs)			
n/ patient median(range) *n/patient mean(sd)* *frequency/patient, % median(range)*	15 (4-57) 19 (14) 91% (67-100%)	7.5 (1-76) 12(13) 55% (8-100%)	**p=0.05** **$p<10^{-7}$** [u]
Non PVL >3mm WMLs			
n,/patient median *(range)* *mean (sd)* *frequency/patient, % median(range)*	2 (0-9) 3 (3) 10% (0-33%)	10 (0-85) 15 (16) 45% (.0-92%)	**$p=10^{-5}$** [u] **$p= 10^{-6}$**
Subcortical/deep (> 3 mm) WMLs5§/patient			
n/patient median*(range)* *frequency/ patient, % median (range)*	6(0-49) 38(0-75)	15 (0-105) 60 (0-100)	**p=0.01**[u] **p=0.01** [u]

Table 5.3. Conventional brain MRI characteristics in MS and in MS plus stratified according to the 52% PVL threshold

Lesion Characteristics	*MS*	MS plus		P values (Mann Whitney U test) adjusted for multiplicity		
		PVL <52% (n= 28)	PVL≥52% (n= 31)	MS plus <52%PVL vs >52%PVL	MS plus <52%PVL vs MS	MS plus >52%PVL vs MS
Total lesion load/ patient (mm³)						
median (range)	4819(1118-18882)	1926 (486-8667)	2432 (539-26781)	p=0,21	**p=0,002**	P=0,05
mean (SD)	6785(5761)	2812 (2461)	4760 (5798)			
WML volume/lesion (mm³)						
median (range)	302 (124-873)	70 (20-159)	131 (54-477)	**$p<10^{-6}$**	**$p<10^{-9}$**	**p<0,00001**
mean (SD)	317 (171)	70(28)	163 (105)			
WML n./ patient,						
Total, n						
median (range)	25 (6-66)	34 (8-122)	22 (3-97)	p=0,05	p=0,08	p=0,91
mean (SD)	25 (15)	40 (29)	28 (22)			
> 3mm, n						
median (range)	25 (6-60)	25 (8-100)	17 (3-97)	p=0,08	p=0,39	p=0,39
mean (SD)	24 (14)	31 (23)	24 (21)			
< 3mm, n						
median (range)	0 (0-7)	7(0-41)	3 (0-13)	**p=0,01**	**$p=10^{-5}$**	**p=0,001**
mean (SD)	1(2)	8 (9)	4 (3)			
Total PVL WML number/ patient						
median (range)	15 (4-57)	5 (1-29)	12 (3-76)	**p=0,002**	**$p<10^{-4}$**	p= 0,22
mean (SD)	19 (13)	7 (6)	16 (15)			
Non PVL >3mm white matter lesions						
median n,/patient (range)	2 (0-9)	18 (5-85)	6 (0-24)	**$p<10^{-5}$**	**$p<10^{-9}$**	**p=0,003**
mean (SD)	3 (3)	24 (18)	7 (6)			
median frequency/patient, % (range)	10% (0-33%)	80 (57-92)	29 (0-50)	**$p<10^{-9}$**	**$p<10^{-9}$**	**p<0,0001**
***PVL frequency** (PVLs/total WMLs)%*						
median(range)	91 (67-100)	22 (8-43)	71 (54-100)	**$p<10^{-6}$**	**$p<10^{-6}$**	**p<0,001**
mean (SD)	88 (12)	24 (11)	73 (12)			
***MS-typical WML location,** % number/total WML/patient*						
% All						
median(range)	63 (25-100)	20 (0-84)	54 (20-100)	**$p<10^{-6}$**	**$p<10^{-6}$**	p=0,15
mean (SD)	61 (20)	22 (19)	58 (21)			
% Juxtaventricular lesions						
median(range)	30 (11-60)	14 (0-50)	42 (0- 100)	**$p<10^{-6}$**	**p=0,0001**	p=0,06
mean (SD)	33 (15)	16 (11)	45 (11)			
% Juxtacortical lesions						
median(range)	20 (0-61)	0 (0-65)	0 (0-48)	p=0,43	**$p<10^{-6}$**	**$p<10^{-6}$**
mean (SD)	22 (17)	5 (13)	5 (9)			
% Infratentorial lesions						
median(range)	2 (0-30)	0 (0-11)	4 (0-100)	**p=0,012**	p<0,06	p=0,81
mean (SD)	6 (8)	1(3)	7 (18)			
subcortical and deep WMLs **,						
***%** number/total WMLs/patient:*						
median(range)	38 (0-75)	81 (16-100)	40 (0- 80)	**$p<10^{-7}$**	**$p<10^{-7}$**	p=0,64
mean (SD)	40 (22)	78 (19)	44 (21)			
patient with majority of subcortical/deep WMLs						
n(%)	7 (25%)	19 (68%)	12 (39%)	**p=0,02**	**p=0,001**	p=0,26

Sensitivity analyses.

In addition, two sensitivity analyses were carried out:

a) median PVL-f in the MS-plus patients or in the patients MS-plus fulfilling (n=15) or not (n=22) the 52% PVL-f threshold aged <50 years (n=37), was not different from the primary analysis for all comparisons (p>0.5, Mann Whitney U test), determining PPV and SP of the MS diagnostic criteria similar to the primary analysis (0.4 and 0,42 respectively); moreover no correlation was found between age at MRI and PVL-fin any of the three groups (typical MS and MS-plus subgroups): Spearman's correlation coefficient r: -0.22 for MS-plus PVL-f<52%, p=0.38; 0.01 for MS-plus PVL-f>52%, p=0.96; 0.12 for typical MS, p=0.58; **figure 5.4**).

b) the other sensitivity analysis, carried out calculating the PVL-f including the small WMLs (<3 mm in maximum diameter) in the total WMLs number assuming them as non-PVLs, showed that including smaller lesions at the denominator of the PVL-f evaluation, a few more MS-plus patients did not fulfil the 52% threshold resulting n=35 (59% of the cases; data not shown), also very close to the primary analysis (22% and 71% respectively, p= NS) and the accuracy of the MS diagnostic criteria in these patients also resulted similar to that in the whole MS-plus population.

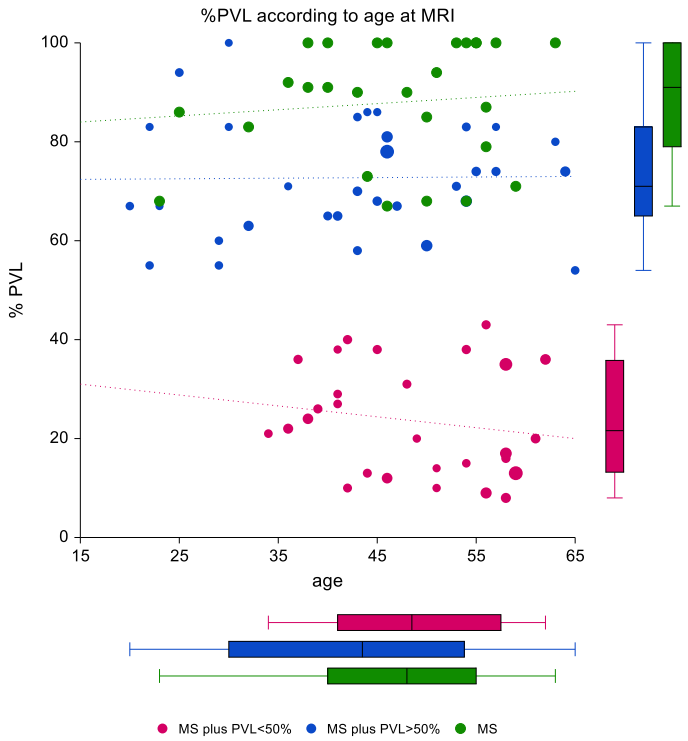


Figure 5.4. Periventricular lesion frequency of each patient plotted according to age in the typical MS group (green) and in the MS-plus fulfilling (blue) or not (red) the 50% rule (PVL-f>50%). Dot size is proportional to the total > 3 mm lesion number/ patient. Vertical and horizontal bars represent median quartiles and range of PVL-f and age, respectively. No correlation between PVL-f and age was observed in the three groups (Spearman's correlation coefficient r: -0.22 for MS-plus PVL-f<50%, p=0.38; 0.01 for MS-plus PVL-f>50%, p=0.96; 0.12 for typical MS, p=0.58). Regression lines of the PVL-f versus age in each group are also reported.

5.3 Secondary Endpoints

Clinical differences

MS and MS-plus cases: All patients fulfilled the MS MRI criteria for DIS (McDonald et al., 2001; Poser & Brinar, 2001; Thompson, Banwell, et al., 2018a; Winsen et al., 2010b). Clinical and demographic characteristics at inclusion of the Typical MS and of MS-plus patients are shown in **Table 5.1.** Remarkably higher ARR of the first decade after disease onset (0.2 vs 0.1; p<0.006) was observed in Typical MS. The longer disease duration in the Typical MS patients instead shouldn't be of note due to the different inclusion criteria (> 5 years for MS).

MS-plus subgroups: The comparison of clinical and brain MRI characteristics in MS-plus subgroups stratified according to the 52% threshold of PVL-f is shown in table **5.3** and **5.4.** The MS-plus not fulfilling the 52% threshold showed a higher rate of cardiovascular risk factors compared to the other (50% vs. 19%, respectively; p=0.03). Arterial hypertension (29% vs. 3%; p=0.027; **Table 5.4**) and patent foramen ovale (p=n.s.) were the most represented risk factor in MS-plus patients not fulfilling the 52% rule. The two groups differed for the frequency of the patients who had received a DMT and for time to first DMT administration. Notably, the number of MS-plus fulfilling the 52% threshold who received DMTs was similar to that in the typical MS group (94% and 96% of patients, respectively; **Table 5.1 and Table 5.4, fig 5.5**). Between the MS-plus group fulfilling or not the 52% threshold, a remarkable difference in the number of patients treated and in time to first treatment was observed as the MS-plus with PVL-f<52% generally received a disease-modifying treatment (DMT) later (median 1.7 years vs. 0.2 years, p< 0.0001) and in lower proportion (54% vs. 94% of patients, p=0.0007), than in those fulfilling the >52% rule (**Table 5.4**). Disease course, in terms of annualized relapse rate, was similar between the two groups (**Table 5.4**).

Differences in red flags distribution in MS-plus subgroups

Distribution in MS-plus patients of the red flags selected is reported in **Table 5.4**. After adjustment for multiplicity the only red flag present differing between the two MS-plus groups categorized by the >52% PVL-f threshold was the number non-MS typical WMLs observed at the conventional MRI, higher in MS-plus with PVL-f<52%.

The total number of any specific category of "red flags" per patient (clinical or laboratory), or the frequency per patient did not differ between the two MS-plus groups.

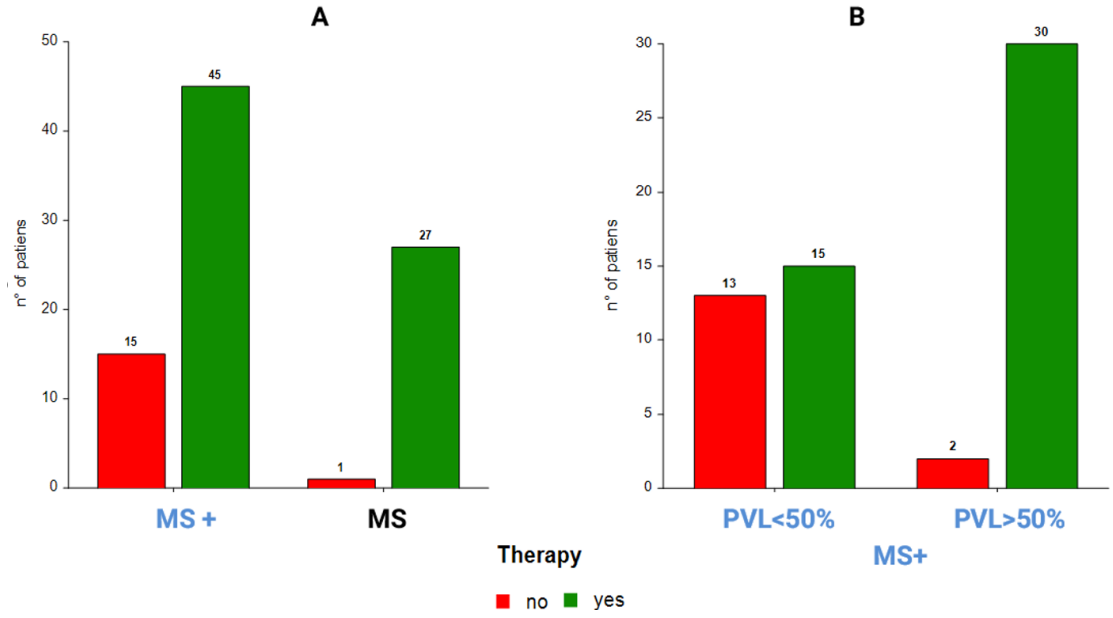


Figure 5,5: A Patients on DMT among MS-plus and MS. 96% (27/28) of patients in the MS group receive a DMTs, which however occurs only in 76% (45/59) of cases in the MS-plus group. The difference between the two groups is significant (p=0.016). **B**. Analyzing the MS-plus subgroups, it can be seen that, however, the MS-plus subgroup with PVL>52% receives a DMTs in 97%(30/31) of cases, similarly to the MS group, while in the MS-plus subgroup with PVL-f<52% treatment occurs only in 46% (13/28) of patients (p<0.001).

Table 5.4 Demographic, clinical, laboratory and MRI characteristics in MS-plus subgroups identified according to the fulfilment of the 52% PVL-f threshold.

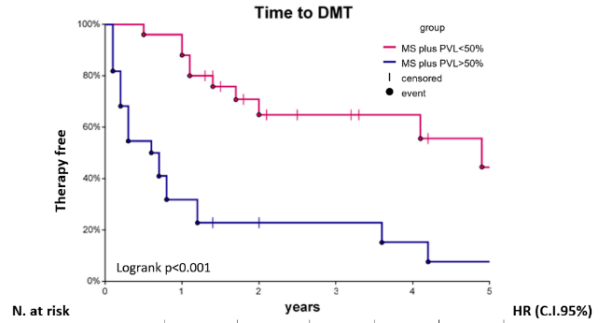
MS-PLUS GROUP Characteristics		**PVL<52% n=28**	**PVL>52% n=31**	**P values** (adjusted for multiplicity)
Clinical-demographic				
Female	*n(%)*	26 (93%)	25 (80%)	p= 0.9 $^{\chi}$
Age at inclusion, years	median *(range)*	48,5 (34-62)	43,5 (20-65)	p= 0.42^{u}
Age at disease onset, years	median,*(range)*	37,8 (23,6-58)	38,2 (14,9-62,4)	p= 1.0 ^{u}
Disease duration, years	median *(range)*	7,4 (0,4-2 8,4)	3,8 (0,2-24,8)	p= 0.9 ^{u}
Patients with onset age ≥50 years	*n(%)*	5 (18%)	5 (16%)	p= 1,00 $^{\chi}$
Patients aged ≥ 50 years at inclusion	*n(%)*	13 (46%)	10 (32%)	p=0.9 $^{\chi}$
EDSS[1] score at onset	median *(range)*	0 (0-2,5)	0 (0-1,5)	p= 0.9 ^{u}
EDSS score at inclusion	median *(range)*	1 (0-5,5)	1 (0-4,5)	p=1.0^{u}
Time to EDSS worsening, years	median *(range)*	4,1 (0-20,8)	2,6 (0-20,8)	p= 1,0 ^{u}
Time to DMT[2] start, years	median *(range)*	1,7 (0-28,4)	0,2 (0-6,2)	p= **0,00003** ^{u}
Patients treated with DMT	*n(%)*	15 (54%)	29 (94%)	p= **0,0007** $^{\chi}$
Time to First Relapse, years	median *(range)*	2,1 (0,5-18,1)	1,6 (0,5-20,8)	p= 1.0 ^{u}
AAR[3] over 10ys from onset	median*(range)*	0,1 (0-0,5)	0,1 (0-0,4)	p= 1,0^{u}
	mean (SE)	0,1 (0)	0,1 (0)	
Cardiovascular risk factors n(%)				
Patient with any CVRF[15]		14 (50%)	6 (19%)	p= **0,03** $^{\chi}$
Arterial Hypertension		8 (29%)	1 (3%)	p= **0,027** $^{\chi}$
Patent foramen ovale		5 (18%)	1 (3%)	P= 0,09
Other*		11 (39%)	6 (19%)	p= 0,27 $^{\chi}$
Red flags				
Red flags/patient	median *(range)*	2 (1-3)	1,5 (1-3)	p= 1.0 ^{u}
Clinical red flags n(%)				
Recurrent abortion		1 (4%)	3 (10%)	p=0.62 $^{\chi}$
Uveitis		1 (4%)	1 (3%)	p=1.00 $^{\chi}$
Arthralgias		4 (14%)	2 (6%)	p=0.40 $^{\chi}$
Sicca sindrome		2 (7%)	1 (3%)	p=0,59 $^{\chi}$
Recurrent aphthosis		3 (11%)	1 (3%)	p=0,33 $^{\chi}$
Raynaud's phenomena		0	3 (10%)	p=0,24 $^{\chi}$
Livedo reticularis		0	1 (3%)	p=1,00 $^{\chi}$
N° patients with clinical red flags		14 (50%)	18 (58%)	p=0,79 $^{\chi}$
Serological red flags n(%)				
ANA[4]+		12 (43%)	11 (35%)	p=0,59 $^{\chi}$
Coagulation factors polymorphisms		2 (7%)	2 (6%)	p=1,00 $^{\chi}$
C3 and/or C4 reduction		2 (7%)	3 (10%)	p=1.00 $^{\chi}$
Persistent increase of ESR[5]/CRP[6]		6 (21%)	4 (13%)	p=0,49 $^{\chi}$
Antiphospholipid-AB[7] + and/or LAC[8]+		5 (18%)	4 (13%)	p=0,72 $^{\chi}$
ANCA[9]+		2 (7%)	2 (6%)	p=1.00 $^{\chi}$
ENA[10]+		2 (7%)	2 (6%)	p=1.00 $^{\chi}$
Other AB (anti GS[11], recoverin, ASMA[12])		1 (4%)	1 (3%)	p=1.00 $^{\chi}$
Patients with serological red flags		9 (31%)	14 (45%)	p= 1.0 $^{\chi}$
CSF[13] red flags *n(%)*				
Negative OCB[14] in 1 spinal tap		7(25%)	4(13%)	p=0,32$^{\chi}$
Negative OCB in ≥ 2 spinal taps		2(7%)	6(19%)	p=0,26 $^{\chi}$
Total N° patients with CSF red flags		9(32%)	10(32%)	p=1,00 $^{\chi}$
MRI red flags				
Non MS-typical lesions**		19 (68%)	12 (36%)	**p=0,02** $^{\chi}$
Patients with MRI red flags *n(%)*		19(68%)	12 (39%)	p= 0,12 $^{\chi}$

$^{\chi}$ Fisher exact χ^2 test; ^{u} Mann-Whitney U test;[1] **EDSS** expanded disability status scale [2] **DMT** disease modifying therapy [3] **AAR** annualized relapse rate; [4]**ANA:** antinuclear antibodies; [5]**ESR**: erythrosedimentation rate; [6]**CRP:** C reactive protein; [7]**AB:** antibodies, [8]**LAC:** lupus anticoagulant; [9]**ANCA**: anti-neutrophilic cytoplasm antibodies, [10]**ENA**: anti extractable nuclear antigens antibodies, [11]**GS** gangliosides, [12]**ASMA**: anti smooth muscle antibodies [13] **CSF:** cerebrospinal fluid; [14]**OCB:** oligoclonal bands; [15] **CVRF** cardiovascular risk factors; * Diabetes, dyslipidemia, hyperhomocysteinemia, polymorphism of coagulation factors, smoking , **prevalence of WML<3mm diameter and/or atypical location of WML (prevalence of subcortical/deep white matter sites) were the only MRI red flags found

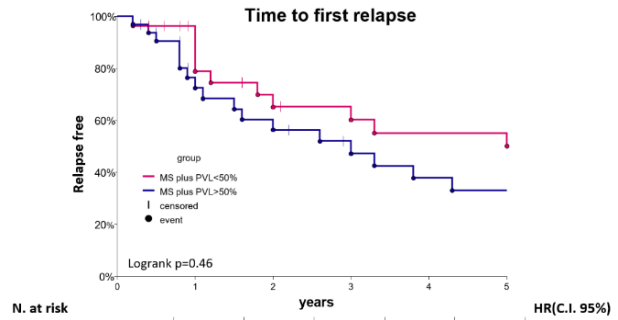
Survival analysis

Survival analyses were conducted among the three groups of patients (typical MS, MS-plus <52%PVL-f, MS-plus >52% PVL-f) to evaluate the time to first relapse, the time to first EDSS worsening and the time to DMTs starting.

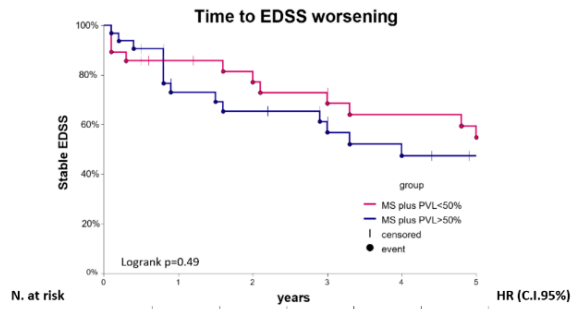
- ***Time to first relapse***: no significative difference emerged for the global comparison nor for the comparison between paired subgroups (Logrank test p=0.1, **Fig**. **5.6**)
- ***Time to first EDSS worsening*** (TEW): in the MS-plus with PVL-f <52% group the TEW resulted longer in the comparison with typical MS (Logrank test p=0.0491). However, no differences were found with MS-plus with PVL-f>52% and in the global comparison of the three groups (**Figure 5.6**)
- ***Time to first treatment with DMT***: at timepoints of 1, 5 and 10 years the percentage of patients not treated with DMT is of 83%, 48% and 48% respectively in the MS-plus with PVL-f <52% and of 39%, 13% and 4% respectively in the MS-plus with PVL-f>52% group, with a significative difference among the two groups (Logrank test p<0.0002, **Figure 5.6**).



N. at risk							HR (C.I.95%)
MS plus PVL<50%	28	24 (88%)	12 (65%)	9 (65%)	7 (65%)	4 (44%)	0.7(0.2-1.3)
MS plus PVL>50%	32	7 (32%)	4 (23%)	3 (23%)	2 (15%)	1 (8%)	2.2(0.8-3.5)



N. at risk							HR(C.I. 95%)
MS plusPVL<50%	28	22 (79%)	15 (65%)	13 (60%)	11 (55%)	11 (50%)	0.7(0.3-1.1)
MS plus PVL>50%	32	19 (72%)	15 (56%)	11 (47%)	8 (38%)	7 (33%)	1.1(0.5-1.6)



N. at risk							HR (C.I.95%)
MS plus PVL<50%	28	21 (86%)	19 (77%)	17 (69%)	14 (64%)	13 (55%)	0.6(0.2-0.9)
MS plus PVL>50%	32	19 (73%)	17 (65%)	14 (57%)	11 (47%)	8 (47%)	0.7(0.3-1.1)

Fig. 5.6 Kaplan Meyer survival analysis for time to first relapse (A), time to DTM start (B) and time to EDSS worsening (C) in MS plus subgroups stratified according to 50% rule. While there are no significant differences in time to first relapse and time to EDSS worsening, MS plus patients with PVL<50% are treated significantly less and later compared to patients with PVL> 50% (p<0.001). *DMT:* disease modifying therapy, *EDSS:* expanded disability status scale; *PVL:* perivenular lesions.

Conventional MRI lesion analysis

Characteristics of the WMLs evaluated by conventional MRI in the Typical MS and in the MS-plus patients and in the MS-plus patients categorized according to the 52% threshold, are shown in **Table 5.2** and **5.3** respectively.

The ≥3 mm (in largest dimension) WMLs number/patient of the whole MS-plus group, did not differ from that of the Typical MS cases (**Table 5.2**), whereas average volume/lesion and total volume/patient was remarkably smaller in the MS-plus ($p<10^{-8}$ and p= 0,004 respectively **Table 5.2**), as expected because of the shorter disease duration (**Table 5.1**). However, in the two MS-plus groups fulfilling or not the 52% PVL-f threshold the ≥3 mm (in largest dimension) WMLs profoundly differed as for lesion size, site, volume/patient, and number/patient, despite the similar age (**Table 5.3**). Then most of the conventional brain MRI parameters examined remarkably differed between these two groups, but noteworthy each overlapping enough to prevent sufficient accuracy to detect true MS cases (data not shown).

The brain distribution of the ≥3 mm (in the largest dimension) WMLs of the MS-plus and of the Typical MS cases, is shown in **Table 5.2**, whereas the same analysis for the MS-plus group fulfilling or not the 52% PVL-f threshold is reported in **table 5.3** and **Figure 5.7**. In the Typical MS patients and in the MS-plus fulfilling the 52% threshold only a little proportion of the WMLs (38 and 40% respectively) were in the subcortical/deep white matter (**Table 5.3, figure 5.7**), the areas relatively less common in the Typical MS cases. Instead in the MS-plus not fulfilling the 52% PVL-f threshold, 81% of the WMLs, were located in these regions ($p<10^{-7}$ for both comparisons). In the MS-plus cases not fulfilling the >52% PVL-f threshold the number of patients with the majority of the WMLs located in the subcortical/deep white matter was higher (19/28; 68%) than in those fulfilling the threshold (12/31; 39% p< 0.02, **Table 5.3**), in this last group being similar to the typical MS cases (**Table 5.3**). In this patient population, atypical brain MRI lesions (majority of subcortical/deep white matter lesions and/or majority of small lesions) were absent in 20/31 (63%) of the MS-plus fulfilling the >52% PVL-f threshold and in 9/28 (27%) of the other MS-plus patients corresponding to a PPV of PVL-f > 52% =0.63.

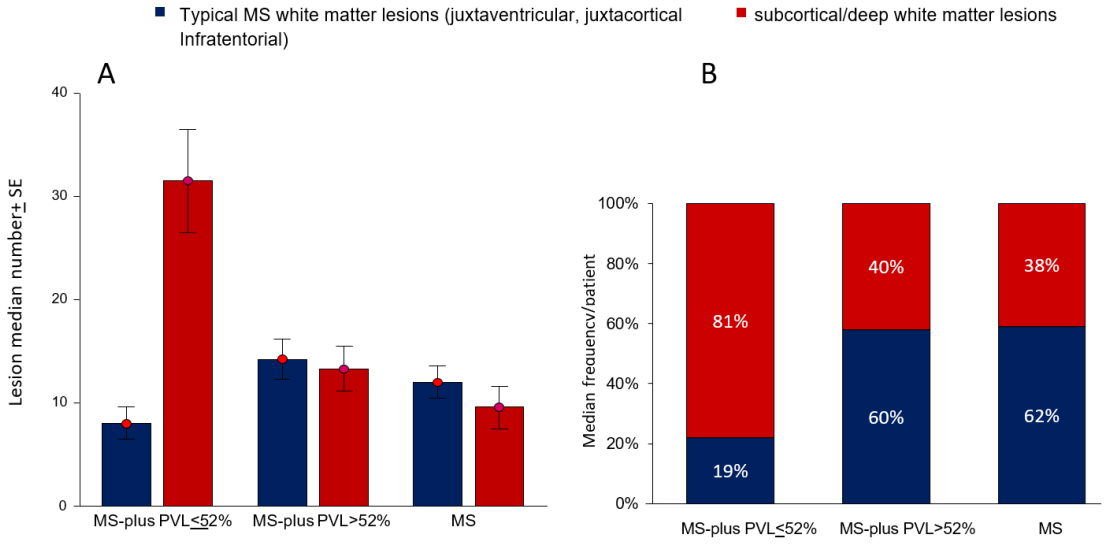


Fig. 5.7. Brain site distribution of white matter lesions (WMLs) in patients fulfilling or not the 52% PVL threshold. WML have been classified topographically into two categories: typical of MS (blue) or not typical (red). Figure **A** shows the median number of MS typical WML/patient. Figure **B** shows the median WML topographical proportion in the different groups. Data show that MS plus patients fulfilling the 52% threshold have a virtually identical topographical distribution of WML to those with typical MS. On the contrary, compared to these two groups, the MS plus patients who did not fulfil the 52% threshold have a large majority of subcortical/deep white matter lesions ($p= 10^{-7}$), not specific for MS, mainly found in case of periarteriolar ischemic lesions.

The number of non-PVL ≥3mm WMLs/patient in the MS-plus cases fulfilling or not the 52% PVL-f threshold was 6 (range 0-24) and 18 (range 5-85) respectively ($p< 0.0006$; **Table 5.3**), in those fulfilling the 52% threshold resulting remarkably closer to the Typical MS (n= 2, range 0-9, **Table 5.3**).

Differences between the two MS-plus groups of patients generated by the 52% threshold were evaluated taking in account age related brain white matter hyperintensities (mainly small vessel disease). A remarkable difference between the MS-plus cases categorized according to the 52% PVL-f threshold was observed in the number of all non PVL lesions (< and > 3 mm; **Table 5.3**) and in the WMLs ≤3 mm (in largest dimension; "small WMLs") number, whereas in the patients older than 50 y of these groups (n= 23), the median number of small WMLs did not differ (**Table 5.2**). As expected, the total number of small WMLs in the patients older than 50 was higher than in younger MS-plus patients (not fulfilling the 52% PVL-f threshold group: median 10; fulfilling the threshold: median 4; p=0.02, Mann Whitney U test) but according to the sensitivity analysis in these patients the difference between the PVL-f >52% threshold stratified groups, remained highly significant: median 5 and 2, respectively, p=0.006). In addition, in the MS-plus cases not fulfilling the >52% PVL-f threshold, there was no correlation between the number of small

WMLs and PVL-f (p=0.7), whereas, in the other MS-plus group, a moderate inverse correlation was observed (ρ=-0.48, p<0.006; **Figure 5.8**).

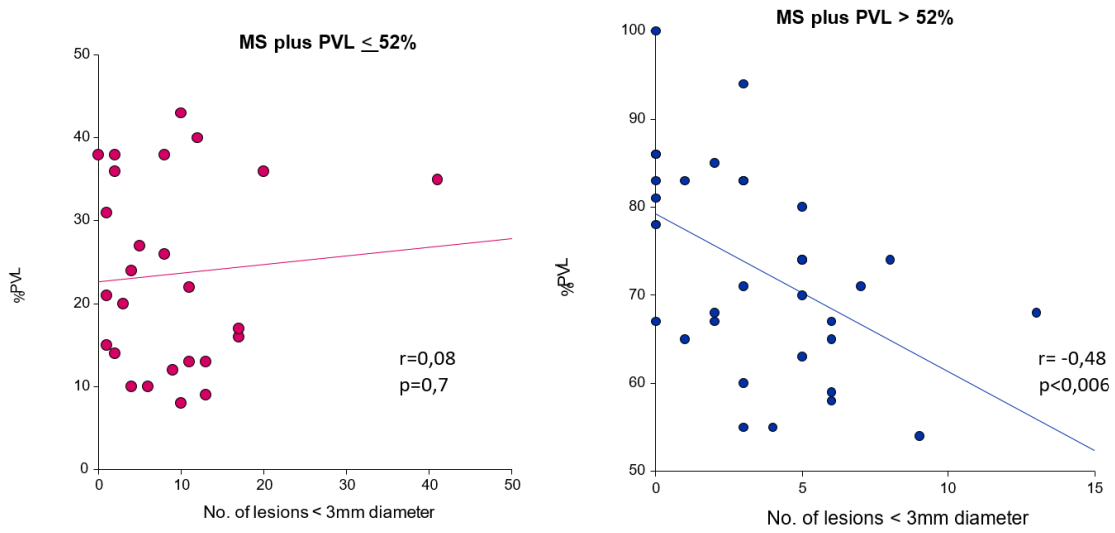


Fig 5.8. Correlation between the PVL-f (%PVL) and the number of WML < 3mm. Inverse correlation was found in the PVL-f>52% MS plus group (r= -0.48, p<0.006, Spearman correlation), indicating that in these patients, some non PVL lesions > 3 mm, probably periarteriolar ischemic lesions, are present explaining why not all the MS patients had 100% PVL-f. Absence of any correlation between PVL-f and WML < 3mm in the PVL-f ≤52% MS-plus patients, suggests that some WML are PVL just by chance and that are not PVLs.

Ophthalmological evaluation

26 eyes of 13 patients affected by typical-MS were evaluated. Four patients had an history of optic neuritis in one eye and two patients developed optic neuritis in both eyes (total 8 eyes). Regarding MS-plus were evaluated 48 eyes of 24 patients. Seven patients of this group had an history of monolateral optic neuritic (total 7 eyes). Out of the MS-plus patients, 12 were included in Group 1 (less than 52% PVL-f) and 12 in Group 2 (>52% PVL-f). The results of ophthalmological evaluation with OCT and OCT-A are shown in **5.5** and **5.6.** A significant difference emerged in the temporal RNFL quadrant, relatively spared in MS-plus with PVL<52% compared to the other two groups (p<0.001, **table 5.5**). Temporal RNFL thickness has already been described in MS (Klistorner et al., 2017): the preferential loss of axons in the temporal quadrant of the RNFL may be due to the fact that this region allows central vision and consists primarily of small parvocellular axons, which are likely more susceptible to damage in MS than larger magnocellular axons (Evangelou et al., 2001). As expected, we found that optic neuritis (ON) was more common in typical MS patients (50% eyes), as compared to MS-plus PVL-f> 52% (33.3%) and MS-plus PVL-f< 52% (16.7%) (p = 0.050) (Table 4). We found having known ON episodes was only marginally associated with temporal RNFL thickness (p = 0.085), and no association with other OCT or OCTA parameters. Such non-significant association was even weaker with

multivariate regression, once MS subgroups were considered, which can be explained by the fact that the actual optic nerve damage was better represented by MS subgroup.

Structural OCT (mean, SD)	Typical MS	**MS-Plus** PVL-f**>52%**	**MS Plus** PVL-f**<52%**	**p-value trend**	**p-value groups**
CFT	270(19)	267(14)	277(17)	0,536	0,604
RNFL superior quadrant	108 (22)	106(18)	110(23)	0,793	0,872
RNFL inferior quadrant	112 (24)	115(18)	118(20)	0,479	0,778
RNFL temporal quadrant	**56(13)**	**67(15)**	**74(13)**	**<0.001**	**<0.001**
RNFL nasal quadrant	62(14)	65(16)	69(15)	0,226	0,469
GCC superior sector	89(12)	86(11)	93(10)	0,446	0,330
GCC inferior sector	93(16)	88(12)	95(10)	0,594	0,275

Table 5.5. CFT (central foveal thickness), RNFL (retinal nerve fiber layer), GCC (ganglion cell complex).

Interestingly, in our sample we found that the deep capillary plexus (DCP) was reduced in the MS-plus group PVL-f<52% both for the whole and outer vessel densities, compared to the MS-plus>52% PVL-f and the typical MS. The vessel density of inner DCP demonstrated a borderline trend of reduction in the MS-plus group 2 compared to the other groups (**Figure 5.9**). This data is interesting, since in previous studies emerged that especially the superficial capillary plexus (SCP) was the one most affected by the vessel density reduction in MS. In particular, the reduction of DCP vessel density in MS-plus patients, suggest an impaired retinal and peripapillary blood flow, and consequently we could hypothesize a different pathogenesis for atypical MS. We also found a significant reduction on optic nerve head (ONH) vessel density for whole vessel density and RPCP vessel density in group 2, compared to group 0 and 1, reflecting a higher involvement of microvascular structures in MS-plus patients PVL-f<50% (**Figure 5.10**).

OCT-Angiography Mean (SD)	Typical MS	**MS-Plus** PVL-f**>52%**	**MS Plus** PVL-f**<52%**	**p-value trend**	**p-value groups**
FAZ area	0,26 (0.09)	0,23(0.09)	0,25(0.04)	0,812	0,66
FAZ perimeter	2,6(0.49)	2,3(0.44)	2,6(0.33)	0,752	0,184
FAZ circularity	0,46(0.08)	0,52(0.14)	0,48(0.1)	0,631	0,39
OCTA SCP whole	17(2.2)	17(3)	15(4.3)	0,154	0,317
OCTA SCP inner	18(2.3)	17(3.3)	15(4.6)	0,111	0,262
OCTA SCP outer	19(2.5)	18(2.6)	17(4.6)	0,213	0,411
OCTA DCP whole	**17(3.6)**	**15(5.4)**	**13(7.4)**	**0,051**	**0,148**
OCTA DCP inner	18(3.7)	15(6.2)	14(7.9)	0,068	0,189
OCTA DCP outer	**20(4)**	**17(5.6)**	**15(7.8)**	**0,029**	**0,09**
Optic disc whole density	**19(1.1)**	**18(1)**	**18(1.1)**	**0,029**	**0,063**
Optic disc RPCP whole density	18 (1.4)	18(1.7)	18(1.7)	0,331	0,459
Optic disc lamina cribrosa	19(1.3)	20(1.3)	19(1.5)	0,998	0,085

Table 5.6. FAZ (foveal avascular zone), OCTA (optical coherenze tomography angiography), SPC (superficial capillary plexus), DCP (deep capillary plexus), radial peripapillary capillary plexus (RPCP).

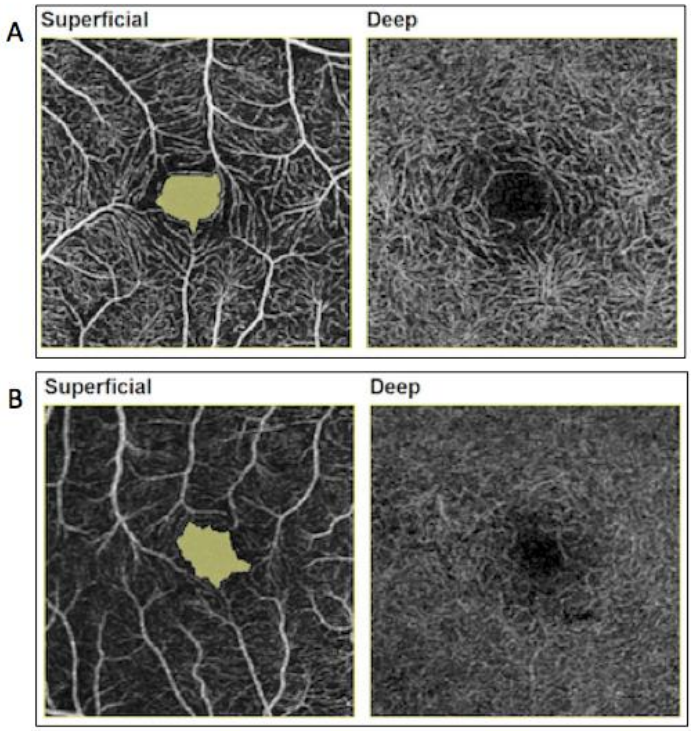


Figure 5.9. Automated FAZ detection on the superficial capillary plexus (SCP). Qualitative evaluation of macular vessel density, which revealed a lower vessel density both of superficial capillary plexus (SCP) and deep capillary plexus (DCP) in a MS-plus patient (B) compared to MS patient (A). In our cohort we only found a statistically significant reduction in DCP vessel density in group 2 (MS-plus < 50% PVLs).

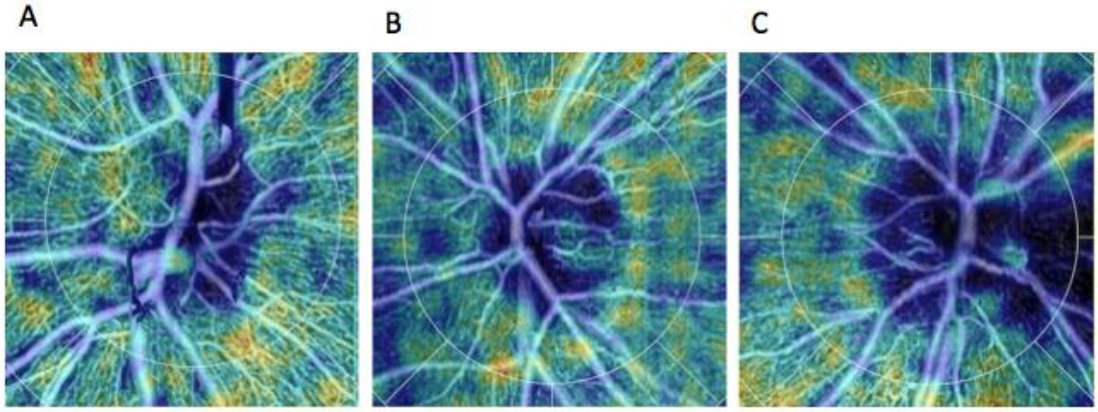


Figure 5.10. OCTA perfusion images of the optic nerve head of the radial peripapillary capillary plexus (RPCP). Vessel density in three different patients with a history of optic neuritis. We can see a vessel rarefaction from image A (group MS) to image B and C (group MS-plus > 50% perivenular lesions and group MS-plus < 50% perivenular lesions, respectively).

CHAPTER 6

DISCUSSION AND CONCLUSIONS

6.1 DISCUSSION

In this study, patients fulfilling the current and most accurate diagnostic criteria for MS carrying clinical, imaging, or laboratory markers of a potential alternative explanation (but not fulfilling diagnostic criteria for a diagnosis different from MS), were evaluated for the frequency of brain PVL, the MRI-identifiable correlate of the same MS pathology and compared with Typical MS patients (with disease duration long enough to allow accurate exclusion of better explanation of the diagnosis) and with non-MS patients carrying MS-like brain lesions.

Our main finding is that in setting a threshold of 52% PVL-f, based on ROC analysis, for Typical-MS cases optimal accuracy of the DIS/DIT based MS diagnostic criteria is observed. However, in atypical "MS-plus" patients, this threshold is met in 52.5% of the patients only, as in these patient population PPV and specificity of MS diagnostic criteria based on DIS and DIT are low (0.53 and 0.52 respectively), thus indicating that in this patients population these criteria may frequently lead to MS misdiagnosis.

As the specificity of the MS diagnostic criteria in MS-plus is about 50%, assuming that "red flags" are present in ~40% of patients with DIS and DIT and MS-like brain lesions visualized by MRI (3-9), the frequency of misdiagnosis expected in the clinical practice using DIS/DIT based diagnostic criteria for MS can be estimated around 20%, a figure very close to that previously estimated by expert opinions (Kaisey et al., 2019a; Midaglia et al., 2021; Solomon & Weinshenker, 2013).

More importantly, the risk of misdiagnosis in new demyelinating syndromes with red flags is also about 50% as this was the positive predictive value of the MS diagnostic criteria observed in MS-plus, a result confirming previously observation (Maggi et al., 2020).

MS-plus patients fulfilling the 52% PVL-f threshold, had a median PVL-f and showed WMLs size similar to that of the Typical MS controls (despite a shorter disease duration) as well as brain location, indicating that these patients are indeed true MS cases, whereas the MS-plus patients not fulfilling the 52% PVL-f threshold had a median PVL-f and showed and WMLs size as well as brain location similar to that of the Not-MS controls indicating that these syndromes are not based on this MS specific pathological marker.

Noteworthy the 52% PVL-f threshold is remarkably close to the 50% PVL-f threshold previously suggested as the "Central Vein Sign", the most accurate PVL threshold for categorizing MS-like demyelinating syndromes as true MS. Indeed, in MS-plus patients other MRI detected pathological marker as brain WMLs size (usually smaller) and location (predominantly the subcortical deep white matter) have low accuracy, for detecting True MS. Furthermore, no other red flag, clinical or laboratory, distinguished typical MS from MS-plus cases, confirming that new pathognomonic markers of true MS were eagerly needed (Solomon, 2019; Thompson, Banwell, et al., 2018a).

Moreover, it is of note that PVL-f assessment was obtained on 1.5T MRI system with optimal inter-rater agreement. 1.5T MRI is a widely available standard device, differing from a 3T setting only as for evaluation time, indicating that this approach could be widely applied in clinical practice.

As for the clinical course at baseline, in the MS-plus cases the ARR of the first 10 years after diagnosis was lower than in Typical MS, despite several patients in MS-plus group don't receive DMT, suggesting clinical differences with typical MS. A possible explanation for this difference is that a group of MS-patients (probably among those with PVL-f<52%) has an underlying alternative disease with a milder course than MS. Accordingly, patients under the 52%-threshold were less likely to be treated with DMT indirectly indicating that in these cases -despite formal fulfilment- the MS diagnostic criteria were correctly applied cautiously by the clinicians in charge.

The PVL-f was not affected by patient age and WMLs size, indicating that age-related small vessel disease did not drive the low PVL-f in the MS-plus not fulfilling the 52% PVL-f threshold. In addition, this group showed a higher rate of cardiovascular risk factors than in the other and the WMLs were more, smaller and more likely to be located in the MS atypic regions (subcortical/deep white matter) (Filippi et al., 2019)). As small brain WMLs result mainly from micro-ischemic mechanisms and are commonly found in the subcortical and deep WM (Cannistraro et al., 2019), it can be hypothesized that in these MS-plus patients the WMLs analysed are mostly due to some form of ischemic and/or inflammatory

microangiopathy, and that the few of them that were found to be perivenular were instead random events not associated with perivenular demyelination.
The red flag more frequently associated to the MS-plus status was atypical WMLs location detected by conventional MRI. This indeed was also one the MRI marker that also differed between the MS-plus groups categorized according to the 52% PVL-f threshold.
Although in the study no definite alternative diagnosis were found in MS-plus patients with PVL-f<52% encouraging data emerged concerning the utility of OCT-A evaluation: only MS-plus patients with PVL-f<52% showed a reduction in vessel density of deep capillary plexus (DCP), suggestive of altered retinal and peripapillary blood flow and thus of a possible microvascular pathogenesis. Accordingly, a different distribution of damage is observed in MS and MS-plus with PVL-f>52% (involvement of superficial capillary plexus-SCP), supporting a different pathological mechanism. A microvascular damage in MS-plus patients with PVL-f<52% was also supported by the finding of significant reduction in ONH and RPCP vessel density, compared to MS-plus with PVL-f>52% and typical MS.

The limit of this study is the sample size that may prevent full generalizability of the new findings. In addition, although the study clearly indicates that with the current MS diagnostic criteria a proportion of MS-plus patients are misdiagnosed, it does not shed light on alternative diagnoses that in the MS-plus patients with PVL-f $\leq$52% may be responsible for the MS-like syndrome. Il can be expected that this will prove to be a heterogeneous group. However, it must be noted that the majority of the WMLs of these patients were small and located in the subcortical deep WM, as commonly found in disorders other than MS such as small vessel arteriopathies, further supported by OCT-A preliminary findings (Maggi et al., 2018b). The possibility that these diseases may represent a source of MS misdiagnosis is supported by the observed segregation in this group of higher cardiovascular risk factors as comorbidities and confirms the observations of a previous study (Guisset et al., 2021).

6.2 CONCLUSIONS

In conclusion, our study demonstrates that in MS-plus patients (demyelinating syndromes carrying red flags of better explanation as atypical clinical MRI, and/or laboratory features but not formally fulfilling the diagnostic criteria of any other neurological disorder), the performance of the MS diagnostic criteria based on MRI DIS and DIT in terms of positive predictive value and of specificity, is low. These data explain the rate of MS misdiagnosis reported in the literature(Sati et al., 2016; Solomon, Naismith, et al., 2019; Solomon, Schindler, et al., 2016; Solomon & Corboy, 2017) and indicate for the first time that this group of patients is

that at higher risk. It also identifies for the first time in these patients the optimal PVL-f threshold representing a simple and feasible tool for detect MS-plus cases that are unlikely to have true MS.

These data provide evidence-based support for including the PVL evaluation in future iterations of the MS diagnostic criteria, as a MS-specific biomarker.

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