SAPIENZA
UNIVERSITÀ DI ROMA

DIPARTIMENTO DI NEUROSCIENZE UMANE

CORSO DI DOTTORATO IN

NEUROSCIENZE CLINICO-SPERIMENTALI E PSICHIATRIA

CURRICULUM NEUROSCIENZE SENSORI-MOTORIE

COORDINATORE: PROF.SSA CRISTINA LIMATOLA

TESI DI DOTTORATO

Effects of disease modifying therapies on anti-JCV antibody status in a large population of Multiple Sclerosis patients

Relatori

Prof. Enrico Millefiorini

Prof. Francesco Patti

Dottoranda

Dott.ssa Eleonora Sgarlata

Anno accademico 2019-2020

Summary

MULTIPLE SCLEROSIS

1.1 Introduction

Multiple sclerosis (MS) is a chronic predominantly immune-mediated disease of the central nervous system (CNS) with inflammatory aetiology, although genetic and environmental factors cannot be discounted, and it is one of the most common causes of neurological disability in young adults globally [1]. MS has a complex aetiology involving a dysregulated immune system with bouts of peripherally mediated inflammation, as well as ongoing CNS compartmentalized inflammation leading to loss of neural tissue and worsening disability due to persistence of immune cells and their activation both around perivascular lesions and in the meninges [2].

The typical MS lesions are multiple perivascular white matter demyelinating plaques associated with various degrees of inflammatory cells [3]. Additionally diffuse neurodegeneration and plaque-like demyelination have also been described in the deep and cortical grey matter [4,5].

MS is clinically characterized by episodes of relapses with worsening of symptoms and subsequently return to a well-being condition that can last also for a long time. Recent research into the complex pathophysiology of MS has yielded several key observations that underscore the need for improved diagnosis leading to early treatment. Disease progression has been shown to occur in the absence of clinical relapses, and even early relapses that appear relatively benign may have permanent neurological consequences. Key opinion leaders have concluded that MS is a neurodegenerative disease associated with a deregulated inflammatory cascade of life-changing symptomatology and cognitive dysfunction resulting in an economic burden that affects patients, providers, and society as a whole. Aggressive and early treatment with disease-modifying therapies (DMTs), as recommended by the National MS Society, has begun to demonstrate positive long-term outcomes, a potential for reduced future disease activity, and improved patient quality of life.

1.2 Epidemiology

The World Health Organization (WHO) estimated that over 2.5 million people globally suffer from MS and with the present global population growing it is estimated to reach 8.5 billion by 2030; the incidence and onset of MS in young adults are expected to rise exponentially. It is estimated that over 400,000 people have MS in the United States, with 700,000 Europeans and 23,000 Australians being also affected with >200 new cases being diagnosed each week in the United States alone [6]. The distribution of MS varies according to geographic location. For example, the further north or south from the equator the higher the prevalence of MS; countries that lie on the equator have extremely low prevalence compared to Scotland, Norway, and Canada [7]. Italy is classified as a high-risk area for MS [8], with the highest rates in the island of Sardinia in which population is characterized by an elevated risk to autoimmune diseases, such as MS, due to an homogeneous genetic background coming from past isolation from other population [9,10].

MS is commonly diagnosed between 20 years and 40 years of age although it can affect younger and older individuals [11]. Females have greater susceptibly to MS, while males have worse disease progression and these two characteristics of the disease are influenced by the immune system and the nervous system, respectively [12]. The female-to-male ratio in MS varies somewhat by geographic region from 2:1 to 3:1, and has increased in the past decades, with a few notable exceptions [13]. Several studies have also showed that being male was associated with shorter time to reaching a moderate/high level of disability progression [14–16].

1.3 Etiopathogenesis

Although the triggers of MS remain unknown, its pathogenesis is best explained by a multifactorial model that incorporates interactions between genetic, epigenetic, and infectious, nutritional, climatic, or other environmental influences including Epstein Barr virus (EBV) infection, sun light exposure and smoking [17]. This array of factors results in the loss of immune homeostasis and self-tolerance manifested in brain and spinal cord infiltration by activated peripheral mononuclear cells, and the development of unregulated pathologic inflammatory responses against structural components of the CNS. Myelin loss, gliosis, and the resulting axonal pathology culminate in progressive, often severe neurological dysfunction [18].

Genetic factors. Epidemiological studies have shown that genetic factors are primarily responsible for SM predisposition [19], and linkage studies in multiplex families have confirmed that variation within the Human Leukocyte Antigen (HLA) region exerts the greatest individual effect on MS risk [20,21]. The strongest consistent linkage and association with MS is with extended haplotypes of the MHC, particularly those containing HLA-DRB1*1501 [22]. The recent use of genome wide association studies provided new tools for a better understanding of MS aetiology. Genome-wide association studies (GWAS) using single nucleotide polymorphisms (SNPs) from the HapMap project allowed the use of an unbiased approach in scanning the whole genome identifying many genes implicated in MS immune regulation [23] and in particular SNPs associated with disease [24,25]. To date, GWASs have defined 194 genetic variants that are associated with MS, with that number likely to rise to over 400 [21].

The effects of genetic sharing, parent of origin, intergenerational versus collinear differences, and gender on the ages of onset were evaluated by Sadovnik et all [26] in heterogeneous relative pairs from a Canadian population base of 30,000 MS index cases: monozygotic or dizygotic twins, siblings, first cousins and parent/child. Results showed that a subject affected by MS had about 15% of probability of having a relative affected by the same disease and that the percentage of concordance in monozygotic twins was significant (20.1%) with a higher incidence in female (34%) that in male twins (6.5%), while in dizygotic twins pairs it is similar to that between non-twin siblings.

Environmental factors. Although genetic susceptibility explains the clustering of MS within families and the sharp decline in risk with increasing genetic distance, it cannot fully explain the geographical variations in MS frequency and the changes in risk that occur with migration, which support the action of strong environmental factors. Among these, vitamin D status, sun exposure, diet, obesity in early life, cigarette smoking and infection with the Epstein-Barr virus are the most consistent environmental predictors of MS risk. The role of vitamin D in MS, its biology, actions, biochemistry and epidemiology studies were demonstrated in several studies in the last years [27–29]. A recent study has investigated whether the influence of low sun exposure on MS risk was mediated by low vitamin D levels and whether low sun exposure or vitamin D deficiency acted synergistically with presence or absence of genetic factors (HLA-

DRB1*15:01 and HLA-A*02:01, respectively) [30]. Result demonstrated that a strong and consistent inverse association of vitamin D with MS risk and clinical score existed and that low sun exposure and vitamin D deficiency were different risk factors that could act synergistically to increase MS risk. Furthermore, Kampman et al. [31] demonstrated that summer outdoor activities in childhood and adolescence were associated with a reduced risk of MS, even in the North of the Arctic Circle where there was a poor sun exposure due to the latitude gradient. A possible explanation could be the particular fatty fish diet of that population, extremely rich in vitamin D, suggesting that supplemental cod-liver oil may be protective when sun exposure is less, so indicating that both climate and diet may interact to influence MS risk at a population level. Dietary habits and lifestyle may influence the course of disease. A hypercaloric and high in saturated fat and sugar diet, refined carbohydrates, fried or processed food (*Western-style diet*) [32] may have proinflammatory effects, then exerting consequences directly on our metabolism or indirectly through their action on gut microbiota. Changes in our metabolism and in microbiota population due to our dietary habits can worsen our health [33]. Proinflammatory dietary habits change the composition of gut microbiota: there is an increase of Western-style diet bacteria, which prevail over saccharolitic bacteria, and a reduced bacterial diversity in the gut. This change leads to an increase in lipopolysaccharide and in the Th17/Treg ratio, expression of proinflammatory cytokines, such as IL-6, IL-1β and tumor necrosis factor-α, and onset of intestinal inflammation and increase of intestinal barrier permeability. Indeed, the gut microbiota has a strong influence on gut barrier integrity and the first consequence of dysbiosis is the increase of gut barrier permeability. An altered permeability of the intestinal barrier has been recently reported in relapsing-remitting MS [34]. Excess body weight during childhood and adolescence has been associated with an increased risk of MS, particularly in the female group of patients [35]. The possible link between MS and obesity has become more interesting in recent years since the discovery of the remarkable properties of adipose tissue, focusing on the role of several adipokines that are able to participate in the mediation of the immune response in MS. Once MS is initiated, obesity can contribute to increased disease severity by negatively influencing disease progress and treatment response, but, also, obesity in early life is highly relevant as a susceptibility factor and causally related risk for late MS development [36]. Cigarette smoking seems to be both an additional risk factor and a powerful aggravating agent for the onset and progression of MS [37]. In fact, smokers have a higher risk than non smokers of developing MS and experiencing related

adverse symptoms and complications. Although the underlying mechanism is not fully understood, researchers suggest that substances in tobacco, such as nicotine, affect the function of the brain and spinal cord through diverse mechanisms, such as immunomodulatory and inflammatory effects and the loss of blood−brain barrier integrity [38].

From the first descriptions of MS, it was suspected that viral infectious factors could be at the origin of the inflammatory process of the disease [39]. In the past, several infectious agents were considered as possible candidates in MS pathogenesis, but not conclusive data about their role were obtained [40]. Microbes considered were Herpes Simplex Virus type 1 (HSV1) and type 2 (HSV2) [40], Herpes Virus (HHV) and HHV6 in particular [41], Clamydia Pneumoniae [42], Human T-lymphotropic Virus type-1 (HTLV1) [43]. To date, Epstein Barr Virus (EBV) is the strongest established risk factor associated with MS [44]. EBV infects more than 90% of all humans, most of whom remain healthy. In contrast, 99% of MS patients have evidence of prior infection with EBV. EBV infects resting B lymphocytes, immortalizing them into long-lived memory B cells that survive largely undetected by the immune system in the peripheral circulation. MS patients show elevated titers to EBV years before developing any neurologic symptoms. Post-mortem pathologic analysis of brains of patients with MS has revealed diffuse EBV-associated B-cell dysregulation in all forms of MS [45]. The “hygiene hypothesis” associated with MS could explain the linkage between MS and EBV infection. Yazdanbakhsh and colleagues [46] summarize the hygiene hypotheses as follows: “.....it has been proposed that the lack of intense infections in industrialized countries owing to improved hygiene, vaccination, and use of antibiotics may alter the human immune system such that it responds inappropriately to innocuous substances (leading to allergy or autoimmunity)....”. The hygiene hypothesis merely holds that a lack of “evolutionary normal” infectious exposures may be a critical factor that contributes to overt disease in an individual who is at risk because of genetic or other predispositions [47]. The three main hypothetical mechanisms linking EBV and MS through B cells involve either the reactivation of EBV within memory B cells in the CNS [48], cross-reactivity of anti-EBV antibodies to human proteins in the CNS (molecular mimicry) [49], or the facilitation of “forbidden” memory B cells recognizing an antigen in the CNS [50]. Confirming this [51], 1) subject with a seropositivity for anti-EBV antibodies have a higher risk for MS, 2) there is at least a 20-fold increase in risk among individuals with a history of mononucleosis compared with those who are EBV-negative, 3) MS patients have a higher titer of anti-EBV antibodies, in

particular antibodies against the EB nuclear antigen (EBNA) expressed in latently infected cells [52], 4) CD4+ T cells specific to EBNA are present at significantly greater frequency and have a broader specificity in MS patients than in control subjects.

1.4 Immunopathogenesis

The pathogenesis of MS involves immune attack against CNS antigens mediated through activated $CD4^+$ myelin-reactive T cells with a possible contribution by B cells. Much of our understanding of immunopathogenesis of MS is derived from the study of experimental autoimmune encephalomyelitis (EAE), an animal model of CNS inflammatory demyelination that can be induced by peripheral immunization with myelin protein components. EAE shares many of the histological features of MS including active demyelination, oligodendrocyte and axonal loss, all of which are presumably mediated by myelin specific T cells [53].

The immunopathogenesis of MS is thought to involve a breach of self-tolerance toward myelin and other CNS antigens resulting in persistent peripheral activation of autoreactive T cells [54]. In a genetically susceptible individual condition, this loss of self-tolerance may be triggered by an environmental antigen, presumably an infectious agent such as a virus. The infection could cause bystander activation of T cells or result in release of autoantigens due to cellular damage, which can then lead to activation of T cells by cross reactivity between an endogenous protein (e.g., myelin basic protein) and the pathogenic exogenous protein (viral or bacterial antigen), a process known as molecular mimicry [55].

Once activated in the periphery, myelin-reactive T cells are able to migrate into the CNS by binding to specific endothelial adhesion molecules. The transmigration process involves interaction between very late antigen-4 (VLA-4) present on T lymphocytes and the vascular cell adhesion molecule-1 (VCAM-1) expressed on capillary endothelial cells; this process is facilitated by expression and upregulation of various adhesion molecules, chemokines, and matrix metalloproteinases (MMPs). Chemoattraction via chemokines and elaboration of matrix metalloproteinases, which may enhance migration by degrading extracellular-matrix proteins, results in invasion of activated autoreactive T cells across the blood–brain barrier (BBB). Inside the CNS, reactivation of T cells by local or infiltrating antigen presenting cells (APCs) results in release of proinflammatory and cytotoxic mediators, recruitment of additional inflammatory cells including

T cells, monocytes and B cells, and persistent activation of microglia and macrophages resulting in myelin damage. The protective myelin sheath may be injured via several mechanisms: cytokine-mediated injury, digestion of surface myelin antigens by macrophages, which may include the binding of antibodies, complement-mediated injury and direct injury by $CD4^+$ and $CD8^+$ T cells [56].

The myelin damage is represented by "plaques", that are focal areas of demyelination associated with variable inflammation and axonal loss that predominantly affect the white matter of the brain, spinal cord, and optic nerves but can also involve the cerebral cortex including subpial regions [57].

The inflammatory infiltrates associated with plaques consist of activated T cells (predominantly $CD8^+$ with variable presence of $CD4^+$ cells), activated macrophages/microglia, plasma cells, and B cells. The evidence based on animal studies suggests that $CD4^+$ T-helper 1 (T_H1) cells which release proinflammatory cytokines such as interferon-gamma, interleukin-2 (IL-2), and tumor necrosis factor-α (TNF-α) are the key players in mediating inflammation in MS with some role for the novel $CD4^+$ T-helper-17 (T_H17) cell subset which secretes IL-17 [54]. The $CD4^+$ T-helper 2 (T_H2) cells, which secret interleukins 4, 5, and 10 are believed to have a counter regulatory role limiting the T_H1-cell-mediated injury [58]. The T_H1/T_H2 paradigm is more apparent in EAE; in MS, indirect evidence exists for a predominant role of Th1 cells based on the success of therapies that shift the cytokine profile away from Th1 toward Th2. CD8+ T cells are believed to be involved as well and can induce axonal pathology by direct injury to MHC I/antigen expressing cells such as neurons and oligodendrocytes [59].

Although T cells have been considered the major contributors to inflammatory activity in MS, growing evidences shed light on B cell role [60]. Indeed, it has been demonstrated that B cells are present in MS lesions, meninges and cerebrospinal fluid (CSF) [61,62] and can contribute to disease progression through antibody-dependent (i.e. secreting intrathecal IgG) [63] and antibody-independent mechanisms [64]. B cells can also stimulate T cells activity through antigen presentation [65] and switching to memory cells which lead to self-proliferation of CD4+ T-cells [66].

The contribution of B cells to MS pathogenesis is supported by observed pathologic heterogeneity of MS lesions, the presence of meningeal inflammation and B-cell follicle-like structures adjacent to subpial cortical lesions, and the success of B-cell-based immunotherapies [67].

MS plaques can be classified histologically as active, chronic and remyelinated. Active lesions are common in RR MS and are characterized by myelin degradation (with relative axonal preservation), macrophage infiltration, reactive astrocytes, and perivascular and parenchymal inflammation [68]. Chronic or inactive plaques are more often seen in patients with progressive disease and are associated with more extensive demyelination, marked axonal depletion, loss of oligodendrocytes, plasma cell infiltrates and relative absence of active inflammation [57]. Remyelinated plaques are seen within or more often at the margins of active plaques and contain thinly myelinated axons and often increased numbers of oligodendrocyte precursor cells [57].

The presence of cortical demyelination and axonal loss has been increasingly recognized in MS, even in early phases of disease. Lucchinetti and colleagues have described four distinct immunopathological patterns of demyelination in active MS lesions [69].

They were defined on the basis of myelin protein loss, the geography and extension of plaques, the patterns of oligodendrocyte destruction and the immunopathological evidence of complement activation. Two patterns (I and II) showed close similarities to T-cell-mediated or T-cell plus antibody-mediated autoimmune encephalomyelitis, respectively. The other patterns (III and IV) were highly suggestive of a primary oligodendrocyte dystrophy, reminiscent of virus- or toxin-induced demyelination rather than autoimmunity. The several patterns of demyelination suggested that there be pathological heterogeneity among MS patients.

Popescu and Lucchinetti [70] have also identify subpial, intracortical and leukocortical lesions as the three main cortical lesion types described in the cerebral and cerebellar cortices of patients with MS. Cortical demyelination may be the pathological substrate of progression, and an important pathologic correlate of irreversible disability, epilepsy and cognitive impairment. Cortical lesions of chronic progressive MS patients are characterized by a dominant effector cell population of microglia, by the absence of macrophagic and leukocytic inflammatory infiltrates, and may be driven in part by organized meningeal inflammatory infiltrates. Cortical demyelination is also present and common in early MS, is topographically associated with prominent meningeal inflammation and may even precede the appearance of classic white matter plaques in some MS patients. However, the pathology of early cortical lesions is different than that of chronic MS in the sense that early cortical lesions are highly inflammatory, suggesting that neurodegeneration in MS occurs on an inflammatory background.

1.5 Clinical symptoms and signs of MS

The clinical symptoms and signs of MS are variable and may result from involvement of sensory, motor, visual, and brainstem pathways. Clinically, MS is characterized by discrete episodes ("attacks" or "relapses") of neurologic dysfunction. The symptoms produced by these episodes vary considerably between patients and depend upon the site of neurologic involvement. Commonly patients may experience numbness, tingling, weakness, vision loss, gait impairment, incoordination, imbalance, and bladder dysfunction [71]. In between these attacks, at least during the relapsing-remitting (RR) phase of the illness, patients are neurologically stable [72]. However, residual symptoms may persist and many patients experience fatigue or heat sensitivity in the interval between attacks. Over several years to decades, many patients who begin with RRMS evolve to the SP phase of the illness, in which they experience an insidious worsening of function and the accumulation of neurologic disability unrelated to any acute attacks that may or may not occur.

Optic neuritis. Acute demyelinating optic neuritis is the presenting symptom in about 20% of MS patients and affects about half of MS patients at some point in the disease course [73].

Optic neuritis is an inflammatory optic neuropathy affecting one or both optic nerves. It is usually unilateral and typically affects young Caucasian women who present with vision loss, dyschromatopsia and painful eye movements. In two-thirds of the cases, the optic disc appears normal on funduscopic examination (retrobulbar optic neuritis or NORB). During the acute phase of optic neuritis, in the other third of cases, the optic nerve appears swollen (papillitis). A relative afferent papillary defect (a Marcus Gunn pupil) is usually present [74]. Visual acuity and visual field usually recover within a few months. In acute optic neuritis, MRI usually demonstrates a hyperintensity on T2-weighted images (i.e., a T2 lesion) of the affected optic nerve as well as contrast enhancement within the nerve, best appreciated on fat-saturated sequences of the orbit [75]. After 15 years, 72% of patients with at least 1 brain lesion on their initial MRI were diagnosed with MS, while only 25% of patients with no brain lesions on initial MRI developed MS [76]. Other well-established methods to elucidate the complex interplay of demyelination, inflammation, axonal loss and neurodegeneration in MS are becoming increasingly available in clinical practice: optical coherence tomography (OCT) and visual evoked potentials (VEPs). OCT is a technique that uses near-infrared light to create images of the retina. It is non-invasive, quick and relatively cheap and easy to use. Furthermore, the

images produced via OCT are of very high resolution and highly reproducible. OCT allows the measurement of the thickness of macular ganglion cell layer (mGCL) and retinal nerve fiber layer (RNFL) [77]. In MS patients both with and without prior optic neuritis is showed a reduction in RNFL and mGCL thickness due to the loss of axons secondary to retrograde degeneration [78] and the distribution of RNFL loss tends to involve the temporal quadrant [79]. Many studies have found correlations between OCT parameters and clinical and paraclinical aspects in MS, suggesting that OCT could be a useful tool for monitoring MS both in clinic and in treatment trials [80]. For example, some studies have found statistically significant reductions in the GCL thickness in eyes of MS patients also without prior optic neuritis, which may reflect subclinical structural damage and help to identify patients with optic neuritis who are at risk of developing MS. OCT could potentially help differentiate between MS subtypes. One multicentre study [81] with 571 MS patients without prior optic neuritis observed a statistically significant lower RNFL thickness in patients with SPMS compared to RRMS ($p = 0.007$), even if these differences disappeared when corrected for expanded disability status scale (EDSS) score. Similarly, Costello et al. [82] found significantly lower RNFL thicknesses in eyes unaffected by prior optic neuritis in patients with SPMS than with RRMS, and with RRMS than with CIS.
VEPs have a role in assessing the extent of demyelination along the optic nerve. Moreover, VEPs testing can be used to predict the extent of recovery after optic neuritis and capture disabling effects of clinical and subclinical demyelination events in the afferent visual pathway [83]. The presence of increased latency with preserved waveform morphology is considered a sign of a demyelinating injury. Early studies showed a prevalence of increased VEP latency in up to 50%–70% of patients with MS without visual complaints and even in about 20%–50% of MS patients without a history of optic neuritis [84].

Myelitis. Transverse myelitis is defined as impairment of motor, sensory and bowel or bladder tracts in the spinal cord secondary to inflammatory-mediated injury. The occurrence of a band-like tightening sensation around the chest or abdomen (the so-called MS “hug”) is a typical symptom of myelitis and suggests involvement of the posterior columns of the spinal cord. It is often accompanied by a horizontal sensory level. The myelitis that occurs in MS is typically partial and usually presents subacutely [85,86], may be the first clinical symptom of MS or may remain a monophasic event. The presence of multifocal and posterior spinal cord lesions is significantly associated with the diagnosis of MS [87].

Brainstem syndromes. The brainstem is commonly affected in MS. The clinical syndromes produced by brainstem involvement in MS include: double vision (cranial nerves III, IV, VI), internuclear ophthalmoplegia (medial longitudinal fasciculus), facial weakness or myokymia (cranial nerve VII), vertigo (cranial nerve VIII), or bulbar (medullary) symptoms such as dysphagia, dysarthria, and tongue weakness (cranial nerves IX, X, XII).

An internuclear ophthalmoplegia (INO) is an eye movement disorder in MS in which the adducting eye movement is slowed down compared to the abducting eye movement. The cause of an INO is demyelination in the medial longitudinal fasciculus (MLF). The MLF connects the abducens nucleus of one side of the brainstem with the oculomotor nucleus of the other side. Conjugacy of horizontal saccades therefore depends on conduction through the MLF [88].

Facial numbness is a relatively common symptom of MS, usually seen during the course of a relapse in patients with RRMS or as a first manifestation in patients with CIS suggestive of MS [89]. Frequencies reported for facial sensory disturbance in MS (excluding trigeminal neuralgia) range from 2.9 to 13.6% [90]. As an initial symptom in patients with CIS it has been reported with a frequency of 3.4% [91]. Facial sensory impairment (cranial nerve V) may arise from multiple localizations, including the brainstem, cervical cord (due to the fact that afferent trigeminal pathways descend from their entry at the pontomedullary junction to the level of the upper cervical spine), subcortical and cortical sensory pathways [71]. Cranial neuralgias, including trigeminal, glossopharyngeal neuralgias, as well as occipital neuralgia, are typical expression of neuropathic pain involving cranial nerves in MS. Neuralgias are characterised by paroxysmal painful attacks of electric shock-like sensation, occurring spontaneously or evoked by innocuous stimuli in specific trigger areas [92].

Involvement of cerebellar networks that connect with the brainstem can lead to unilateral ataxia, dysmetria, or dysdiadochokinesia. Cerebellar manifestations in MS can be present at any time of the clinical course. Early cerebellar findings are a predictor of disability and disease progression. Most patients have cerebellar manifestations once they enter the progressive stages of the disease. Of the cerebellar findings, tremor is by far the most common [93].

Motor symptoms. Weakness affects up to 89% of MS patients at some point in the disease course. Focal weakness in the limbs in MS is usually due to involvement of the corticospinal tract [94] and, thus, it is often accompanied by other signs of the upper motor neuron syndrome, such as hyperreflexia, spasticity, and an extensor plantar response on Babinski or Chaddock testing. Spasticity, a velocity-dependent increase in resistance to passive muscle stretch, is associated with stiffness, spasms, cramping, and gait impairment and can occur also in the absence of weakness. Muscle spasms are often associated with spasticity.

Sensory impairment. Numbness and paresthesias are common symptoms experienced by MS patients. When these symptoms are transient, lasting only seconds to minutes, they are unlikely to be due to an acute relapse in MS. Conversely, when they last many hours to days they may well reflect an acute inflammatory-demyelinating injury. Sensory complaints affect 87% of MS patients at some point in the disease course and in particular abnormalities in sensibility to temperature and pain are the most prominent sensory disturbances [95]. Chronic pain is a common symptom in MS [96]. Nociceptive pain, that occurs when nociceptors are activated in response to tissue damage, in MS patients can be provoked by abnormalities in the musculoskeletal system, for example, spasms. Pain in MS usually has neuropathic features such as burning, electrical or sharp sensations. Lhermitte's symptom – an electrical-shock-like sensation running down the spine upon neck flexion – occurs in up to one-third of MS patients at some point in the disease (Kanchandani and Howe, 1982). The neuroanatomic localization of Lhermitte's symptom is the posterior column in the cervical or upper thoracic spinal cord. Neuropathic pain may include both central and peripheral neuropathic pain and can be caused by lesions in the brain or spinal cord [95]. As demonstrated in patients with spinal cord injury for other disease different from MS, both impaired spinothalamic tract and via the dorsal column medial lemniscal pathway mediated functions are necessary for the development of neuropathic pain in MS [97].

Imbalance. MS patients often describe the sensation of being off-balance, unsteady or uncoordinated. The overwhelming majority have abnormalities of postural control and gait even early in the disease course. In all, 50-80% have balance and gait dysfunction and over 50% fall at least once each year. Balance dysfunction in MS is conceptualized as three interrelated problems: decreased ability to maintain position,

limited and slowed movement towards limits of stability, and delayed responses to postural displacements and perturbations [98]. In addition, functional balance performance may be affected by impaired dual-task integration. Walking changes in MS include reduced gait speed, impaired walking balance, and reduced walking-related physical activity. Imbalance appears to be related to the disconnection between the spinal cord, cerebellum and cerebral cortex, which in turn produces atrophy of the sensory motor cerebellar regions that are functionally connected with specific cortical areas [99].

Cognitive impairment. Though often neglected, cognitive impairment is a common feature of MS that affects 43-70% of patients [100]. MS can lead to frank dementia, but this is rare and usually occurs in the context of extensive and progressive disease [101]. The most common domains affected in MS are slowed information processing, executive dysfunction and impairment of long-term verbal and visual memory [102]. Cognitive impairment in MS is associated with brain atrophy, white-matter involvement (especially periventricular lesions) and cortical demyelinating plaques detectable at MRI with double inversion recovery (DIR) sequences [103,103]. Recommendations for cognitive screening and management in MS care endorsed by the Consortium of Multiple Sclerosis Centres and the International Multiple Sclerosis Cognition Society are: 1) to increase professional and patient awareness/education about the prevalence, impact and appropriate management of cognitive symptoms; 2) in MS patients with clinical or MRI evidence of neurologic damage consistent, to perform early baseline screening with the Symbol Digit Modalities Test (SDMT) or similarly validated test, and to repeat annual re-assessment in order to determinate treatment effects (e.g. starting/changing a disease-modifying therapy) or relapse recovery or progression/new-onset of cognitive impairment [104].

Depression. Major depression affects about 30–45% of MS patients depending on the screening methodology used [105,106]. Aetiology of depression is unclear. Several medical and psychiatric comorbidities, as pain, fatigue, anxiety or cognitive impairment, may contribute to the strong association between depression and MS [107]. Studies on MRI scan in MS patients affected by major depression showed a possible association with to injury of fronto-temporal networks. Confirming this, in a study by Zorzon et al. [108], brain atrophy was significantly more conspicuous in the left frontal lobe (P=0.039), in both frontal

lobes (P=0.046) and showed a trend towards a difference in the right frontal lobe (P=0.056), in the right temporal lobe (P=0.057) and in both temporal lobes (P=0.072) of depressed patients. Interestingly, elevated levels of proinflammatory cytokines, which are known to correlate with depression severity [109], have been reported in both the CNS and peripheral circulation in patients with MS. Increased concentrations of proinflammatory cytokines are thought to induce depressive symptoms by reducing the release of serotonin at synapses [110,111] and, consequently, might also lead to the malfunctioning of noradrenergic and serotonergic circuits that represent the pathways targeted by several antidepressant drugs [107].

Fatigue. Fatigue is one of the most debilitating symptoms in MS and was reported as a current symptom in 83% of patients in a large survey (Minden et al., 2006). Patients often describe the fatigue of MS as a general sense of low energy, 'a feeling arising from difficulty in initiation of or sustaining voluntary effort' or 'an overwhelming sense of tiredness that is out of proportion (in relation to the performed activity)' [112].
In an attempt towards standardisation, a recent taxonomy distinguishes two major dimensions of fatigue: perception of fatigue and performance fatigability [113]. The latter refers to objectively measurable aspects of fatigue, for example, the observable decrease in performance during a cognitive or motor task. By contrast, the perceptual dimension is inherently subjective and cannot be assessed directly by an external observer. From a physiopathological perspective, these two dimensions are distinct: explanations of fatigability can, in principle, be derived from physiological and biochemical principles. By contrast, understanding the subjective perception of fatigue requires a cognitive perspective, in particular, concepts of introspection and metacognition [114]. It is important to distinguish complaints of fatigue from complaints of motor weakness. Fatigue can persist between clinical relapses, but often worsens in association with disease activity, and can be exacerbated by other disease, such as depression, hypothyroidism, adrenal insufficiency, anaemia and sleep disorders.

Bladder and bowel dysfunction. Lower urinary tract (LUT) dysfunction is common in patients with MS and is a major negative influence on the quality of life of these patients. About 20–25% of MS patients with bladder symptoms exhibit findings of bladder under-activity from low contractility on urodynamic testing, which leads to the symptoms of urinary frequency and incomplete emptying. The most frequent reported

urodynamic abnormality is that of detrusor hyperreflexia – "overactive bladder" [115]. In healthy subject, the detrusor reflex involves the coordinated contraction of the detrusor muscle with a simultaneous relaxation of the urethral sphincter. The detrusor reflex is inhibited voluntarily through pathways originating in the cortex and travelling in the spinal cord and is controlled by muscarinic cholinergic innervation. Loss of this inhibition leads to over-activation of the reflex at small bladder volumes and automatic emptying, resulting in symptoms of urinary urgency, frequency, and incontinence. [116].

The management of these patients requires a multidisciplinary approach [115]. Intermittent self-catheterization is the preferred option for management of incomplete bladder emptying and urinary retention. Antimuscarinics are the first-line treatment for urinary symptoms B in neurological patients due to their favourable cost-benefit ratio [117]. They reduce symptoms by blocking muscarinic receptors distributed throughout the detrusor and suburothelium, thus blocking parasympathetic-mediated activation of the detrusor [118]. Even if the M3 muscarinic receptor is of greatest significance functionally, most of the antimuscarinics non-selectively bind with muscarinic receptors of different sub-types across several organs. This is responsible for the side effect profile of these medications including dry mouth, blurred vision, and constipation [119], which influence adherence to these medications. If antimuscarinics are ineffective, or poorly tolerated, a range of other approaches are available, with varying levels of evidence. Intradetrusor botulinum toxin A injections are a highly effective and minimally invasive treatment for patients with treatment-refractory neurogenic detrusor overactivity owing to MS or spinal cord injury [120]. In addition to inhibiting the release of vesicular neurotransmitters from parasympathetic nerve terminals of the detrusor smooth muscle, this toxin is also likely to inhibit the release of transmitters involved in afferent signalling pathways in the bladder mucosa [121]. Stimulation of the tibial nerve or sacral nerve root S3 has proven to be successful in managing overactive bladder symptoms. The exact mechanisms of action of this approach remain uncertain but are thought to be a result of modulation of spinal pelvic reflexes through activation of inhibitory interneurons [122]. Surgical options include augmentation cystoplasty, cutaneous continent diversion and ileal conduit surgery, and should be performed only after careful selection of patients. Stress urinary incontinence owing to sphincter deficiency remains a therapeutic challenge, and is only managed surgically if conservative measures have failed [123].

The most difficult urinary condition to manage in MS is that of detrusor-sphincter dyssynergia [124], that arises from the loss of coordination between the detrusor and sphincter muscles, and leads to urinary hesitancy, interruptions of the urinary stream, and incomplete emptying. It usually requires clean intermittent catheterization.

Acute urinary retention can be a presenting symptom of acute myelitis. A postvoid residual, or straight catheterization, is an important measure of neurogenic bladder function in this context [125].

Bowel dysfunction in MS is less common than bladder involvement, and constipation is the most frequent complaint. Bowel incontinence from MS usually occurs in the context of severe spinal cord injury [126].

Sexual dysfunction is reported to affect up to one third of patients, up to 80% of men and 50–70% of women [127]. The most common pathologies identified are erectile dysfunction in men and loss of libido and/or fatigue in women [128].

Other clinical presentations. There are several other less common clinical syndromes that may be consistent with a first presentation of MS [129]. Cerebral hemisphere lesions, particularly large tumefactive brain lesions, can present as a hemispheric syndrome with symptoms that include aphasia, encephalopathy, and manifestations of increased intracranial pressure, in addition to motor and sensory symptoms. Paroxysmal symptoms (with recurrence over at least 24 h) are transient, recurrent, stereotyped symptoms such as vibrating or shock-like sensation with neck flexion (Lhermitte phenomenon), tonic spasms, trigeminal neuralgia, or paroxysmal dysarthria.. Other less common symptoms include seizures and symptoms related to disorders of thermoregulation or sleep.

Psuedo-relapse. Transient worsening or recrudescence of MS symptoms can occur in the context of infection or other stressors [130]. A common culprit is a urinary tract infection, which may otherwise be asymptomatic, particularly in women [131].

1.6 Measurements of clinical assessment in MS

The Expanded Disability Status Scale (EDSS). It is the most popular and widely used instrument is MS clinical evaluation [132]. The EDSS is a clinician-administered assessment scale evaluating the functional systems of the central nervous system (Figure 1). The EDSS is used to describe disease progression in patients with MS and to assess the effectiveness of therapeutic interventions in clinical trials. It consists of ordinal rating system ranging from 0 (normal neurological status) to 10 (death due to MS) in 0.5 increments interval (when reaching EDSS 1). The lower scale values of the EDSS measure impairments based on the neurological examination, while the upper range of the scale (> EDSS 6) measures handicaps of patients with MS. The determination of EDSS 4 – 6 is heavily dependent on aspects of walking ability. The literature reveals that the EDSS is the most widely used and best-known instrument to assess disease progression in MS [133] (Figure 2). The great advantage of the EDSS is its international acceptance (including the EMA) as a primary endpoint in clinical trials. Because it is so commonly used, studies that use the EDSS can easily compare results to other findings. However, the mean limit is represented by the weakness in reliability and sensitivity to change of disease status, and a clear recommendation on interpreting changes in EDSS value does not yet exist. EDSS changes by 1.0 points from a baseline EDSS less than or equal to 5.5 and 0.5 points over a baseline 5.5 are commonly recognized as a clinically increase in disability. However, it is now understood that it is more accurate to define disability change as a sustained change for 12 weeks or, even more reliably, for 24 weeks.

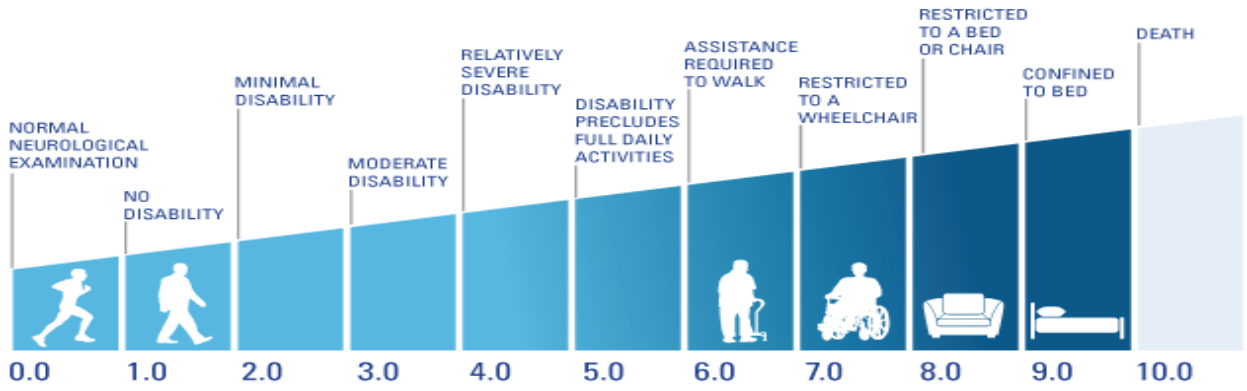
Figure 1. Summary table of EDSS values

0.0	Normal neurologic examination
1.0	No disability, minimal signs in one FS
1.5	No disability, minimal signs in more than one FS
2.0	Minimal disability in one FS
2.5	Mild disability in one FS or minimal disability in two FS
3.0	Moderate disability in one FS, or mild disability in three or four FS. Fully ambulatory
3.5	Fully ambulatory but with moderate disability in one FS, and more than minimal disability in several others
4.0	Fully ambulatory without aid, self-sufficient, up and about some 12 h/day despite relatively severe disability; able to walk without aid or rest for about 500 m
4.5	Fully ambulatory without aid; up and about much of the day; able to work a full day; may otherwise have some limitation of full activity or require minimal assistance; characterized by relatively severe disability; able to walk without aid or rest for about 300 m
5.0	Ambulatory without aid or rest for about 200 m; disability severe enough to impair full daily activities (e.g., work a full day without special provisions)
5.5	Ambulatory without aid or rest for about 100 m; disability severe enough to preclude full daily activities
6.0	Intermittent or unilateral constant assistance (cane, crutch, brace) required to walk about 100 m with or without resting
6.5	Constant bilateral assistance (canes, crutches, braces) required to walk about 20 m without resting
7.0	Unable to walk beyond approximately 5 m even with aid; essentially restricted to wheelchair; wheels self in standard wheelchair and transfers alone; up and about in wheelchair some 12 h/day
7.5	Unable to take more than a few steps; restricted to wheelchair; may need aid in transfer; wheels self but cannot carry on in standard wheelchair a full day; may require motorized wheelchair
8.0	Essentially restricted to bed or chair or perambulated in wheelchair, but may be out of bed itself much of the day; retains many self-care functions; generally has effective use of arms
8.5	Essentially restricted to bed much of day; has some effective use of arms and retains some self-care functions
9.0	Confined to bed; can still communicate and eat
9.5	Totally helpless bed patient; unable to communicate effectively or eat/swallow
10	Death due to MS

FS: functional system; m: meters. Source: Reference 21.

Medscape Source: US Pharm © 2009 Jobson Publishing

Figure 2. Disability progression according to EDSS score.



The Multiple Sclerosis Functional Composite (MSFC). It is another important instrument in MS disease clinical assessment [134], developed as an additional clinical measure of MS disability progression in order to improve the standard measure of MS disability for clinical trials and to develop a multidimensional metric of overall MS clinical status [134]. The MSFC is a three-part performance scale for evaluating the degree of impairment in MS patients. It includes the assessment of leg function by moving a short walking distance ("Timed 25-Foot Walk", T25FT), the assessment of arm function using breadboard test ("9-Hole Peg Test", 9HPT) and an attention/concentration test to assess cognitive functions ("Paced Auditory Serial Addition test", PASAT). An integrated MSFC score is calculated using z-scores. In recent years, the MSFC is increasingly used in clinical trials and its use as clinical trial endpoints is recommended to provide information on dimensions no covered in the EDSS, such as upper limb function or cognitive skills [133]. Although the MSFC was developed rigorously, its weaknesses include interpreting the z-scores, the learning effects of the PASAT, low acceptance by patients and lack of a visual dimension [133].

The multiple sclerosis severity scale (MSSS). It is a powerful method for predicting disease progression over time. The scale was created by Roxburgh et al. in 2005 [135] in order to assign a severity score to the patient's type of MS base on the degree of disability accumulated over a given number of years (*disease progression rate*) [136].

The score is obtained calculating the EDSS score and the number of years of disease: higher EDSS score and fewer number of years will determinate a higher score of MMSS, so revealing a more severe course of MS with more disability progression (Figure 3).

Figure 3. Global Multiple Sclerosis Severity Scores (MSSS). The MSSS for an individual patient is ascertained by finding the column corresponding to the patient's EDSS and the row corresponding to the number of years since the onset of MS.

	0	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5	EDSS
0	0.89	2.94	4.79	6.51	7.76	8.25	8.73	9.18	9.44	9.54	9.62	9.78	9.90	9.94	9.95	9.95	9.99	10.00	10.00	
1	0.64	2.34	4.12	5.75	7.04	7.76	8.38	8.91	9.23	9.42	9.57	9.73	9.88	9.93	9.95	9.98	9.99	9.99	10.00	
2	0.53	1.99	3.65	5.25	6.47	7.21	7.90	8.47	8.83	9.06	9.26	9.50	9.75	9.86	9.93	9.97	9.98	10.00	10.00	
3	0.45	1.73	3.28	4.79	5.98	6.80	7.55	8.14	8.55	8.82	9.05	9.34	9.63	9.78	9.87	9.92	9.97	9.98	9.99	
4	0.33	1.40	2.81	4.23	5.36	6.19	6.95	7.61	8.06	8.37	8.66	9.04	9.45	9.68	9.81	9.88	9.95	9.98	9.99	
5	0.29	1.24	2.54	3.85	4.88	5.73	6.53	7.21	7.69	8.02	8.32	8.80	9.31	9.60	9.77	9.87	9.94	9.97	9.99	
6	0.24	1.10	2.30	3.51	4.51	5.36	6.13	6.79	7.27	7.61	7.95	8.49	9.09	9.46	9.70	9.82	9.92	9.97	9.99	
7	0.23	1.02	2.08	3.14	4.08	4.91	5.70	6.42	6.93	7.29	7.64	8.25	8.93	9.34	9.60	9.76	9.89	9.95	9.99	
8	0.20	0.92	1.90	2.88	3.74	4.48	5.27	6.03	6.54	6.90	7.29	7.96	8.72	9.22	9.55	9.74	9.88	9.95	9.99	
9	0.20	0.86	1.72	2.60	3.38	4.09	4.85	5.59	6.09	6.48	6.90	7.66	8.54	9.09	9.46	9.70	9.86	9.94	9.99	
10	0.19	0.77	1.53	2.34	3.09	3.77	4.51	5.24	5.74	6.12	6.58	7.40	8.32	8.93	9.34	9.61	9.83	9.93	9.99	
11	0.17	0.70	1.38	2.11	2.81	3.42	4.13	4.89	5.40	5.82	6.32	7.18	8.15	8.77	9.22	9.51	9.77	9.91	9.98	
12	0.15	0.64	1.29	1.99	2.65	3.25	3.92	4.63	5.15	5.56	6.06	6.95	7.95	8.63	9.13	9.43	9.72	9.89	9.97	
13	0.14	0.58	1.17	1.83	2.48	3.08	3.72	4.43	4.97	5.38	5.86	6.77	7.84	8.55	9.01	9.34	9.67	9.86	9.96	
14	0.11	0.51	1.07	1.75	2.39	2.96	3.61	4.36	4.92	5.32	5.77	6.62	7.64	8.36	8.84	9.20	9.58	9.82	9.95	
15	0.10	0.46	0.99	1.64	2.25	2.83	3.46	4.21	4.78	5.18	5.58	6.40	7.47	8.20	8.70	9.12	9.53	9.79	9.95	
16	0.09	0.38	0.86	1.42	1.98	2.56	3.20	3.93	4.50	4.87	5.23	6.08	7.24	8.03	8.55	9.05	9.50	9.76	9.94	
17	0.06	0.31	0.76	1.28	1.77	2.31	2.99	3.74	4.28	4.65	5.01	5.85	7.03	7.84	8.39	9.00	9.52	9.79	9.96	
18	0.04	0.24	0.63	1.10	1.54	2.08	2.70	3.36	3.89	4.26	4.60	5.47	6.74	7.63	8.27	8.95	9.49	9.77	9.96	
19	0.05	0.26	0.60	0.99	1.38	1.89	2.49	3.15	3.67	4.06	4.41	5.35	6.68	7.60	8.29	9.01	9.57	9.81	9.97	
20	0.05	0.25	0.59	0.96	1.31	1.73	2.31	2.96	3.45	3.86	4.23	5.15	6.51	7.51	8.25	8.99	9.57	9.80	9.95	
21	0.05	0.29	0.64	1.02	1.40	1.79	2.34	2.95	3.40	3.79	4.16	5.08	6.39	7.40	8.17	8.92	9.52	9.79	9.96	
22	0.04	0.23	0.55	0.92	1.31	1.69	2.21	2.81	3.26	3.66	4.04	4.99	6.35	7.39	8.18	8.89	9.45	9.74	9.95	
23	0.05	0.28	0.61	0.95	1.29	1.67	2.20	2.81	3.24	3.69	4.16	5.13	6.44	7.47	8.24	8.90	9.45	9.76	9.95	
24	0.05	0.25	0.55	0.91	1.29	1.65	2.14	2.73	3.14	3.54	4.01	5.01	6.32	7.37	8.15	8.83	9.41	9.75	9.96	
25	0.05	0.24	0.50	0.80	1.18	1.58	2.05	2.56	2.90	3.28	3.81	4.92	6.28	7.31	8.12	8.80	9.39	9.77	9.98	
26	0.06	0.22	0.49	0.81	1.18	1.57	2.05	2.59	2.96	3.35	3.87	4.95	6.32	7.41	8.22	8.89	9.48	9.80	9.96	
27	0.06	0.24	0.49	0.77	1.13	1.55	2.02	2.53	2.85	3.21	3.74	4.80	6.20	7.33	8.17	8.92	9.56	9.85	9.98	
28	0.05	0.18	0.40	0.71	1.13	1.50	1.87	2.40	2.79	3.07	3.48	4.56	6.00	7.10	7.95	8.79	9.48	9.81	9.98	
29	0.04	0.20	0.47	0.77	1.15	1.48	1.79	2.30	2.75	3.08	3.47	4.40	5.70	6.78	7.68	8.64	9.40	9.76	9.96	
30	0.03	0.16	0.44	0.78	1.14	1.43	1.70	2.27	2.81	3.15	3.48	4.29	5.50	6.57	7.46	8.43	9.27	9.67	9.92	
Years																				

A number of other instruments are available to assess MS: the Ambulation Index (AI) [137], the Scripps Neurological Rating Scale (SNRS) [138] and the Illness Severity Scale (ISS) [139]. Specific instruments for measuring health-related quality of life in MS patients are the Multiple Sclerosis Quality of Life-54 (MSQOL-54) and the Multiple Sclerosis Quality of Life Inventory (MSQLI) [140]. Only a few of these instruments meet the requirements of methodological standards (e.g. validity, reliability, responsiveness), particularly for use in clinical trials. None of these instruments is recognized to use in clinical trials without any restrictions.

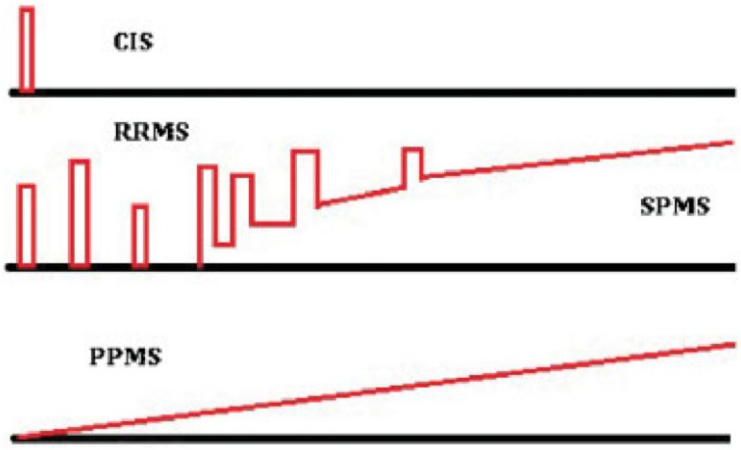
1.7 Clinical phenotypes

MS has been categorized into four distinct clinical phenotypes: relapsing– remitting (RRMS), secondary-progressive (SPMS), primary progressive (PPMS), and progressive relapsing (PRMS) [141] (Figure 4). The increasing use of MRI has led to renewed interest in the concept of "preclinical" MS – the incidental identification of imaging abnormalities indicative of MS in patients who have never had a clinical attack. This clinical scenario has been called the "radiologically isolated syndrome" (RIS) [142]. Moreover, about 85% of patients have onset of MS with a relapse consisting of a single episode of neurological disturbance known as a clinically isolated syndrome (CIS) [143]. The term CIS is typically applied in a young adult (aged 20–45 years) with an episode of acute or sub-acute onset, which reaches a peak quite rapidly (within 2–3 weeks). To be termed CIS, the episode should last for at least 24 h and occur in the absence of fever or infection, with no clinical features of encephalopathy. Furthermore, by definition, CIS is always isolated in time (i.e., monophasic) and in space (i.e., monofocal). The first clinical event in these patients can be optic neuritis (the most common presentation in many reported CIS studies), incomplete myelitis, or brainstem or cerebellum syndrome [144].

Although useful from a theoretical standpoint, in clinical practice, this categorization is often unable to adequately capture the complexity of disease phenotypes as there is often overlap between clinical phenotypes, the transition between RR and SP multiple sclerosis is unclear, and classification is often based on a patient's recollection and description of historical events. Further, all MS disease phenotypes share common characteristics, and although there can be relative differences in a number of imaging and laboratory markers between specific MS subtypes (CSF and serum neurofilament levels, rate of new lesion formation, rate of brain and spinal cord atrophy [1,145–148]; none of these markers can definitively permit an accurate differentiation. As a result, MS disease subtype classification is still largely based on clinical characteristics.

Figure 4. MS classification and clinical types.

CIS, clinically isolated syndrome; *RRMS*, relapsing remitting MS; *PPMS*, primary progressive MS; *SPMS*, secondary progressive MS.



Relapsing remitting MS. RRMS is the commonest form of MS, and about 80–90% of all MS patients will fall into this category at some point in their disease course. Relapses ("attacks" or "flares") are discrete episodes of neurologic dysfunction that typically evolve over hours to days and then persist for days to weeks before remitting.

Secondary progressive MS. Most, but not all, patients with RRMS will go on to develop insidious neurologic worsening and accumulation of disability – "secondary progression" – that is not directly related to discrete attacks. This phenotype is called SPMS. The median time to development of secondary progression varies between 10 and 20 years, depending on "aggressive" disease course [149,150]. Time to development of secondary progression is shorter when the age at clinical onset is greater [151]. Progression, and not relapse activity, accounts for most of the long-term disability burden in MS [152]. The transition from RRMS to SPMS is often a continuum, with insidious progression sometimes occurring in the background of clinical relapses and inflammatory disease activity on MRI.

Primary progressive MS. About 10–20% of patients with MS never experience a discrete relapse, but instead present with insidious neurologic worsening and disability accumulation – "progression" [150]. This

phenotype is called PPMS. The age at which clinical progression begins in patients with PPMS and SPMS is nearly identical in most large MS epidemiologic cohorts [153]. PPMS can be diagnosed when a patient has experienced at least 1 year of clinical progression plus two out of the following three criteria: (1) evidence for dissemination in space on MRI based on > or equal to 1 T2 hyperintense lesion in at least one area characteristic for MS (periventricular, juxtacortical, or infratentorial), 2) > or equal to 1 T2 lesion in the spinal cord and 3) positive oligoclonal bands and/or elevated immunoglobulin G (IgG) index in the CSF as evidence of intrathecal inflammation [154].

Progressive relapsing MS. About 5% of MS patients present with a hybrid course characterized by prominent progression at onset (what appears to be primary progressive disease initially), but then develop a few superimposed relapses [155].

1.8 Diagnosis

The diagnosis of MS is based on demonstrating evidence of inflammatory-demyelinating injury within the central nervous system that is disseminated in both time and space. Diagnosis is made through a combination of the clinical history, neurologic examination, magnetic resonance imaging and the exclusion of other diagnostic possibilities. Other so-called "paraclinical" tests, including the examination of the cerebrospinal fluid, the recording of evoked potentials, urodynamic studies of bladder function, and ocular coherence tomography, may be helpful in establishing the diagnosis for individual patients, but are often unnecessary [71].

Diagnostic criteria for MS have evolved over the past several decades, with each revision impacting the apparent prevalence and prognosis of the disorder. The result has been to encourage earlier diagnosis without compromising accuracy. In the pre-MRI era, the diagnosis of MS was only based on clinical history and examination and required demonstration of at least two clinical attacks disseminated in time and space. In 1983 a working group chaired by Poser allowed to use “paraclinical” evidence, specifically neuroimaging or electrophysiological abnormalities, as evidence of dissemination in space for diagnosis of “clinically definite” MS. In 2001, an international panel chaired by McDonald (McDonald criteria), allowed MRI evidence of disease activity to serve as evidence of dissemination in time (DIT) and space (DIT) [156]. MRI

criteria for DIS required satisfying three of the following four features: 1) > or equal to 1 gadolinium enhancing lesion or > or equal to 9 T2 hyperintense lesions, 2) > or equal to 1 infratentorial lesion, 3) > or equal to 1 juxtacortical lesion and 4) > or equal to 3 periventricular lesions. The panel also allowed for two T2 hyperintense lesions in a patient with oligoclonal bands on CSF examination to satisfy the criteria for dissemination in space. In a revision by Polman et al. in 2005 [157], the use of a new subclinical T2 hyperintense lesion occurring at least 1 month after a reference scan obtained >1 month after the onset of the first clinical episode as new diagnostic criteria allowed to alternatively satisfy the requirement for DIT. These new diagnostic criteria were also useful to better clarify the role of spinal cord lesions and to simplify diagnosis of PPMS. In 2011 [154] MRI criteria for DIS were simplified in the identification of > or equal to 1 T2 hyperintense lesion in two of four of the following regions: 1) periventricular white matter, 2) juxtacortical white matter, 3) infratentorial white matter and 4) spinal cord.

The International Panel on Diagnosis of MS reviewed the 2010 McDonald criteria and recommended revisions to simplify and improve their diagnostic utility [158]. The 2017 McDonald criteria continue to apply primarily to patients experiencing a typical CIS, define what is needed to fulfil DIT and DIS of lesions in the CNS, and stress the need for no better explanation for the presentation. The main changes are: 1) in patients with a typical CIS and clinical or MRI demonstration of DIT, the presence of CSF-specific oligoclonal bands allows a diagnosis of MS even in absence of DIT, 2) both symptomatic or asymptomatic lesions can be considered for showing DIT and DIS and 3) cortical lesions can be used to demonstrate DIS. Regarding PPMS, the diagnostic criteria remain largely unchanged, the only exception being that the distinction between symptomatic and asymptomatic lesions are no longer needed.

Other paraclinical test used to confirm MS diagnosis, especially in those cases of suspected disease (for example, CIS) are: 1) evoked potentials; 2) cerebrospinal fluid (CFS) studies; 3) research or specific biomarkers.

Visual-evoked potentials (VEPs) are abnormal in 30% of patients with CIS, regardless of clinical symptoms and in >50% of patients with MS who have no history or clinical evidence of optic nerve dysfunction. Somatosensory-evoked potentials (SSEPs) and brain stem auditory-evoked potentials (BAEPs) may also be used as evidence of demyelination that is non-detectable clinically or on MRI. Pelayo et al. [159] showed

that if all 3 evoked potentials (VEP, SSEP, and BAEP) are abnormal at the time of CIS, there is an increased risk of developing moderate disability from MS that is independent of MRI findings.

About 60-70% of patients with CIS and up to 90% of those with MS have 2 or more immunoglobulin G (IgG) OCBs uniquely to the CSF [160]. Some 70-90% of MS patients will have an elevated IgG index [161] and this may be in conjunction with or independent of the presence of OCB in the CSF. On one hand, the presence of 2 OCBs in the CSF has a positive predictive value of 97%, a negative predictive value of 84%, a sensitivity of 91%, and a specificity of 94% for developing relapsing remitting MS (RRMS) after a CIS [162]. Tintorè et al. [163] showed that the presence of OCBs within 3 months of CIS nearly doubled the risk of a second clinical attack over 50 months. On the other hand, CSF with >50 white blood cells (WBCs)/mm3 or >100 mg/dl protein is rarely observed in MS, and this should raise the possibility of an alternative diagnosis [164].

Biomarkers in conjunction with other prognostic criteria, such as MRI, may help in the early identification of MS and stratify CIS patients according to their risk for progression to CDMS. Antibodies targeting myelin antigens are, naturally, one of the most extensively studied serum biomarkers in MS. The presence of IgM against the extracellular domain of myelin oligodentrocyte protein (MOG), together with antibodies specific for myelin basic protein (MBP) in CIS patients was shown to be highly predictive for CDMS [165]. However, other studies revealed controversial results ranging from highly significant to totally non significant, so their use is controversial.

Biomarkers of axonal degeneration have also the potential to improve our capacity to predict and monitor neurological outcome in MS patients. Neurofilament proteins, one of the major proteins expressed within neurons and axons, have been detected in cerebrospinal fluid and blood samples from MS patients and are now being actively investigated for their utility as prognostic indicators of disease progression in MS [166]. Neurofilament light chains (NfL) are unique to neuronal cells, are shed to the CSF and are detectable at low concentrations in peripheral blood. Several study results support their value as a sensitive and clinically meaningful blood biomarker to monitor tissue damage and the effects of therapies in MS [167].

Additional novel biomarkers for MS include osteopontin, TNFa, various cytokines and chemokines and ab-crystalin, but their value is debated.

1.9 Differential diagnosis

Diagnosis of MS requires exclusion of diseases that could mimic or better explain the clinical and paraclinical findings, so an International Panel of MS experts developed consensus perspectives on MS differential diagnosis [168].

Atypical MS related demyelinating syndromes are: 1) tumefactive MS, characterized by presence at least of one large (>2 cm) acute demyelinating lesion, with accompanying edema, mass effect and ring enhancement; 2) Schilder's disease, Marburg type and Balò concentric sclerosis, considered as variants of tumefactive MS with a more severe and rapidly negative evolving course.

The most common disease that can be considered as MS mimics are the neuromyelitis optica (NMO) and NMO spectrum disorders (NMOSD), and the Acute Disseminated Encephalomyelitis (ADEM).

NMO is an inflammatory CNS syndrome distinct from MS characterized by optic nerve and spinal cord involvement, usually in monophasic not relapsing course, and associated with serum aquaporin-4 immunoglobulinG antibodies (AQP4-IgG). The new nomenclature [169] defines the unifying term NMO spectrum disorders (NMOSD), which is stratified further by serologic testing (NMOSD with or without AQP4-IgG). The core clinical characteristics required for patients with NMOSD with AQP4-IgG include clinical syndromes or MRI findings related to optic nerve, spinal cord, area postrema, other brainstem, diencephalic, or cerebral presentations. More stringent clinical criteria, with additional neuroimaging findings, are required for diagnosis of NMOSD without AQP4-IgG.

ADEM has been historically recognized as distinct from MS based on its monophasic course and presence of encephalopathy (manifest either as altered level of consciousness, behavioural change, or altered cognitive function) in combination with multifocal symptoms (e.g., cerebellar signs, cerebral motor or sensory features, optic neuritis or myelitis) often following an infectious illness [170]. MRI typically shows usually symmetrical multifocal or diffuse brain lesions [171]. However, an initial diagnosis of ADEM is often revised to prototypic MS after evidence emerges for continuing clinical activity consistent with MS [172].

Other more rarely disease need to be considered as MS mimics are Cerebral Autosomal Dominant Arteriopathy with Subcortical infarcts and Leukoencephalopathy (CADASIL), Susac syndrome, Vasculities as Systemic Lupus Erythematosus (LES) and Sjogren syndrome, sarcoidosis.

1.10 Therapies

The gold standard strategies in the management of MS are focused on (1) treating acute attacks, (2) ameliorating symptoms and (3) reducing biologic activity through disease-modifying therapies [173].

Treatment of acute relapses. Glucocorticoids are used as first-line treatment for attacks as they provide short-term clinical benefit by reducing the severity and shortening the duration of attacks. Typically intravenous (IV) methylprednisolone 1 g/day for 3– 5 days is given, often followed by an oral course of prednisone beginning at a dose of 60–80 mg/day and then tapered over 2 weeks [174]. Second-line treatment for patients resistant or refractory to glucocorticoid treatment includes plasmapheresis, IV immunoglobulin (IVIG), and adrenocorticotropic hormone (ACTH) [174]. The use of plasmapheresis (plasma exchange) is reserved for cases of severe symptoms refractory to glucocorticoids and generally involves five to seven exchanges (40–60 ml/kg per exchange) every other day for 14 days. IVIG is not approved in this indication but is sometimes used off-label in steroid-unresponsive patients as second- or third-line treatment; notably, this is the preferred treatment for postpartum patients. ACTH is another FDA-approved option but is rarely used because of high cost and uncertain advantages over glucocorticoids. In individuals who are unable to tolerate oral corticosteroids, ACTH is given intramuscularly at 80-120 units for two to three weeks and can be tapered.

Symptomatic treatment. The specific treatment of symptoms is an essential component of the overall management of MS. Symptomatic treatment is aimed at the elimination or reduction of symptoms impairing the functional abilities and quality of life of the affected patients. Moreover, with symptomatic treatment the development of a secondary physical impairment due to an existing one may be avoided.

The most common MS symptoms need to be treated are disorders of motor function and coordination, of cranial nerve function, of autonomic, cognitive, and psychological functions as well as MS-related pain syndromes and epileptic seizures. A consensus paper containing proposals for their treatment has been processed [175].

Spasticity is one of the most important symptoms complained by MS. Even if physiotherapy is generally accepted as a basic treatment option, spasticity requires to be also treated with oral medications comprising

centrally acting agents, such as baclofen, clonidine and tizanidine, as well as anticonvulsants such as benzodiazepines and gabapentin [176]. The efficacy and safety of 9-delta-tetrahydrocannabinol:cannabidiol (THC:CBD) oromucosal spray for treatment of MS spasticity has been demonstrated in several trials [177,178]. Observational studies and registry data subsequently confirmed the effectiveness and tolerability of THC:CBD oromucosal spray under everyday practice conditions. Among patients who respond to treatment, THC:CBD oromucosal spray has been shown to produce positive improvements in gait parameters and to normalize muscle fibers. Interventional procedures include focal injections of botulinum toxin, phenol or alcohol, and an intrathecal baclofen pump [179]. For the other symptomatic conditions, the more frequent treatment used are amantadine for fatigue, oxybutinin for urinary incontinence, antidepressants such as selective serotonin reuptake inhibitors (SSRIs) for depression.

Disease-Modifying Therapies (DMTs).

There is still no curative treatment for MS, but during the last 20 years different therapies have become available including interferon beta (IFN), glatiramer acetate (GA), teriflunomide (TFN), dimethylfumarate (DMF), natalizumab (NTZ), fingolimod (FTY), alemtuzumab (ALM), mitoxantrone (MTX) and cladribrine (CLD) and several new compounds are in development. All the approved medications have mainly anti-inflammatory effects by modifying the course of MS through suppression or modulation of immune function, and increasing evidence indicates that all of them are more effective in the early phases of disease development.

With the availability of highly efficacious therapies, the goal of treatment has changed dramatically in the last decades, from simply reducing relapse rates and slowing of disability progression to preventing all evidence of new disease activity and reducing progressive disability. Particularly in relapsing disease, a novel treatment strategy has emerged, where the aim is to achieve no evidence of disease activity (NEDA). The concept of NEDA, based on a stabilization of the condition on therapy such that there are no clinical (relapse or progression of disability) or radiological evidence (new T2 or contrast-enhancing lesions) of activity over a period of observation, has come to the forefront. In broad terms, once a diagnosis of MS is made, an assessment of disease (expected risk and frequency of relapse, clinical worsening or radiological progression activity) must be formulate for a given patient. To make this, it could be necessary the

determination of the long-term risk of progression, including incomplete recovery from first clinical relapse, more than one relapse in the first year of diagnosis, multifocal presentation and higher EDSS score prior to treatment, as well as imaging features at diagnosis. This prognostication then leads to a simpler therapeutic approach, choosing from a small handful of appropriate treatments rather than considering all agents, since many of the more efficacious therapies for aggressive disease are balanced against more onerous side-effect profiles.

It is possible to distinguish two therapeutic lines, based on the severity of the disease and on numerous side-effects that may occur.

Therapies as Interferon-beta, Glatiramer Acetate, Teriflunomide and Dimethylfumarate belong to the list of first-line drugs in MS.

Interferon-beta (IFNβ) is a naturally polypeptide predominantly produced by fibroblasts. Its anti-inflammatory effects are largely believed to result from the inhibition of T-lymphocyte proliferation, a shift of cytokine response from an inflammatory response to an anti-inflammatory profile, and a reduced migration of inflammatory cells across the BBB [180]. IFNβ is available for MS treatment in recombinant forms, as interferon beta-1a (Rebif®, Avonex®) or peginterferon beta-1a (Plegridy®) or interferon beta-1b (Extavia®, Betaferon®). All the IFNβ preparations have shown beneficial effects in reducing the annualized relapse rate (ARR), the progression of disability in RRMS as well as the magnetic resonance imaging (MRI) disease activity [181,182]. Besides these advantages, most patients (50%–75%) experience flu-like symptoms, including muscle aches, fever, chills, headache and back pain, that usually appear 2–8 h after an injection and resolve within 24 h [183]. Isolated cases of severe injection-site reactions involving infection or necrosis as well as severe cases of acute liver failure and pancreatitis [184] have demonstrated that periodic surveillance of liver function and blood counts before starting therapy and every 6 months thereafter is recommended. IFNβ treatment may induce formation of specific neutralizing antibodies (NABs). The NABs usually appear within 6–18 months of treatment, so reducing the efficacy of treatment. Accordingly, it is recommended to test all patients for the presence of NABs every 6 months during the first 2 years of therapy, and treatment should be switched in patients who are confirmed to be NAB positive [185].

Glatiramer acetate (GA) (Copaxone®) is a pool of synthetic peptides, resembling sequences of myelin basic protein, with an average length of 40–100 residues. Its mechanism of beneficial effect in MS remains

incompletely understood, but it is thought to augment TH2-cell function and inhibit myelin basic protein-specific T-cell activity, and to promote neuronal repair through the stimulation of neurotrophin secretion [186]. In RRMS patients GA determinates a significant reduction in ARR and in gadolinium-enhanced MRI activity [187], besides to significantly prolong time to a second relapse and to reduce the risk of new MRI lesions in patients with a possible MS [188]. GA is usually well tolerated, but most patients (65%) experience injection-site reactions (pain, erythema, swelling and pruritus). About 15% report a transient self-limited systemic reaction (immediately after injection) of facial flushing and chest tightness, accompanied at times by palpitation, anxiety and dyspnea. Rare and severe reported side effects are lobular panniculitis and skin necrosis [189].

Teriflunomide (TFN) (Aubagio®) is an immunomodulatory agent that selectively and reversibly inhibits the mitochondrial enzyme dihydro-orotate dehydrogenase, a key mitochondrial enzyme in the de novo pyrimidine synthesis pathway, leading to a reduction in proliferation of activated T and B lymphocytes. [190]. The therapeutic effect in MS is not fully understood but it is probably mediated by a reduced number of circulating lymphocytes. In the TEMSO and TOWER studies, TFN 14 mg was associated with a lower ARR and less disability accumulation compared with placebo [191]. TFN was also investigated as add-on therapy to IFN-b in patients with relapsing forms of MS and first clinical episode suggestive for MS, showing similar effects on reduction of ARR, time to a new/second relapse, presence of new MRI lesions [192,193]. Common adverse events include upper respiratory tract infection, urinary tract infection, paresthesia, diarrhea, nausea, hair thinning, liver enzymes increase and hypertension. TFN is associated with increased liver enzyme levels and ttreatment should be stopped if liver enzyme levels increase three times above upper normal levels [194]. TNF is also contraindicated in pregnant patients because of a potential risk of teratogenicity.

Dimethylfumarate (DMF) (Tecfidera®) is an oral first-line therapy for RRMS patients with strong efficacy and neuroprotective and immunomodulatory effects, and a favourable benefit-risk profile. It is believed that the mode of action of DMF involves both nuclear factor erythroid-derived 2-related factor (Nrf2)-dependent and independent pathways, leading to a downregulation of inflammatory cytokines and an overall shift from a proinflammatory Th1/Th17 response to an anti-inflammatory/regulatory Th2 response [195]. In the pivotal, placebo-controlled phase III DEFINE and CONFIRM trials in adults with RRMS, twice-daily DMT reduced

clinical relapse and MRI measures of disease activity and improved some aspects of health-related quality of life [196,197]. Both trials have been extended in the ENDORSE study, which was conducted for more than 5 years, confirming data about efficacy and safety highlighted in the previous trials [198]. DMT had an acceptable tolerability profile. The most common adverse events are flushing and gastrointestinal events, usually of mild or moderate severity and largely manageable [199]. Because DMT reduce absolute lymphocyte counts, the monitoring of DMF treated patients is an important consideration regarding the risk to develop opportunistic infections such as PML. Generally, an absolute lymphocyte counts less than 500 mm3 that persisted for > 6 months in DMT treated patients should warn clinicians about the possibility of stopped treatment.

Fingolimod, Natalizumab and Alemtuzumab are newer drugs that are currently considered as second-line therapies, administered in adult patients with RRMS when the initial treatment has proven to be inadequate. There is uncertainty in clinical practice regarding how and when to switch from the first-line to a second-line therapy, so the ECTRIMS/EAN guideline development group produced a set of recommendations regarding switching from IFN or GA to a second-line therapy in patients with RRMS [200]. The guideline strongly recommends that patients that are currently treated with IFN or GA who show evidence of disease activity should be offered a more efficacious drug. The authors did not specify which drugs were more efficacious in the recommendation section but reported that all analyzed studies in the guideline consistently showed a benefit in switching to NTZ, FTY or ALM compared with IFN or GA.

Fingolimod (FTY) (Gylenia®) is an oral sphingosine 1-phosphate receptor (S1PR) modulator that subsequent to its phosphorylation binds with high affinity to S1PR, leading to internalization and degradation of S1PR, mainly on T lymphocytes, but also microglia, astrocytes, and endothelial cells [201]. FTY also increases the production of brain-derived neurotrophic factor and improves neurodegenerative processes [202]. Its therapeutic activity could be due to regulation of the migration of selected lymphocyte subsets into the central nervous system leading to reduction of reactive activation of glia (which may favour naturally occurring remyelination) [203]. It has been the first orally active immunomodulatory drug used in MS, that has opened up new approaches to the treatment of the disease. In the phase III TRANSFORMS and FREEDOMS trials, FTY was more effective than IFNβ-1a [204] and placebo [205] in reducing both clinical and MRI outcome measures, including brain volume loss. FTY seems to have also a significant beneficial

impact on ameliorating cognitive impairment [206]. Common adverse events include upper respiratory tract infection, headache, cough, diarrhea and back pain [207]. FTY may also cause a transient bradycardia and atrioventricular block. It is therefore recommended to monitor patients continuously with an electrocardiogram for 6 h after the first dose, and to extend the monitoring of patients who develop specific clinically relevant signs of heart arrhythmia. Generally, FTY should not be used by patients with known cardiac arrhythmias or patients using other medications known to induce bradycardia. Rare adverse events of elevated liver enzymes and macular -edema may occur.

Other sphingosine-1-receptor modulators such as Siponimod are under development. Encouraging results on efficacy of Siponimod in reducing disease progression and MRI activity in relapsing MS [208] have also led to the initiation of the EXPAND study in SPMS, which is expected to be completed on 30 June, 2023 [209].

Natalizumab (NTZ) (Tysabri®) is a humanized anti–α4 integrin monoclonal antibody; it binds the α4 subunit of α4β1 and α4β7 integrins, blocking the binding to their endothelial receptors, thereby attenuating the CNS inflammation [210]. In addition, NTZ acts also by inhibiting the interaction between α4-positive leukocytes, fibronectin, and osteopontin. NTZ was initially approved by the US Food and Drug Administration (FDA) in 2004 for RRMS, upon the interim results of two phase-III trials, the AFFIRM and the SENTINEL [211,212], but in 2005 it was taken off the market after the occurrence of three cases of Progressive Multifocal Leukoencephalopathy (PML), until that moment considered only as a secondary complication in patients affected by human immunodeficiency virus (HIV) infection or other rare forms of immunodeficiency: two cases in MS patients and one in a Crohn's disease (CD) patient [213,214].

In consideration of NTZ great efficacy, it was released on the market in the European Union in 2006 in association to a Global Risk Management Plan. The "TOUCH®" program (TYSABRI Outreach: Unified Commitment to Health Prescribing Program) [215] has been developed in order to facilitate its appropriate use and to focused on its safety.

Final results of the two longitudinal clinical trials after two years have showed the efficacy and safety of NTZ in monotherapy versus placebo (AFFIRM) or in combination with IFN β-1a versus IFN alone (SENTINEL). Primary endpoints of the two studies were (Table 1) annualised relapse rate (ARR) and risk of cumulative EDSS progression for at least 12 weeks [216,217]. Secondary endpoints were: rate of relapse-free patients, number of new T2 lesion on MRI, presence of new gadolinium-enhancing (Gd+) lesions, ARR,

disability progression measured by the Multiple Sclerosis Functional Composite (MSFC), number of new T1 lesion and volume lesions in T2. Tertiary endpoints concerned about NTZ efficacy on reduction of atrophy, impact on quality of life and visual functions.

Table 1. Mean endpoints of AFFIRM and SENTINEL trials.

ENDPOINT	**AFFIRM** (*NTZ vs placebo*)	**SENTINEL** (*NTZ + IFN vs IFN*)
↓ risk of EDSS progression ≥12 weeks at 2 years	42%	24%
↓ ARR at 1 years	68%	54%
↓ ARR at 2 years	68%	55%
% of relapse-free patients at 2 years	39%	40%
↓ n° of new T2 lesion at 2 years	83%	83%
↓ n° of new gadolinium-enhancing (Gd+) lesions at 2 years	92%	89%
↓ n° of new T2 lesion at 2 years	76%	44%
↓ volume lesions in T2 at 2 years	18%	20%

The safety and tolerability of NTZ when added to GA in patients with RRMS were evaluated in a phase 2, randomized, double-blind, placebo-controlled study on 110 RRMS patients (GLANCE) [218]. The primary outcome assessed whether this combination would increase the rate of development of new active lesions on cranial MRI scans versus GA alone. Results showed that the mean rate of development of new active lesions was 0.03 with combination therapy versus 0.11 with GA alone ($p = 0.031$). Combination therapy resulted in lower mean numbers of new gadolinium-enhancing lesions (0.6 versus 2.3 for GA alone, $p = 0.020$) and new/newly enlarging T2-hyperintense lesions (0.5 versus 1.3, $p = 0.029$).

The efficacy and tolerability of NTZ have also been confirmed in several post-marketing studies [219–221] and in a prespecified subgroup analysis of AFFIRM and SENTINEL trials [222], demonstrating that NTZ is effective in reducing disability progression and relapses in patients with relapsing MS, particularly in patients with highly active disease.

To date, NTZ is recommended as monotherapy in adult relapsing-remitting MS patients with highly active disease despite an adequate and complete cycle with one of the DMTs or in RRMS patients with a severe disease course defined by at least two or more disabling relapse in 1 year or one or more Gd+ lesions on MRI scan or an increase of number of T2 lesions compared to a previous MRI scan [223].

Alemtuzumab (ALM) (Lemtrada®) is a humanized monoclonal antibody against CD52 (cluster of differentiation 52) and is approved as escalation therapy for relapsing MS. The application of ALM leads to a rapid but long-lasting depletion predominantly of CD52-bearing B and T cells with reprogramming effects on immune cell composition resulting in the restoration of tolerogenic networks [224]. Compared with the IFN-b1a treated group, in CARE-MS I and II trial [225,226] ALM demonstrated efficacy in treatment-naïve patients with active RRMS and those relapsing on prior DMTs, with a consistent and manageable safety and tolerability profile, with maintenance of efficacy over years from the start of treatment [227,228].

After depletion of circulating T and B lymphocytes, with the lowest observed values occurring within days, lymphocytes repopulated over time, with B cell recovery usually complete within 6 months, while T lymphocytes recovered more slowly and generally did not return to baseline by 12 months post-treatment [229]. For this reason, routine monthly monitoring is required for up to 48 months after the last infusion to promptly identify potentially serious autoimmune adverse events. In fact, despite its effectiveness ALM treated patients can experiment frequent and significant adverse events. Besides to infusion-associated reactions, approximately 30%–40% of patients develop mild to moderate infections and secondary autoimmune diseases, predominantly affecting thyroid, kidney and thrombocytic function [230]. In summary, although a highly effective medication, long-term side effects of ALM can be severe and might limit the therapeutic spectrum. To date, within the European Union ALM is indicated for the treatment of adult patients with RRMS with active disease defined by clinical or imaging features; therefore, ALM may be an appropriate treatment choice across a broad range of patients with RRMS, including treatment-naïve patients with active disease, patients with highly active disease or for patients relapsing on prior DMTs. Generally, ALM is administered in a unique dosing regimen via intravenous infusion on 5 consecutive days at baseline and on 3 consecutive days 12 months later, and as-needed retreatment (3 consecutive days at least 12 months after the last course) in cases of disease recurrence [231].

With the emerging interest in determination of B-cells role as active player in MS pathogenesis, several B cell-targeted therapies have been developed.

Rituximab (RTX) (®) is a chimeric monoclonal B-cell-depleting anti-CD20 antibody that causes rapid and complete depletion of B cells both in blood and with a lesser degree in CSF [67]. RTX is approved as therapy for lymphoma and in combination with methotrexate for the treatment of rheumatoid arthritis.

Although not approved as therapy for MS, the medication is widely used in MS centers in Europe and has been further developed to the humanized follow-up medication ocrelizumab, discussed below.

The exact mechanism of RTX by which depletion of B and T cells contrasts inflammatory activity in MS patients is not fully understood. It has been speculated that it could be linked to indirect effects depending by B cells, such as cytokine or modulators of T cell activity production [232]. RTX also induces apoptosis in small subgroups of proinflammatory CD3+ T cells expressing CD20 [233]. Efficacy of RTX was evaluated both in RRMS and PPMS patients (HERMES study [234] and OLYMPUS study [235], respectively), demonstrating a significant reduction in the ARR and number of new Gd-enhancing lesions on cranial MRI. Although OLYMPUS trial failed to show any significant beneficial impact on disease progression in patients with PPMS, subgroup analyses suggested a possible benefit in younger patients (≤51 years of age) with active inflammatory lesion components [235]. As known for IFNs, RTX therapy is also associated with the risk of developing infusion-related antibodies, which correlate with incomplete B-cell depletion and possible failure of the treatment [236].

Ocrelizumab (OCRE) (Ocrevus®) is a monoclonal antibody directed against CD20. The antibody binds to the large extracellular loop of CD20, which leads to the depletion of CD20-expressing B cells. Importantly, plasma cells and antigen-specific antibody titers are not influenced as plasma cells do not carry CD20 [237]. OCRE has been investigated in pivotal trials both in relapsing MS and PPMS. The OPERA I and II trials compared OCRE against IFNb1a (44 lg) [238], showing a reduction in the ARR, the percentage of patients with disability progression was and the number of new gadolinium-enhancing lesions. Although n the ORATORIO trial, OCRE has a role in reducing disability progression, the effect seems be limited to younger patients with high disease activity [239]. The main side effects of OCRE include mild infusion-related reactions, such as pruritus, rash, throat irritation, and flushing. Low immunogenicity has been observed in OCRE treated patients as the incidence of antidrug antibodies in the peripheral blood is not relevant [237].

Cladribine (CLD) (Mavenclad®) is a purine nucleoside and is phosphorylated in cells with a high amount of deoxycytidine kinase, which leads to nuclear accumulation and cell death. CLD reduces the proliferation of microglia and selectively depletes lymphocytes, with a predilection for B lymphocytes and especially memory B lymphocytes [240]. Instead, CLD induced only modest depletion of T cells, which may not be consistent with a marked influence on MS, based on previous $CD4^+$ T-cell depletion studies. The therapeutic

drug-response relationship with CLD is more consistent with lasting B-cell depletion and, coupled with the success seen with monoclonal $CD20^+$ depletion, suggests that B-cell suppression could be the major direct mechanism of action [241]. The approval process for CLD was interrupted in 2011 was due to reports about a possible increase in the risk of malignancies. However, a recent meta-analysis of 11 phase III trials comparing the malignancy risk of several DMTs seems to not confirm this increased risk, claiming that the cancer rate in the CLD group (0.34%) was the same as with the other therapies [242]. The CLARITY study is the most important pivotal trial on efficacy and safety of CLD [243], in which both relapse rate and the risk of disability progression associated with the brain lesion count and atrophy were significantly reduced. In the ORACLE MS study, CLD has also proven to be able to potentially prevent the conversion to clinically definite MS in CIS patients with a first demyelinating event regarding the potential to prevent [244]. The most common side effect included lymphopenia, explained by the mechanism of action, and in 2.3% of treated patients typical herpes zoster infections could be documented [243].

Other potential therapies for MS management has been evaluated in the recent years, although results on their efficacy and safety are still not promising.

Daclizumab (DAC) is a monoclonal antibody directed against the CD25 subunit of the interleukin-2 receptor. Its high-yield process activates immunoregulatory CD56-bright natural killer cells, which inhibit the survival of CD4 and CD8 T cells [245]. DAC has shown efficacy in slowing the inflammatory process of MS, but the appearance of potentially serious side effects (including fulminant hepatopathy and encephalitis) has not allowed its use to significantly impact current clinical practice [246].

Laquinimod (LAQ) is a quinoline carboxamide showing structural similarities with kynurenic acid, whose immunomodulatory properties have been deciphered from studies in the animal model of MS, experimental autoimmune encephalomyelitis (EAE). Data indicates that LAQ exerts beneficial activities both on the peripheral immune system and within the CNS, modulating the function of various myeloid antigen presenting cell populations, which then down-regulate proinflammatory T cell responses. Further, data also indicate that LAQ acts directly on resident cells within the CNS to reduce demyelination and axonal damage [247]. However, this drug did not reach the primary endpoint of reduction in brain volume in a phase 2 trial and in disability progression in a phase 3 trial of patients with RRMS, respectively; as a consequence the development of this drug will probably not be continued in MS [248].

Table 2. Summary of all MS available drugs and mode and frequency of administration.

DRUG	MODE AND FREQUENCY OF ADMINISTRATION
IFNβ 1a	im injection, 1 time a week (Avonex) or 3 times a week (22 mcg or 44 mcg)
IFNβ 1b	sc injection, every other day
pegIFNβ 1a	sc injection, 1 time every 14 days
GA	sc injection, 1 time a day
TFN	Oral, tablet, 14 mg 1 time a day
DMF	Oral, capsule, 240 mg 2 times a day
FTY	Oral, capsule, 0.5 mg 1 time a day
NTZ	iv infusion, 1 time every 4 weeks
ALM	iv infusion, first year 5 infusions in 5 days (at 12 mg), second year 3 infusions
RTX	iv infusion, 500 or 1000 mg, 1 time every 6 months
OCRE	iv infusion, first dose two 300 mg infusions, 2 weeks apart, second dose after 6 months
CLD	Oral, tablet, 3.5 mg/kg bodyweight for 2 years, 2 treatment week/ year

IFNβ-1a Interferonβ-1a, *IFNβ-1b* Interferonβ-1b, *pegIFNβ 1a* Peginterferone β 1a, *GA* Glatiramer Acetate, *TFN* Teriflunomide, *DMT* Dimethylfumarate, *FTY* Fingolimod, *NTZ* Natalizumab, *ALM* Alemtuzumab, *RTX* Rituximab, *OCRE* Ocrelizumab, *CLD* Cladribine, *sc* subcutaneous, *qd* once daily, *bid* twice daily, *IV* intravenous.

JOHN CUNNINGHAM VIRUS (JCV) AND RISK OF PML ASSOCIATED WITH MS TREATMENTS

The reduced immune surveillance of the tissues with the use of monoclonal antibodies is responsible for an increase in the incidence of infections, especially viral or intracellular parasites, both in the CNS and in other districts.

Despite the large number of individuals with MS treated with a wide variety of broadly immunosuppressive regimens, including high dose corticosteroids, AZA, and cyclophosphamide, prior to the era of DMTs in 1993, no cases of PML in MS had been observed until 2005 when two individuals who had received both NTZ and interferon-β1a in a pivotal trial of NTZ were described [249,250]. Subsequently, it was recognized that NTZ alone was sufficient to cause PML.

Progressive Multifocal Leukoencephalopathy (PML) is an opportunistic infection caused by John Cunningham virus (JCV) reactivation [251,252], which has a severe impact on patients' disability course, functional outcome, and quality of life [253].

The JCV is a DNA virus of the human polyomavirus family [254]. Serum antibodies to JCV are found in 50% to 70% of the healthy general population, indicating that JCV is ubiquitous in humans [255]. The passage of the JCV through the body can be stratified into several steps including primary viremia, latency, and reactivation. Two portals of entry for JCV into the body have been suggested: the tonsils and the gastrointestinal tract [256]. Primary infection with JCV typically occurs in early life and it is frequently asymptomatic; after primary infection, JCV presumably remains latent in various tissues, such as the kidneys, bone marrow, and lymphoid tissue [257,258]. Most of the studies observed a prevalence of around 60–70% of detectable antibodies against JCV in the general population, with seroprevalence increasing with the age [257]. Polyomavirus-induced disease typically occurs as a result of resurgence of the persistent infection when immune control is compromised. PML probably occurs as the interaction between JCV seropositivity and patient's features: the disease typically affects heavily immunosuppressed patients [258] or patients previously treated with immunosuppressant drugs or other monoclonal antibodies [259,260]. By reactivation of latent JCV, the virus spreads into the CNS crossing through the BBB and causes infection of oligodendrocytes, resulting in demyelination [261,262]. The lack of myelin leads to axonal dysfunction and

ultimately can result in the permanent loss of neurons. PML lesions in the brain affect the white matter, and are often multifocal, suggesting that the virus spreads to the brain by a haematogenous route [263].

The first cases of PML were reported in 1958 in three patients with chronic lymphocytic leukemia (CLL) and Hodgkin's lymphoma [264]. Up until the early 1980s, only about 200 cases were reported and they were all associated with lymphoproliferative disorders.

With the advent of the Acquired Immunologic Deficiency Syndrome (AIDS), PML became more prevalent. Neurological signs of PML was first described by Igor Koralnik et al. [265] in a patients with Human immunodeficiency Virus (HIV) infection in which JCV infected granule cell neurons of the cerebellum leading to severe atrophy. Subsequently, in the years between 1970s and 1990s about 85% of cases occurred in patients with HIV infection. The introduction of Highly Active Antiretroviral Therapy (HAART) helped increase the life expectancy of individuals diagnosed with PML [266], but the mortality rate in AIDS-associated PML cases is still approximately 50% [267].

From an immunopathogenetic point of view, the decrease in CD4+ T cells is thought to be the major determinant for developing PML because they are required for maintenance of CD8+ T cells, the most important player in the overall immune control of JCV infection [268].

From a symptomatic point of view, PML can be supposed on the basis of the clinical presentation. Berger et al. [269] found that hemiparesis, visual impairment, and altered mentation were the 3 most common initial manifestations. Symptoms referable to the posterior fossa as ataxia, dysmetria, and dysarthria, are usually indicative of involvement of the cerebellum and brain stem [270]. Other signs and symptoms associated with PML include headache, vertigo, seizures, sensory deficits, parkinsonism, aphasia, and neglect syndromes [271].

More recently PML has been diagnosed in groups of individuals taking monoclonal antibody (mAb) based drugs to treat lymphoproliferative disorders or autoimmune diseases as MS.

No PML cases have been reported in MS patients before the introduction of NTZ [272]. The mechanism by which NTZ treatment increases the risk of developing PML is not yet known but it has been hypothesized that the combination of low immune-surveillance of the CNS and the increased presence of B cells and $CD34^+$ progenitor cells can favor viral replication, ultimately leading to PML.

In addition, PML has been observed also in few MS patients treated with other drugs, such as DMT [273] and FTY [253]. In the case of DMT treatment, persistent lymphopenia seems likely to identify a higher-risk group of patients. In fact, prolonged lymphopenia with absolute lymphocyte counts of less than 750 lymphocytes per mL accounts was observed in most cases of PML associated with DMT, due to the loss of CD8+ cells that are crucial to control of JCV [274].

To the best of our knowledge, no cases of PML attributable to OCRE have been described so far in MS patients, while PML has been reported in one patient treated with TFN [275] and in one treated with ALM [276], but in both cases patients have developed PML after switching from NTZ to the new drug and PML was finally attributed to NTZ. An increase in the risk of PML development has been recorded in patients treated with RTX and PML is listed as a potential side effect of the treatment [277]. PML has not yet been associated with CLD treatment in MS, but has been documented in patients treated for hematological disorders, including follicular lymphoma [278] and systemic mastocytosis [279].

In NTZ-treated MS patients, brain MRI can detect signs of PML at very early stages [280]. In comparison to classical HIV-associated cases of PML, NTZ-PML displays a higher frequency of MRI signs suggestive of inflammation at the time of diagnosis, including contrast enhancement and punctuate lesions with a perivascular distribution pattern, reported in approximately 30% of the patients [281–283].

Suspected diagnosis should be confirmed by the detection of the virus in the CSF through the Polymerase Chain Reaction (PCR) test; however, definitive diagnosis of PML requires neuropathologic demonstration of the typical histopathologic triad (demyelination, bizarre astrocytes, and enlarged oligodendroglial nuclei) [284,285].

PML risk is associated with duration of exposure to NTZ treatment, prior immunosuppresant (IS) use and presence of high titer of anti-JCV antibody (JCV index) [286–288]. Therefore, determination of antibodies against JCV is an important tool for risk stratification in NTZ treated patients with RR–MS. According to these data, European Medicines Agency (EMA) has updated the estimate risk for PML in seropositive JCV antibody patients treated with NTZ and a risk stratification algorithm has been created with the aim to evaluated the PML risk for each patient [287].

On September 2019, the global overall incidence of PML in NTZ treated patients was 4.08 per 1000 patients (95% CI 3.80–4.36 per 1000 patients). There have been 825 confirmed PML cases, 822 of which in patients

with MS (224 cases in US, 515 in European Economic Area, 86 in rest of world, respectively) and in the 76% of patients a moderate level of disability remained [Global Natalizumab (TYSABRI) Post-marketing PML Update. September 2019]. The mortality rate among NTZ-treated MS patients is around 30% [289]. When a diagnosis of PML is made, the treatment is suspended in order to restore immune surveillance of the CNS. As a consequence, IRIS can occur and can be fatal [290].

The first use of serum antibodies to JC virus for PML risk stratification able to provide qualitative information (positive/negative status) was introduced in 2010 [291]. Since 2013, a second-generation test that delivers a JC antibody index value in addition to positive or negative serostatus has been validated and used [292].

The PML risk estimate for patients with an index value less than 0.9 has been reported to be significantly lower than the PML risk for patients with an index greater than 1.5 [288]. Prolonged use of NTZ is known to be associated with higher risk of PML [252,286] because patients on therapy undergo to a JC virus seroconversion more frequently than control patients not on NTZ [293].

For these reasons, in patients who are negative or low positive for anti-JCV antibodies, at least six-monthly follow-up tests are recommended by consensus [294].

Following EMA recommendations , patients with high levels of JCV index treated for over 2 years should be informed about the risk of PML related to NTZ and the need to be vigilant for up to six months after its discontinuation.

At the 24th NTZ dose, patients should evaluate again with the neurologist the opportunity to continue treatment or to switch to any other first or second line MS therapies.

Generally, patients with high levels of JCV index treated for over 2 years tend to discontinue NTZ treatment, despite the risk of disease reactivation peaking during a “high risk period” between the second and the eighth month since stopping the drug [295]. In fact, several studies showed that after NTZ discontinuation disease activity got worse than pre-NTZ status [295–297], indicating a rebound effect. The hypothesis of a “drug holiday after NTZ Withdrawal” cannot be recommended, since this choice could lead the patient to experience severe disease reactivation and even a rebound condition, dangerous in terms of disability accumulation and also life-threatening and cognitive functions [298].

A possible strategy may consists in a Pulsed Corticosteroid Treatment after NTZ discontinuation. Evangelopoulos et coll. [299] analyzed effects of corticosteroid treatment on 20 MS patients during a six-month washout period after NTZ discontinuation. Patients received monthly intravenous methylprednisolone (1000 mg/infusion) and received regular clinical and radiological assessment. During the six-month washout period, only one patient out of 10 had a mild sensory relapse associated with MRI activity in the group treated with methylprednisolone. In the control group of untreated 10 patients, one developed several active lesions in brain MRI and another one had a severe relapse. These data suggest that monthly methylprednisolone treatment in the washout period could determine a clinically stable disease phase, allowing a more safe transition to another therapy .

Another alternative strategy could be the administration of NTZ with extended interval dosing (EID) [276]. A single study [300] tried to evaluate the best way to interrupt treatment, showing a higher rate of relapses in the group with sudden NTZ discontinuation compared to the one who tapered it down slowly. These interesting data suggests a way to reduce the return to high activity after NTZ withdrawal, but more data on larger cohorts are needed. However, in absence of unanimous consensus, the suspension of treatment and the shifting to a safer therapy represents the most common strategy to limit the risk of PML.

AIM OF THE STUDY

PML diagnosis and management in MS patients treated with various DMTs represent one of the most difficult challenges and several efforts are being made to determinate PML risk stratification for each patient related to specific MS treatment and to prevent PML infection and its consequences on disease course.

Some studies focused on the impact of DMTs on the longitudinal evolution of anti-JCV antibody index [301], but no studies have considered the interference of NTZ discontinuation on JCV status modification over an extended time, in order to refine treatment strategies. Moreover, although the risk of PML is also present in MS patients treated with other drugs, few studies have evaluated the JCV index profile during treatment with DMTs different from NTZ.

This project consists of two different studies. The aim of the first study was to investigate the anti-JCV antibody status pre, during and post NTZ treatment and describe the trend of JCV index after a long period of NTZ discontinuation in order to identify possible alternative therapeutic strategies.

The aim of the second study was to evaluate JCV status modification during treatment with currently used DMTs different from NTZ in order to possibly define the best possible therapeutic approach, especially in those patients with high JCV index at the beginning of the new treatment or with a higher risk of PML, and to explore if there exists further exit strategies in patients treated with NTZ and high JCV index score.

MATERIALS AND METHODS

4.1 Study population.

This retrospective-prospective observational study enrolled patients with diagnosis of RR–MS treated with several DMTs and followed at the MS Centre of Catania University Hospital between January 2010 and December 2018. Data about patients was obtained retrospectively from the database iMED, a computerized medical record in which at each clinical follow-up physician of the MS centre collect demographic, clinical and laboratory information. This study protocol was approved by the local Ethical Committee of the University of Catania (Catania 1). Each patient participating to the study signed an Informed Consent specifically designed for the study.

Two different datasets with demographic and clinical data were created. For the first study, inclusion criteria were: (1) diagnosis of RR–MS according to the Mc Donald criteria 2010 [154], (2) at least six NTZ administrations, (3) at least two determinations of JCV Index during the follow-up period, (4) NTZ discontinuation period for more than 6 months. Patients eligible prospectively underwent to the anti-JCV antibody determination after a period of NTZ discontinuation.

For the second study, inclusion criteria were: (1) diagnosis of RR–MS according to the Mc Donald criteria 2010 [154], (2) treatment for at least six months with rituximab-ocrelizumab (RTX-OCR), fingolimod (FTY), interferons-glatiramer acetate (IFN-GA), teriflunomide (TFN), dimethylfumarate (DMF), alemtuzumab (ALM) or cladribine (CLD), (3) at least two determinations of JCV Index during the follow-up period. Main outcomes were the JCV index changes and the rate of seroconversion.

4.2 Sampling.

In the first group of NTZ treated patients, JCV status was evaluated at baseline (T0), at the time of the NTZ discontinuation (T1) and at the last follow-up (T2).

In the second group of patients treated with other DMTs, JCV status was evaluated at baseline before starting treatment (T0) and during treatment (T1). Evaluation of JCV index score at further timepoints (T2) has been already planned but data were not enough to be included in this study.

At each timepoint, patients were divided into two subgroups based on their JCV status: negative JCV index and positive JCV index, with a JCV value between 0.9 and 1.5 (low positive JCV index) or > 1.5 (high positive JCV index) respectively. Blood samples were collected by peripheral venous puncture. JCV index was determined only through a qualitative result (positive or negative) for patients screened before 2011 (STRATIFY JCV Dx Select, [288]) and by a two-step enzyme-linked immunosorbent assay (STRATIFY II) for patients screened after 2011. Analysis was centrally performed at Unilabs in Copenhagen, Denmark. Qualitative (negative/positive) and, for anti-JCV antibody positive patients, semi quantitative results were obtained. An index value of less than 0.9 was considered as negative and equal to or greater than 0.9 as positive. Seroconversion was defined as changing status of serum JCV antibody.

4.3 Statistical analysis.

Statistical analysis was performed using STATA 16.1 software packages (StataCorp. 2011; Stata Statistical Software: Release 12; College Station, TX: StataCorp LP). The numerical data sets were tested for normal distribution with the Shapiro-Wilk test. Student's t tests will be applied for parametric variables, while for non parametric variables, differences between subgroups are analyzed with a $\chi 2$ test. Nominal data were analyzed by Pearson's Chi Square or Fisher's exact test, where applicable. Differences in terms of JCV index value in the first analysis at each time point were calculated by Kruskall-Wallis test and one-way ANOVA, where applicable. The correlation between clinical and laboratory variables was carried out using a bivariate correlation (Pearson's or Spearman's correlation). Univariate analyses were carried out in order to correct for age, sex, disease duration, previous use of immunosuppressant drugs or NTZ and mean interval time between starting DMTs and determination JCV index. We considered a two-sided p-value of < 0.05 as statistically significant.

RESULTS

In the first study, out of 332 patients screened referring to the MS centre who had a history of NTZ treatment during their disease course, 285 patients met inclusion criteria (mean age 41 years; female 201 [70.5%]). In Fig. 5, the flow-chart shows how patients have been screened at different timepoints based on their JCV status. Demographic and clinical characteristics of the whole cohort at baseline are summarized in Table 3. Out of 285 patients, at T0 208 (73%) were JCV negative, and 67 patients (23.5%) were positive [33 (11.6%) in the low positive JCV index group and 34 (11.9%) in the high positive JCV index group, respectively]. Data were not available for the remaining 10 (3.5%) patients (female 7 and male 3, respectively). Out of JCV positive patients at baseline, 3 (9.1% of 33) in low positive JCV index group and 9 (26.5% of 34) in high positive JCV index group were naive, while 55 (82.1% of 67) had already been treated with a DMT; in the group of JCV negative patients, 60 (28.8% of 208) were naive and 148 (71.2% of 208) had already been treated with a DMT. There were not statistically significant differences between groups in term of age and sex, years of disease, number of previous DMTs and previous use of immunosuppressant drugs. Out of 285 patients, 170 patients who remained with a negative JCV serology continued treatment, while after a mean of NTZ treatment of 31.1±14.6 months (average of number of NTZ administrations: 26), 115 patients stopped NTZ because of high level of JCV index and progressive risk of PML, some of them shifting to a safer DMTs. Discontinuers stopped treatment for the following reasons: adverse events (2 patients, 1.7%), inefficacy as appearance of clinical or radiological relapse (4 patients, 3.5%), growing PML risk (89 patients, 77.4%), progression of disease (9 patients, 7.8%) or lost to follow-up (11 patients, 9.6%). After a mean time of follow-up of 110.6±115.1 days from NTZ discontinuation, most of the patients shifted to another DMTs, and in particular 34 (29.6% of 115) switched to a first-line therapy [16 (13.9%) to Interferon/Glatiramer Acetate (IFN/GA), 12 (10.4%) to Dimethilfumarate (DMF) and 6 (5.2%) to Terifunomide (TFN), respectively] and 60 (52.2%) switched to a second-line therapy [44 (38.3%) to Fingolimod (FTY), 9 (7.8%) to Alemtuzumab (ALM), 2 (1.7%) to Azathioprine (AZA), 2 (1.7%) to Daclizumab (DAC), 2 (1.7%) to Ocrelizumab (OCRE) and 1 (0.9%) to Rituximab (RTX), respectively]. For the remaining 21 (18.3%) patients, neurologists decided to stop every therapy due to progression course of the disease. At T1 JCV index data were available for 107 patients (93% of 115). Out of 115 subjects, 69 (60% of 115) were JCV-positive and 38 (33.0% of 115) were JCV negative. In the subgroup of JCV positive, 48 (69.6% of 69) were

already positive at baseline and remained positive (18 patients in low positive JCV index group switched in the high positive JCV index group), 19 (27.5%) seroconverted from a negative to a positive status during the treatment period and no patients seroconverted from a positive to a negative status. In the subgroup of JCV negative, 27 (71.1% of 38) were already negative at baseline, 7 (18.4%) seroconverted from a positive to a negative status during the treatment period and no patients seroconverted from a negative to a positive status. For 6 patients (2 in JCV positive group and 4 in JCV negative group, respectively) it was not possible to match JCV data between T0 and T1 due to not available index values in one of the timepoints. After a mean time of NTZ suspension of 35.8±23.6 months (T2), 80 (69.6%) of 115 patients underwent to a new JCV index determination (remaining 35 patients were already followed in other centres). Out of 80, 63 (78.8%) patients were discontinuers due to high JCV index, while 17 patients (21.3%) interrupted treatment for other reasons (disease progression, adverse events). From T1 to T2, 72 (90% of 80) patients did not change their respective JC status, while 5 (6.3% of 80) secoconverted [2 patients (2.5% of 80) to a positive status and 3 patients (3.8% of 80) to a negative status, respectively]. For the remaining 3 patients matching data were not available. Table 4 also shows the variations of JCV status during the follow-up in all NTZ patients divided into subgroups (negative JCV index, low positive JCV index and high positive JCV index). Table 5 shows JCV index changes among different timepoints. In the whole cohort of NTZ treated patients, there was a statistically significant difference in JCV index values between T0 and T2 (0.60±0.98 vs 0.88±1.39, p=0.007). Dividing into subgroups, there was not a statistically significant increment of JCV index value in the group of continuers (0.14±0.39 vs 0.19±0.52, p=0.1), while the increase of JCV index score was statistically significant in the group of patients who stopped treatment (1.29±1.20 vs 2.35±1.51, p=0.008). Moreover, evaluating the JCV index changes at each timepoint, in the subgroup of discontinuers the JCV index increase was greater during NTZ treatment period (T0 vs T1, p< =0.0009) and kept on increasing also during the period of discontinuation (T1 vs T2, p=0.04). As showed in Fig. 6, the analysis of variance (ANOVA) confirmed that the JCV index increase was statistically significant between T0 and T1 and T0 and T2 (p=0.004 and p < 0.001, respectively) while the variation of JCV index between T1 and T2 was not statistically significant (p=0.09).

Considering the rate of seroconversion of JCV index during the follow-up period, the percentage of patients who seroconverted to a positive status during NTZ treatment was greater than those seroconverted after NTZ

discontinuation and it was statistically significant (p=0.008) (Table 6). There was no difference in the percentage of patients who seroconverted to a negative status during the follow-up. There were no correlations between seroconversion to a positive status and years of disease, duration of NTZ treatment, previous immunosuppressant drugs or number of previous DMTs.

Considering the type of exit strategy used after NTZ discontinuation, patients who did not shift to an another drug remained with stable high level of JCV index, in line with our results previously reported, while patients already JCV positive who switched to another DMTs had a progressive increase of JCV index. This increase was statistically significant in the group who shifted to FTY, with a gain of 0.5 point of index in 11 patients ($p < 0.05$) and of 1 point of index in 5 patients ($p < 0.05$) compared with other DMTs (Fig. 7).

In the second study, out of 443 patients screened, 190 met inclusion criteria and were finally enrolled. The mean age was 43.2 ±11.3 years and 122 (64.2%) were females. 16 (8.4%) patients were treated with IFN/GA, 34 (17.9%) with RTX/OCRE, 32 (16.8%) with DMF, 18 (9.5%) with TFN, 14 (7.4%) with ALM, 68 (35.8%) with FTY and 8 (4.2%) with CLD. Demographic and clinical characteristics of the whole cohort are summarized in Table 7. There were not statistically significant differences between groups in term of age, sex, disease duration, previous use of immunosuppressant drugs or NTZ and JCV index at T0. FTY group had a longer DMT duration compared with CLD group ($p< 0.002$). Mean interval time between start of DMTs and determination of JCV index was 25.9±18.4 months.

Comparing JCV value between T0 and T1 (Figure 8), FTY group showed a statistically significant increase in JCV index during the follow-up (2.11±1.28 vs 2.57±1.40, p=0.0001), while patients treated with ALM had a significant reduction of their JCV index (2.93±1.02 vs 2.33±1.32, p=0.005). A reduction, even if not statistically significant, was also found in RTX/OCRE (1.90±1.58 vs 1.82±1.63, p=0.54) and in CLD (2.04±1.44 vs 1.90±1.33, p=0.44) groups. In other treatment groups no differences were found in terms of JCV index. Applying a multivariate analysis corrected for disease duration, the use of previous immunosuppressant drugs or NTZ treatment did not modify our results.

During the follow-up, 16 (8.4% of 190) patients seroconverted to a positive JCV status, while 7 (3.7% of 190) patients seroconverted to a negative status. Stratifying the analysis for each treatment, there were not statistically significant differences in rate of seroconversion.

Figure 5. Flow chart of NTZ treated patients screened and relative JCV index.

JCV index − John Cunningham (JC) virus index negative (titer of anti-JCV antibody below 0.9), *JCV index* + John Cunningham (JC) virus index positive (titer of anti-JCV antibody above 0.9), *T0* before NTZ treatment initiation, *T1* at the time of the NTZ discontinuation, *T2* last follow-up, *n.a.* not available, *Tot.* Total.

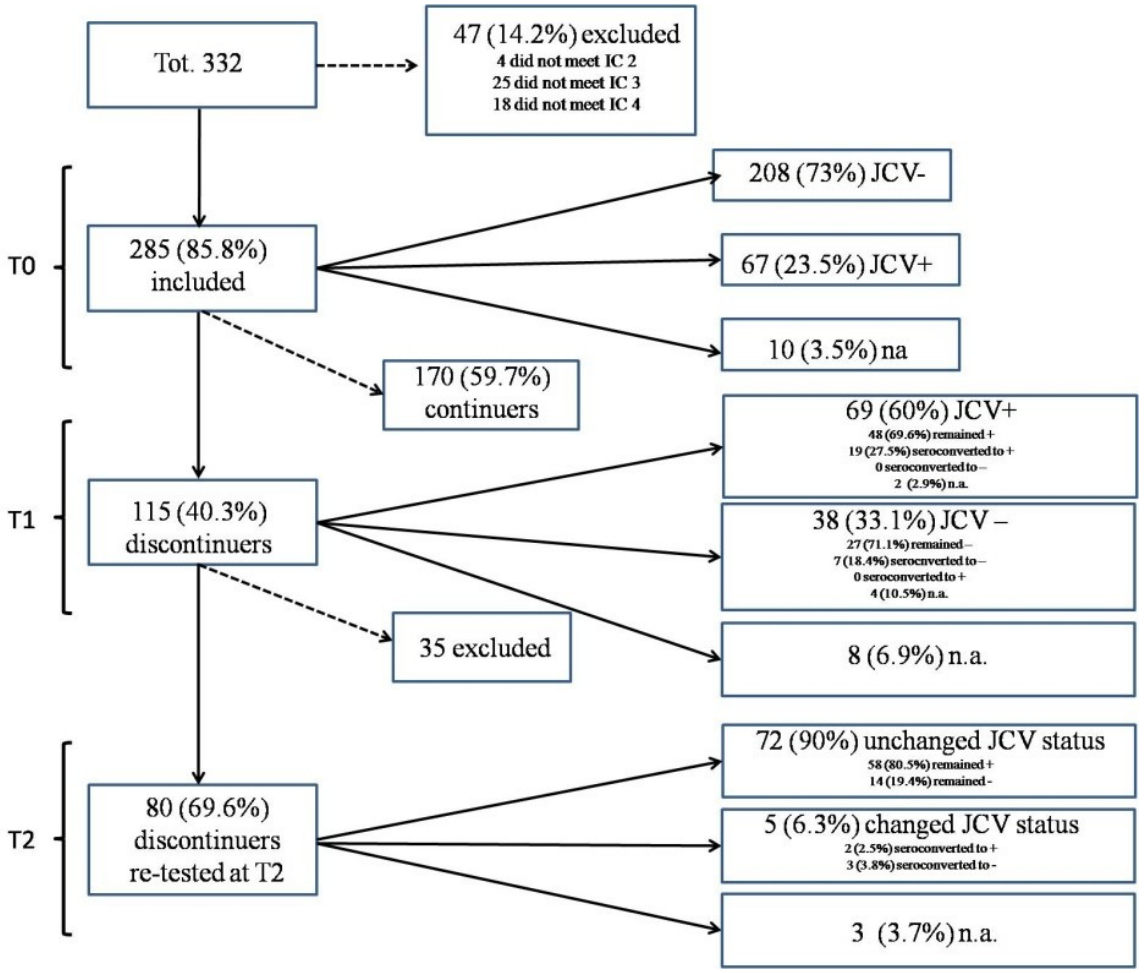


Figure 6. Percentage of NTZ treated patients correlated with changes in JCV index score (JCV index >1.5, between 0.9 and 1.5 or <0.9) among the three timepoints.

JCV index John Cunningham (JC) virus index, *T0* before NTZ treatment initiation, *T1* at the time of the NTZ discontinuation, *T2* last follow-up, *n.a.* not available data.

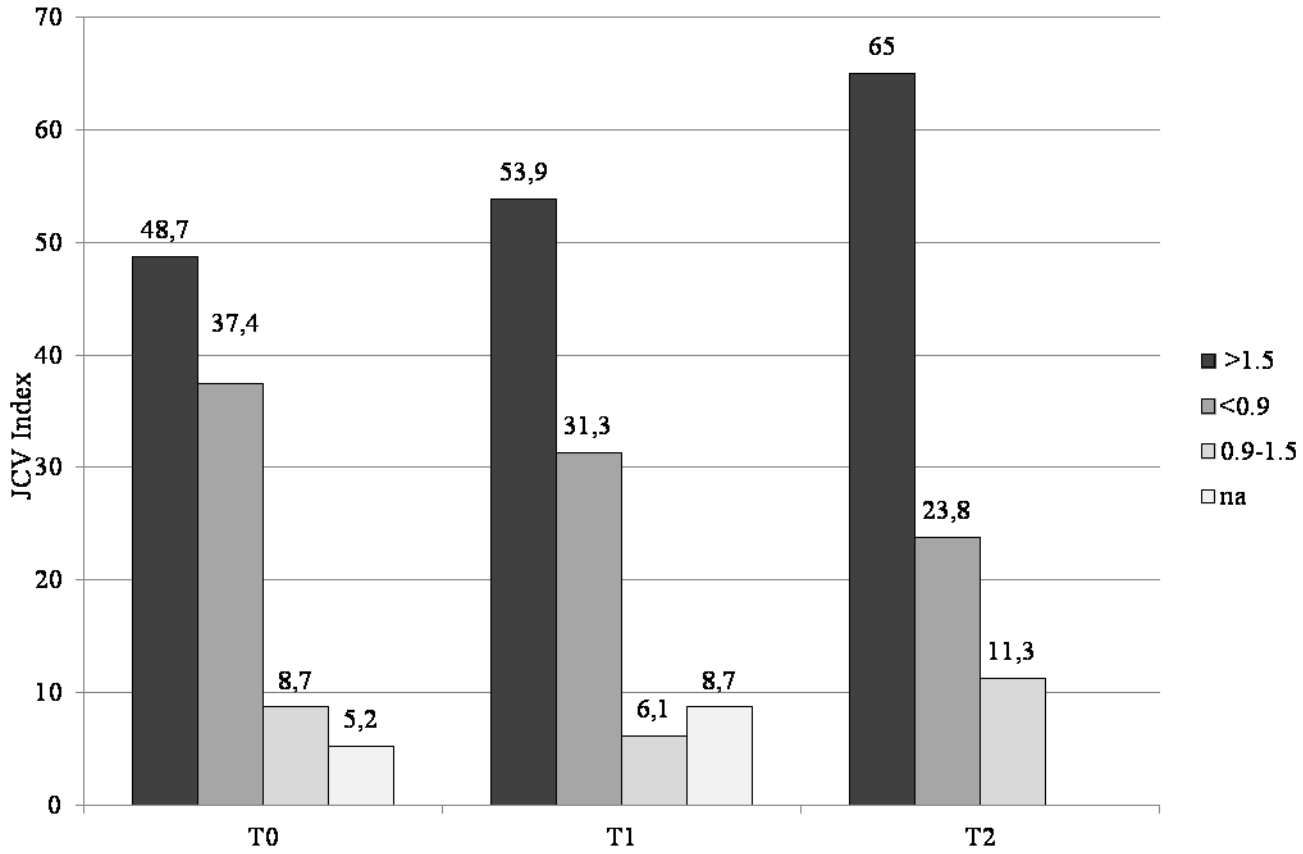


Figure 7. Percentage of patients with Δ variations of JCV index equal or greater than 0.5 and 1.0 in NTZ discontinuers according to the different exit strategies.

JCV index John Cunningham (JC) virus index, *IFN/GA* Interferon/Glatiramer Acetate, *DMF* Dimethylfumarate, *TFN* Terifunomide, *FTY* Fingolimod, *ALM* Alemtuzumab, *AZA* Azathioprine, *DAC* Daclizumab, *OCRE* Ocrelizumab, *RTX* Rituximab, , *DMTs* disease modifying treatments.

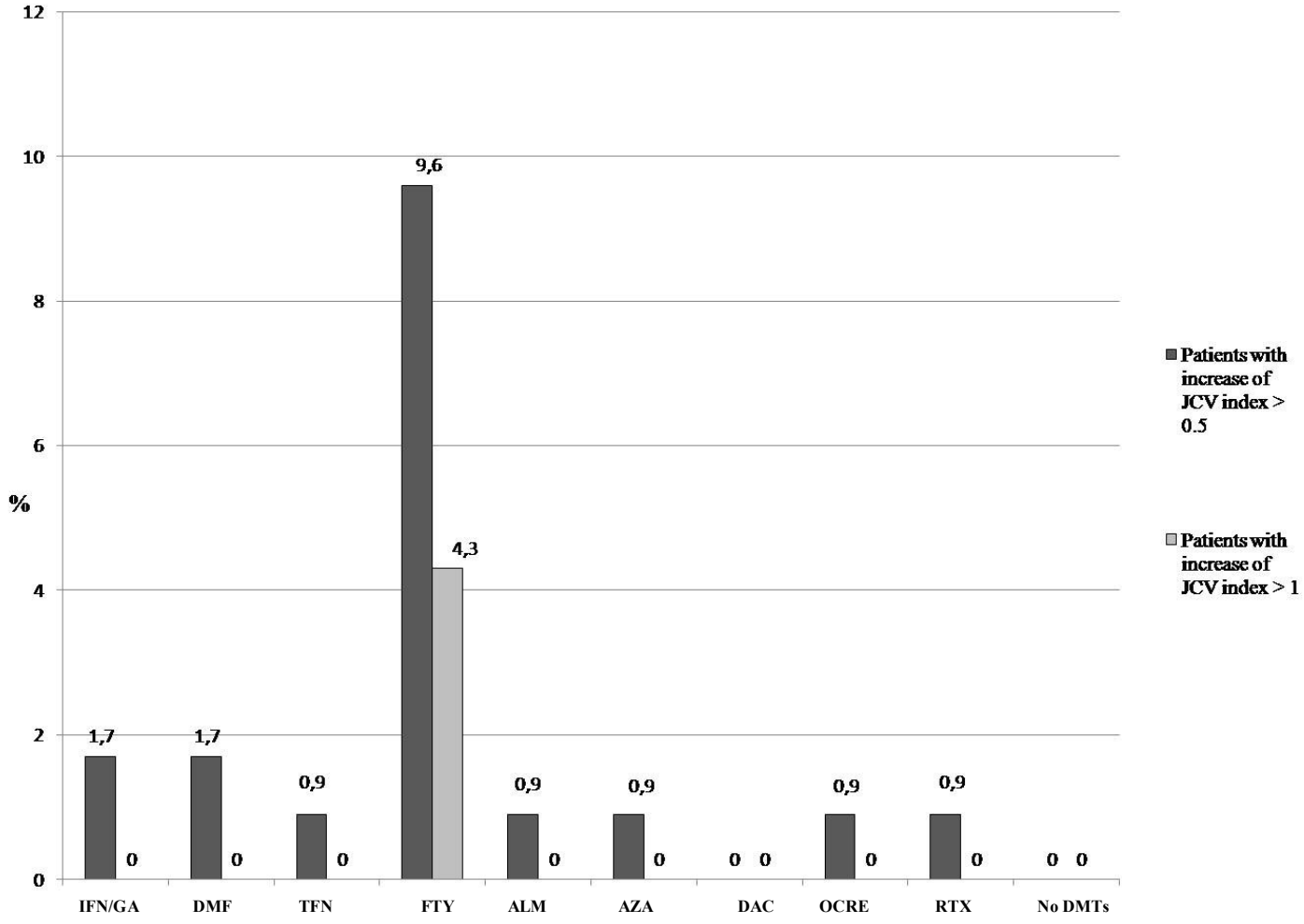


Figure 8. Statistically significant variations in JCV index status during the follow-up in FTY (p<0.0001) and ALM (p<0.005) patient's groups compared with other DMTs.

FTY Fingolimod, *ALM* Alemtuzumab, *RTX/OCRE* Rituximab/Ocrelizumab, *CLD Claddribine, DMF* Dimethylfumarate, *TFN* Terifunomide, *IFN/GA* Interferon/Glatiramer Acetate, *JCV index at T0* John Cunningham (JC) virus index at baseline before starting treatment, , *JCV index at T1* John Cunningham (JC) virus index during treatment.

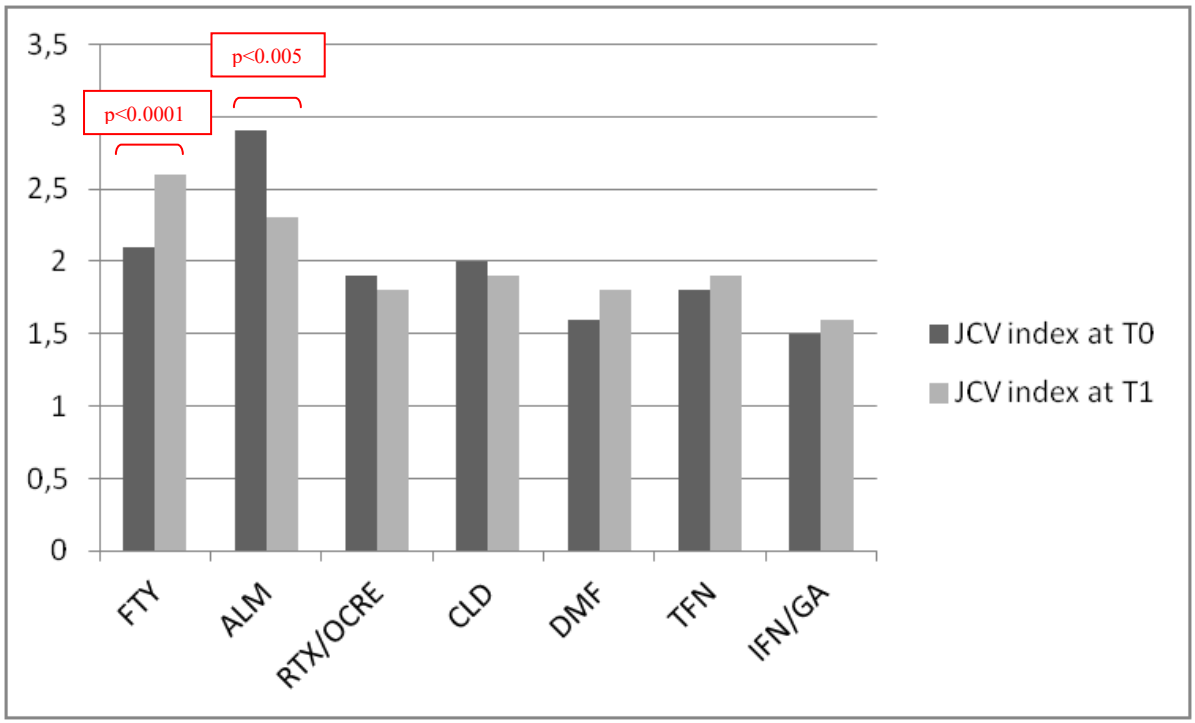


Table 3. Demographical and clinical characteristics of the NTZ population at baseline.

	JCV index - N= 208 (73%)	**Low + JCV index (0.9<JCV<1.5) N= 33 (11.6%)**	**High + JCV index (>1.5) N= 34 (11.9%)**
Age (mean±SD)	40.3±10.8	45.2±8.4	41.4±11.5
Female	n=152 (73.1%)	n=19 (57.6%)	n=23 (67.6%)
Disease duration years (mean±SD)	12.4±8.1	18.4±8.2	13.4±6.8
No. naïve patients (%)	n=60 (28.8%)	n=3 (9.1%)	n=9 (26.5%)
No. previous DMTs	1.2±1.1	1.8±1.1	1.3±1.1
No. patients with previous use of IM)%	41 (19.7)	n=13 (39.4%)	n=11 (32.3%)

JCV index − John Cunningham (JC) virus index negative (titer of anti-JCV antibody below 0.9), *Low+JCV index* John Cunningham (JC) virus index positive (titer of anti-JCV antibody between 0.9 and 1.5), *High+JCV index* John Cunningham (JC) virus index positive (titer of anti-JCV antibody above 1.5), *DMT* disease modifying treatment, *IM* immunosuppressant drugs, *SD* standard deviation.

Table 4. The variations of JCV status during the follow-up in NTZ group of patients.

	TOT	**JCV index -**	**Low + JCV index (0.9<JCV<1.5)**	**High + JCV index (>1.5)**	**N.A.**
No. patients at T0	n=285	n=208 (73%)	n=33 (11.6%)	n=34 (11.9%)	n=10 (3.5%)
JCV index at T0 (mean±SD)	0.60±0.99	0.11±0.24	1.42±1.0	2.81±1.04	
No. patients at T1	n=115	n=38 (33.1%)	n=7 (6.1%)	n=62 (53.9%)	n=8 (6.9%)
JCV index at T1 (mean±SD)	1.92±1.45	0.21±1.46	1.29±1.45	2.96±1.45	
No. patients at T2	n=80	n=19 (23.7%)	n=9 (11.3%)	n=52 (65%)	n=0
JCV index at T2 (mean±SD)	2.35±1.51	0.28±1.52	1.23±1.52	3.31±1.51	

T0 before NTZ treatment initiation, *T1* at the time of the NTZ discontinuation, *T2* last follow-up, *JCV index* − John Cunningham (JC) virus index negative (titer of anti-JCV antibody below 0.9), *Low+JCV index* John Cunningham (JC) virus index positive (titer of anti-JCV antibody between 0.9 and 1.5), *High+JCV index* John Cunningham (JC) virus index positive (titer of anti-JCV antibody above 1.5), *N.A* not available, *SD* standard deviation.

Table 5. JCV index changes in the whole cohort of NTZ treated patients among different timepoints.

	JCV index at T0 (mean±SD)	**JCV index at T1 (mean±SD)**	**JCV index at T2 (mean±SD)**	**p-value**
Whole cohort 285	0.60±0.98	n.a.	0.88±1.39	0.007
Continuers 170 (59.6%)	0.14±0.39	n.a.	0.19±0.52	0.1
Discontinuers 115 (40.4%)	1.29±1.20	1.90±1.45	2.35±1.51	T0 vs T1 : 0.0009 T1 vs T2: 0.04 T0 vs T2: 0.008
	Patients at T0	Patients at T1	Patients at T2	
JCV > 1.5 *0.9<JCV<1.5* *JCV <0.9* *n.a. patients*	n= 56 n= 10 n= 43 n= 6	n= 62 n= 7 n= 36 n= 10	n= 52 n= 9 n= 19 n= 35	

JCV index − John Cunningham (JC) virus index, *T0* before NTZ treatment initiation, *T1* at the time of the NTZ discontinuation, *T2* last follow-up, *SD* standard deviation, *n.a.* not available.

Table 6. Rates of seroconversion in NTZ treated patients at different timepoints.

	From T0 to T1	**From T1 to T2**	**p-value**
Patients seroconverted to a positive status	16.5% (19 of 115)	2.5% (2 of 80)	0.008
Patients seroconverted to a negative status	6.1 % (7 of 115)	3.8% (3 of 80)	0.1

T0 before NTZ treatment initiation, *T1* at the time of the NTZ discontinuation, *T2* last follow-up.

Table 7. Demographic and clinical characteristics of the whole cohort of patients treated with DMTs different from NTZ.

Drug (n= patients)	**IFN/GA n=16**	**RTX/OCRE n=34**	**DMF n=32**	**TFN n=18**	**ALM n=14**	**FTY n=68**	**CLD n=8**	**p value**
Age (mean±sd)	38.6±11.1	44.9±13.1	45.6±11.6	45.6±10.3	37.6±10.9	43.8±9.7	34.1±9.8	ns
Sex (%)	F=13 (81.3%) M=3 (18.7%)	F=19 (55.9%) M=15 (44.1%)	F=21 (65.6%) M=11 (34.4%)	F=12 (66.7%) M=6 (33.3%)	F=7 (50%) M=7 (50%)	F=43 (63.2%) M=25 (36.8%)	F=7 (87.5%) M=1 (12.5%)	ns
Disease duration* (mean±sd)	8.1±6.9	9.7±7.4	11.6±8.6	13.1±7.8	15.1±5.9	17.0±7.8	6.0±3.9	ns
patients with previous use of IM (%)	0	5 (14.7%)	5 (15.6%)	5 (27.8%)	5 (35.7%)	21 (30.9%)	0	ns
patients with previous use of NTZ (%)	0	12 (35.3%)	7 (21.9%)	2 (11.1%)	13 (92.3%)	41 (60.3%)	1 (12.5%)	ns
DMT duration (mean±sd)**	24.8±10.1	22.5±13.6	36.9±19.1	23.8±10.4	35.1±14.3	46.9±22.9	18.0±7.0	FTY vs CLD 0.002
JCV index at T0 (mean±sd)	1.51±1.33	1.90±1.58	1.58±1.36	1.83±1.60	2.93±1.02	2.11±1.28	2.04±1.44	ns

*years
**months

IFN/GA Interferon/Glatiramer Acetate, RTX/*OCRE* Rituximab/Ocrelizumab, *DMF* Dimethylfumarate, *TFN* Terifunomide, *ALM* Alemtuzumab, *FTY* Fingolimod, *CLD* Cladribine, *JCV index at T0* John Cunningham (JC) virus index determination at baseline, *DMT* disease modifying treatment, *sd* standard deviation, *ns* not significant.

DISCUSSION

We retrospectively investigated anti-JCV antibody status changes in patients with MS during NTZ treatment and the JCV index longitudinal modification after NTZ discontinuation. Moreover, our project also evaluated JCV index variations in patients treated with other DMTs.

According to our results, during NTZ treatment patients showed a statistically significant increase in their JCV index (1.20 vs 1.90, p=0.0009) and NTZ treatment was associated with a significant percentage of patients (16.5%) seroconverted to a positive status. Similar results have been reported from other studies in literature in which the annualized seroconversion rate has been described as 6–7% in the first year of NTZ therapy, progressing to 10–25% after 4 years of continuous treatment with this drug [302–306].

The median worldwide prevalence of JCV [307] and the seropositivity rates [308] among adults with MS has been found to be of 58% and between 50% and 90%, respectively. Seropositivity for JCV may be subject to a variety of influences and the values reported by different authors may therefore be somewhat skewed. For example, some large studies did not find any association between JCV positivity and the previous use of NTZ or other immunosuppressive drugs [302]. Conversely, other authors reported that seroconversion rates increased by more than 8% per year of use of NTZ [309]. A recent meta-analysis of JCV seroconversion during treatment with NTZ established that the rate of change of serological status was 10.8% per year [310]. In our study cohort, patients with a negative JCV serology at baseline (170 of 209, 81.3%) remained negative during the follow-up, confirming data in other studies showing that JCV negative patients or patients with antibody levels below or equal to 0.9 both have a low risk of seroconversion [311]. During NTZ treatment, in our cohort of patients 7 subjects seroconverted to a negative status. Analyzing the data in detail, for 3 patients the presence of JCV antibody was tested before 2011 when Stratify II Test was not available and only qualitative test (negative/positive) was performed, so it could be speculated that these patients had a JCV value slightly higher than 0.9 before starting NTZ (and for this reason they had been screened as positive) and their seroconversion to a negative status could be considered as a false positive value. The remaining 4 patients had a JCV positive value only in the first determination of index and subsequent values were negative, however it not possible to define if a laboratory error may be considered or not. In those patients who were already JCV index positive before starting NTZ, JCV index values progressively increased during treatment (doubling in about 40% of patients after a mean time of NTZ treatment of 32±14

months and an average of 26 NTZ administrations), so much to be the main reason of discontinuation. It could seem that this group of patients would be more willing to stop therapy than patients who seroconverted to positive status during treatment. Possible explanation is that they had a longer time of NTZ exposure and a greater number of NTZ infusions, so clinicians recommend the shift to a safer DMT based on recently approved guidelines, on their professional experience and on patients' clinical features and concerns [200]. The JCV index score increase or the seroconversion rate to a positive status remained consistently high also after NTZ discontinuation in about 80% of patients, therefore reducing the possibility of restarting treatment.. Our results suggest that NTZ could act as an indelible signature on the therapeutic history of patients, influencing not only the disease course of MS due to improvement of the clinical condition, but also the longitudinal JCV antibody serology.

In patients with MS treated with NTZ for more than 24 months who have anti-JCV antibody positivity, at least six-monthly follow-up tests are recommended by consensus in order to detect seroconverters and to discuss treatment continuation in patients with increased risk of PML [253,294,312]. Usually clinicians prefer to stop therapy and to shift to a safer DMT [200]. Since NTZ is considered one of the most effective DMTs in MS, during the post-NTZ follow-up period it is believed that testing JCV index at frequent intervals could be a good strategy to identify possible JCV index reduction, to better stratify patient's risk and to adopt different therapeutic strategies (alternative therapies or more frequent MRI scanner to detect early PML development). Results obtained in this study seem to demonstrate that PML risk imprinting related to NTZ cannot be mitigated with treatment discontinuation. However, further studies are needed to uphold our data.

Furthermore, analyzing in detail the group of JCV index patients after NTZ suspension and correlating the variations of their JCV values with the type of DMTs chosen as exit strategy post NTZ treatment, our results demonstrated that there was a statistically significant progressive increase of JCV index in the group of patients who shifted to FTY. This result is in line with another study published in 2018 by Aoyama e coll. [313] in which an association between increased JCV index and therapy with FTY is reported, especially related to duration of treatment. In the last years, FTY has become a common exit strategy choice in patients previously treated with NTZ, particularly those who have been on treatment for more than 24 months and JCV antibody positivity, due to the easy handling of the drug and the few side effects related.

FTY acts as a functional antagonist of sphingosine 1-phosphate (S1P), regulating lymphocyte egress from secondary lymphoid organs to the circulation. As result, FTY reduces the amount of circulating lymphocytes and thereby the transmigration of pathogenic immune cells into the CNS [203]. FTY has also a role on modification of B cell subsets, reducing circulating memory B cells, while increasing the proportions of transitional B cells and B regulatory cells (B-regs) [314]. Despite severe lymphopenia, this is the reason because FTY treated patients have only a mild elevated risk of infectious complications and maintain their immunocompetence. Based on this immune profile, in our study the increase of JCV index in patients who stopped NTZ and shifted to FTY could be explained by the presence of new circulating transitional B cells and B-regs induced by FTY. These series of cells, created after NTZ discontinuation, has not yet come into contact with JCV, so it would react to virus as a new infectious agent, thereby expanding B cell population and promoting the role of antibody-producing B cells. It could be the reason because the titer of JCV antibody is increased with the use of FTY. However, from our analysis NTZ treatment does not seem to have the main role in determining JCV changes in a long time period.

Results of the second part of the study seemed to confirm these data, because FTY treated patients showed a significant increase in the JCV index (2.11±1.28 vs 2.57±1.40, p=0.0001), independently from previous treatment with NTZ, so suggesting that other mechanisms involving B and T cells regulation during FTY treatment may play a role and should be investigated in further studies.

Concerning JCV index changes in patients treated with the most frequently used DMTs in MS, we demonstrated that drugs with a B and T depleting mechanism of action, as ALM, induced a statistically significant reduction of anti-JCV antibodies titer (2.93±1.02 vs 2.33±1.32, p=0.005). Even if not significant, there was a similar trend also in patients treated with CLD and specific B cell profile drugs, as RTX and OCRE.

It is well known that B cell can directly contribute to the development and progression of MS, both being the source of antibody-producing plasma cells and acting as potent Antigen-Presenting Cells (APC). In fact, in peripheral blood as well as in CNS, B cells show signs of chronic inflammation along with a shift towards antigen-experienced memory B cells [315], indicative of an antigen-mediated activation of B cells. In MS patients, B cells express higher level of co-stimulatory molecules with the potential to promote pro-inflammatory differentiation of responding T cells [316].

For these reasons, in the last years, several drugs targeting B cells are developed [173,317]. AML is directed against CD52, a molecule highly expressed on B and T surface, resulting in a rapid and profound depletion of T and B cells. RTX and OCRE are monoclonal antibodies directed against CD20, a molecule expressed on B cells from the late pro-B cell through the memory cell stages. They induce cell apoptosis through via complement-dependent cytotoxicity (CDC) or via antibody-dependent cellular cytotoxicity (ADCC), respectively. Finally, the most striking action of CLD is selective, long-lasting, depletion of B lymphocytes with a particular predilection for memory B cells.

According to our results, reduction of JCV index in those patients treated with ALM, RTX/OCRE and CLD could be explained by the rapid depletion in B circulating cells, consequently responsible for the decrease in CD4+ T cells. It has been demonstrated that CD4+ T cells have a major role in the developing of PML because they are required for maintenance of CD8+ T cells, the most important player in the immune control of JCV infection [268]. Therefore, our results could suggest that in those patients treated with drugs with a B profile, the rapid depletion in B circulating cells and the associated reduction of JCV index could lead to a lower risk of PML. In line with this hypothesis, in literature a single case of a patient with clinical symptoms and features suggestive of PML has been reported during ALM therapy [276] after switching from NTZ, while no cases of PML attributable to OCRE or CLD have been described in MS patients. The maintenance of pathogen clearance in ALM treated patients could seem to be related to a less profound depletion of lymphocytes in lymphoid organs rather than in periphery. The functional preservation in the residual B and T cells might explain the relatively low incidence of infections and the apparent normal response to vaccinations in ALM treated patients [318]. Therefore, it could be speculated that the use of treatment acting selectively on B cells could be considered a valid therapeutic strategy in those patients with a high anti-JCV antibodies titer before starting treatment and/or with previous exposure to therapies with a high risk of PML, in order to minimize the potential risk.

If our results were confirmed by other studies, future therapeutic scenarios might foresee the possibility to discontinue NTZ in those patients with high anti-JCV antibodies titer and to switch to another B cell profile drug. Once obtained a reduction of JCV index below 1.5 or a seroconversion to a negative status, NTZ treatment could be restarted.

In patients treated with ALM, lymphocytes repopulated over time, with B cell recovery usually complete within 6 months, while T lymphocytes recovered more slowly and generally did not return to baseline by 12-18 months post-treatment [229]. In our study, the mean interval time between the first course of therapy (T0) and the last JCV determination (T1) in the group of ALM treated patients was 20.3±11.3 months (range 12-26). The reduction of JCV after more the one year of treatment in our group of patients could be explained by the fact that the rapid B lymphocyte recovery consists mainly of restored immature B cells, preferentially confined in the lymphoid organs and potentially reacting to a new JCV presentation with a low immunogenic profile. However, further studies on JCV index changes after a longer time period from the beginning of ALM therapy are needed.

According to its mechanism of action, CLD's selective depletion on memory B cells might explain because CLD would reduce JCV index and consequently the risk of PML without predisposing to immunosuppression-related complications. However, the small number of CLD patients enrolled in the study may have prevented to demonstrate such reduction. Hence, further studies should take into account a larger sample size.

About the mechanism of action of RTX and OCRE treatments, in several studies it was demonstrated that CD20 + B cells were markedly depleted, while the CD4 and CD8 T cells populations were partially depleted by about 10–25% in the peripheral blood [319]. Thus, the functional preservation of residual T cells could explain because in our study in RTX/OCRE group we did not find statistically significant reduction of JCV index status but only a trend.

The main limitation for this study was the retrospective design. Firstly, in our study the cut-off of 0.9 instead of 0.4 was used as the threshold for positive JCV index status. However, this choice has been justified by the fact that patients in treatment with NTZ were stratified for PML risk based on the previous study [288] in which it was demonstrated that JCV index value equal or higher of 0.9 was associated with a higher risk of PML. Recently, it was suggested that the risk-stratification algorithm used by clinicians in their clinical practice could be inadequate, because according to recent estimates PML seems occur more frequently than expected [320]. For this reason, there is a growing interest regarding new measures to potentially improve the PML risk stratification, in particular the L-selectin blood-test [321] and lipid-specific Immunoglobulin M (IgM) bands in cerebrospinal fluid (CSF) [322]. Furthermore, a better management of NTZ treatment

through the extension of the time interval between infusions [323] or customizing the dosage based on the patient's weight [324] could further reduce the PML incidence. Further prospective studies should apply a cut-off of 0.4 in order to improve the knowledge about JCV index variations during MS treatments.

As a further consequence of the retrospective design of the study, for some patients data about JCV index were available only as a qualitative result (negative/positive) because they collected before 2011 when Stratify II Test was not used. For this reason, the seroconversion to a negative status observed during NTZ treatment for a very small number of patients could be considered as a false positive data.

Moreover, a more frequent rate of NTZ discontinuation in JCV positive than in JCV negative patients was found. This is in line with the current literature and confirmed by common clinical practice that suggest to consider NTZ discontinuation in JCV positive patients after 24 NTZ doses, as the growing risk of PML is related to high number of NTZ administrations. However, we cannot exclude that the high frequency of JCV positive found in NTZ discontinuers group could have biased our results, influencing treatment duration and size of discontinuers subgroup.

Finally, secondary to the retrospective profile of our study, the blood samples were collected at different times during the therapy courses. For those treatments involving a single administration every 6/12 months as RTX/OCRE and CLD, or 5 days of infusion the first year and 3 days of infusion 12 months apart as ALM, the different rates of B and T cells repopulation should be carefully considered. However, future studies with longitudinal profile may address these issues.

CONCLUSION

Our results demonstrated that treatment with DMTs may influence JCV status in MS patients.

In particular, an high percentage of patients shift to or remain in a positive JCV status during NTZ treatment, while no patients seroconvert to a negative status after suspension. NTZ discontinuers patients who stopped treatment because of high level of JCV index tend to remain in the same JCV status during the follow-up period, independently from the time interval between discontinuation and start of new DMTs. NTZ discontinuation seems not to be able to interfere on JCV status modification on long time period. It makes non-sense to repeat a JCV determination every six months to expect a seroconversion to a negative value in order to restart treatment.

The finding of an increase of JCV index in those patients subsequently treated with FTY may rise the question if the use of this kind of treatment as exit strategy after NTZ discontinuation would represent the safest choice. Moreover, the reduction of anti-JCV antibodies titer in those patients treated with drugs with a B depleting profile, as ALM or RTX/OCRE or CLD, could suggest a different approach in the treatment decision making process. Notably, since an increase of JCV index would expose patients to a higher risk of PML, our data may suggest that the use of treatments acting selectively on B cells could represent a valid therapeutic approach in those patients who stopped NTZ or in those with an high JCV index before starting a new therapy. It is known that NTZ is one of the most efficacious drug for treating MS, and the decision of suspending NTZ is mainly driven by the PML risk stratification; thus, according to our results, several treatments sequencing scenarios in which switching to different highly-effective DMTs could maximize disease control and minimize PML risk based on the mechanism of action.

REFERENCES

1. Oh J, Vidal-Jordana A, Montalban X. Multiple sclerosis: clinical aspects. Curr Opin Neurol. 2018;31:752–9.

2. Bar-Or A, Pender MP, Khanna R, Steinman L, Hartung H-P, Maniar T, et al. Epstein-Barr Virus in Multiple Sclerosis: Theory and Emerging Immunotherapies. Trends Mol Med. 2020;26:296–310.

3. Lassmann H. Pathogenic Mechanisms Associated With Different Clinical Courses of Multiple Sclerosis. Front Immunol. 2018;9:3116.

4. Haider L, Simeonidou C, Steinberger G, Hametner S, Grigoriadis N, Deretzi G, et al. Multiple sclerosis deep grey matter: the relation between demyelination, neurodegeneration, inflammation and iron. J Neurol Neurosurg Psychiatry. 2014;85:1386–95.

5. Calabrese M, Magliozzi R, Ciccarelli O, Geurts JJG, Reynolds R, Martin R. Exploring the origins of grey matter damage in multiple sclerosis. Nat Rev Neurosci. 2015;16:147–58.

6. Katsara M, Apostolopoulos V. Editorial: Multiple Sclerosis: Pathogenesis and Therapeutics. Med Chem Shariqah United Arab Emir. 2018;14:104–5.

7. Dargahi N, Katsara M, Tselios T, Androutsou M-E, de Courten M, Matsoukas J, et al. Multiple Sclerosis: Immunopathology and Treatment Update. Brain Sci [Internet]. 2017 [cited 2019 Jan 16];7. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5532591/

8. Rosati G. The prevalence of multiple sclerosis in the world: an update. Neurol Sci Off J Ital Neurol Soc Ital Soc Clin Neurophysiol. 2001;22:117–39.

9. Lampis R, Morelli L, Congia M, Macis MD, Mulargia A, Loddo M, et al. The inter-regional distribution of HLA class II haplotypes indicates the suitability of the Sardinian population for case-control association studies in complex diseases. Hum Mol Genet. 2000;9:2959–65.

10. Mg M, M L, E C, V L, G S, Mp P, et al. Genetic factors and the founder effect explain familial MS in Sardinia [Internet]. Neurology. Neurology; 2002 [cited 2020 Sep 24]. Available from: https://pubmed.ncbi.nlm.nih.gov/11805258/

11. Antel J, Antel S, Caramanos Z, Arnold DL, Kuhlmann T. Primary progressive multiple sclerosis: part of the MS disease spectrum or separate disease entity? Acta Neuropathol (Berl). 2012;123:627–38.

12. Golden LC, Voskuhl R. The importance of studying sex differences in disease: The example of multiple sclerosis. J Neurosci Res. 2017;95:633–43.

13. Koch-Henriksen N, Sørensen PS. The changing demographic pattern of multiple sclerosis epidemiology. Lancet Neurol. 2010;9:520–32.

14. Weinshenker BG, Rice GP, Noseworthy JH, Carriere W, Baskerville J, Ebers GC. The natural history of multiple sclerosis: a geographically based study. 3. Multivariate analysis of predictive factors and models of outcome. Brain J Neurol. 1991;114 (Pt 2):1045–56.

15. Savettieri G, Messina D, Andreoli V, Bonavita S, Caltagirone C, Cittadella R, et al. Gender-related effect of clinical and genetic variables on the cognitive impairment in multiple sclerosis. J Neurol. 2004;251:1208–14.

16. Vukusic S, Confavreux C. Prognostic factors for progression of disability in the secondary progressive phase of multiple sclerosis. J Neurol Sci. 2003;206:135–7.

17. Ascherio A, Munger KL. Epidemiology of Multiple Sclerosis: From Risk Factors to Prevention-An Update. Semin Neurol. 2016;36:103–14.

18. Baranzini SE, Oksenberg JR. The Genetics of Multiple Sclerosis: From 0 to 200 in 50 Years. Trends Genet TIG. 2017;33:960–70.

19. De Silvestri A, Capittini C, Mallucci G, Bergamaschi R, Rebuffi C, Pasi A, et al. The Involvement of HLA Class II Alleles in Multiple Sclerosis: A Systematic Review with Meta-analysis. Dis Markers. 2019;2019:1409069.

20. Hollenbach JA, Oksenberg JR. The immunogenetics of multiple sclerosis: A comprehensive review. J Autoimmun. 2015;64:13–25.

21. International Multiple Sclerosis Genetics Consortium, Wellcome Trust Case Control Consortium 2, Sawcer S, Hellenthal G, Pirinen M, Spencer CCA, et al. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. Nature. 2011;476:214–9.

22. Ramagopalan SV, Ebers GC. Genes for multiple sclerosis. Lancet Lond Engl. 2008;371:283–5.

23. Axisa P-P, Hafler DA. Multiple sclerosis: genetics, biomarkers, treatments. Curr Opin Neurol. 2016;29:345–53.

24. International HapMap Consortium, Frazer KA, Ballinger DG, Cox DR, Hinds DA, Stuve LL, et al. A second generation human haplotype map of over 3.1 million SNPs. Nature. 2007;449:851–61.

25. Welter D, MacArthur J, Morales J, Burdett T, Hall P, Junkins H, et al. The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. Nucleic Acids Res. 2014;42:D1001-1006.

26. Sadovnick AD, Yee IM, Guimond C, Reis J, Dyment DA, Ebers GC. Age of onset in concordant twins and other relative pairs with multiple sclerosis. Am J Epidemiol. 2009;170:289–96.

27. Ascherio A, Munger KL. Environmental risk factors for multiple sclerosis. Part II: Noninfectious factors. Ann Neurol. 2007;61:504–13.

28. Ascherio A, Munger KL, Simon KC. Vitamin D and multiple sclerosis. Lancet Neurol. 2010;9:599–612.

29. Kc S, Kl M, A A. Vitamin D and multiple sclerosis: epidemiology, immunology, and genetics [Internet]. Curr. Opin. Neurol. Curr Opin Neurol; 2012 [cited 2020 Sep 24]. Available from: https://pubmed.ncbi.nlm.nih.gov/22547098/

30. Hedström AK, Olsson T, Kockum I, Hillert J, Alfredsson L. Low sun exposure increases multiple sclerosis risk both directly and indirectly. J Neurol. 2020;267:1045–52.

31. Kampman MT, Wilsgaard T, Mellgren SI. Outdoor activities and diet in childhood and adolescence relate to MS risk above the Arctic Circle. J Neurol. 2007;254:471–7.

32. Riccio P, Rossano R, Liuzzi GM. May diet and dietary supplements improve the wellness of multiple sclerosis patients? A molecular approach. Autoimmune Dis. 2011;2010:249842.

33. Riccio P, Rossano R. Diet, Gut Microbiota, and Vitamins D + A in Multiple Sclerosis. Neurother J Am Soc Exp Neurother. 2018;15:75–91.

34. Buscarinu MC, Cerasoli B, Annibali V, Policano C, Lionetto L, Capi M, et al. Altered intestinal permeability in patients with relapsing-remitting multiple sclerosis: A pilot study. Mult Scler Houndmills Basingstoke Engl. 2017;23:442–6.

35. Liu Z, Zhang T-T, Yu J, Liu Y-L, Qi S-F, Zhao J-J, et al. Excess Body Weight during Childhood and Adolescence Is Associated with the Risk of Multiple Sclerosis: A Meta-Analysis. Neuroepidemiology. 2016;47:103–8.

36. Guerrero-García J de J, Carrera-Quintanar L, López-Roa RI, Márquez-Aguirre AL, Rojas-Mayorquín AE, Ortuño-Sahagún D. Multiple Sclerosis and Obesity: Possible Roles of Adipokines. Mediators Inflamm. 2016;2016:4036232.

37. Cummings IG, Lucchese G, Garg S, Soni M, Majid AF, Marczin N, et al. Ten-year improved survival in patients with multi-vessel coronary disease and poor left ventricular function following surgery: A retrospective cohort study. Int J Surg Lond Engl. 2020;76:146–52.

38. van der Mei I a. F, Simpson S, Stankovich J, Taylor BV. Individual and joint action of environmental factors and risk of MS. Neurol Clin. 2011;29:233–55.

39. Jf K. Epidemiologic evidence for multiple sclerosis as an infection [Internet]. Clin. Microbiol. Rev. Clin Microbiol Rev; 1993 [cited 2020 Sep 24]. Available from: https://pubmed.ncbi.nlm.nih.gov/8269393/

40. Ascherio A, Munger KL. Environmental risk factors for multiple sclerosis. Part I: the role of infection. Ann Neurol. 2007;61:288–99.

41. Knox KK, Brewer JH, Henry JM, Harrington DJ, Carrigan DR. Human herpesvirus 6 and multiple sclerosis: systemic active infections in patients with early disease. Clin Infect Dis Off Publ Infect Dis Soc Am. 2000;31:894–903.

42. Bagos PG, Nikolopoulos G, Ioannidis A. Chlamydia pneumoniae infection and the risk of multiple sclerosis: a meta-analysis. Mult Scler Houndmills Basingstoke Engl. 2006;12:397–411.

43. Oger J. HTLV-1 infection and the viral etiology of multiple sclerosis. J Neurol Sci. 2007;262:100–4.

44. Almohmeed YH, Avenell A, Aucott L, Vickers MA. Systematic review and meta-analysis of the sero-epidemiological association between Epstein Barr virus and multiple sclerosis. PloS One. 2013;8:e61110.

45. Bagert BA. Epstein-Barr virus in multiple sclerosis. Curr Neurol Neurosci Rep. 2009;9:405–10.

46. Yazdanbakhsh M, Kremsner PG, van Ree R. Allergy, parasites, and the hygiene hypothesis. Science. 2002;296:490–4.

47. J F, Z F. The hygiene hypothesis and multiple sclerosis [Internet]. Ann. Neurol. Ann Neurol; 2007 [cited 2020 Sep 25]. Available from: https://pubmed.ncbi.nlm.nih.gov/17315205/

48. Serafini B, Rosicarelli B, Franciotta D, Magliozzi R, Reynolds R, Cinque P, et al. Dysregulated Epstein-Barr virus infection in the multiple sclerosis brain. J Exp Med. 2007;204:2899–912.

49. Jh V, T R, Gh R, Md N, E B-C, H N. An Epstein Barr virus-related cross reactive autoimmune response in multiple sclerosis in Norway [Internet]. J. Neuroimmunol. J Neuroimmunol; 1996 [cited 2020 Oct 10]. Available from: https://pubmed.ncbi.nlm.nih.gov/8823380/

50. Pender MP. Infection of autoreactive B lymphocytes with EBV, causing chronic autoimmune diseases. Trends Immunol. 2003;24:584–8.

51. Laurence M, Benito-León J. Epstein-Barr virus and multiple sclerosis: Updating Pender's hypothesis. Mult Scler Relat Disord. 2017;16:8–14.

52. Massa J, Munger KL, O'Reilly EJ, Falk KI, Ascherio A. Plasma titers of antibodies against Epstein-Barr virus BZLF1 and risk of multiple sclerosis. Neuroepidemiology. 2007;28:214–5.

53. Gold R, Linington C, Lassmann H. Understanding pathogenesis and therapy of multiple sclerosis via animal models: 70 years of merits and culprits in experimental autoimmune encephalomyelitis research. Brain J Neurol. 2006;129:1953–71.

54. Rc S, B H. Update on immunopathogenesis and immunotherapy in multiple sclerosis [Internet]. ImmunoTargets Ther. Immunotargets Ther; 2013 [cited 2020 Sep 29]. Available from: https://pubmed.ncbi.nlm.nih.gov/27471685/

55. Gran B, Hemmer B, Vergelli M, McFarland HF, Martin R. Molecular mimicry and multiple sclerosis: degenerate T-cell recognition and the induction of autoimmunity. Ann Neurol. 1999;45:559–67.

56. O'Connor KC, Bar-Or A, Hafler DA. The neuroimmunology of multiple sclerosis: possible roles of T and B lymphocytes in immunopathogenesis. J Clin Immunol. 2001;21:81–92.

57. Popescu BFG, Lucchinetti CF. Pathology of demyelinating diseases. Annu Rev Pathol. 2012;7:185–217.

58. Tzartos JS, Friese MA, Craner MJ, Palace J, Newcombe J, Esiri MM, et al. Interleukin-17 production in central nervous system-infiltrating T cells and glial cells is associated with active disease in multiple sclerosis. Am J Pathol. 2008;172:146–55.

59. Batoulis H, Addicks K, Kuerten S. Emerging concepts in autoimmune encephalomyelitis beyond the CD4/T(H)1 paradigm. Ann Anat Anat Anz Off Organ Anat Ges. 2010;192:179–93.

60. Magliozzi R, Howell O, Vora A, Serafini B, Nicholas R, Puopolo M, et al. Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. Brain J Neurol. 2007;130:1089–104.

61. Lovato L, Willis SN, Rodig SJ, Caron T, Almendinger SE, Howell OW, et al. Related B cell clones populate the meninges and parenchyma of patients with multiple sclerosis. Brain J Neurol. 2011;134:534–41.

62. Owens GP, Ritchie AM, Burgoon MP, Williamson RA, Corboy JR, Gilden DH. Single-cell repertoire analysis demonstrates that clonal expansion is a prominent feature of the B cell response in multiple sclerosis cerebrospinal fluid. J Immunol Baltim Md 1950. 2003;171:2725–33.

63. Obermeier B, Lovato L, Mentele R, Brück W, Forne I, Imhof A, et al. Related B cell clones that populate the CSF and CNS of patients with multiple sclerosis produce CSF immunoglobulin. J Neuroimmunol. 2011;233:245–8.

64. Wanleenuwat P, Iwanowski P. Role of B cells and antibodies in multiple sclerosis. Mult Scler Relat Disord. 2019;36:101416.

65. Lisak RP, Benjamins JA, Nedelkoska L, Barger JL, Ragheb S, Fan B, et al. Secretory products of multiple sclerosis B cells are cytotoxic to oligodendroglia in vitro. J Neuroimmunol. 2012;246:85–95.

66. Jelcic I, Al Nimer F, Wang J, Lentsch V, Planas R, Jelcic I, et al. Memory B Cells Activate Brain-Homing, Autoreactive CD4+ T Cells in Multiple Sclerosis. Cell. 2018;175:85-100.e23.

67. Piccio L, Naismith RT, Trinkaus K, Klein RS, Parks BJ, Lyons JA, et al. Changes in B- and T-lymphocyte and chemokine levels with rituximab treatment in multiple sclerosis. Arch Neurol. 2010;67:707–14.

68. Frischer JM, Bramow S, Dal-Bianco A, Lucchinetti CF, Rauschka H, Schmidbauer M, et al. The relation between inflammation and neurodegeneration in multiple sclerosis brains. Brain J Neurol. 2009;132:1175–89.

69. Lucchinetti C, Brück W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. Ann Neurol. 2000;47:707–17.

70. Popescu BFG, Lucchinetti CF. Meningeal and cortical grey matter pathology in multiple sclerosis. BMC Neurol. 2012;12:11.

71. Jm G. Multiple sclerosis: diagnosis, differential diagnosis, and clinical presentation [Internet]. Handb. Clin. Neurol. Handb Clin Neurol; 2014 [cited 2020 Sep 25]. Available from: https://pubmed.ncbi.nlm.nih.gov/24507522/

72. Lublin FD, Reingold SC, Cohen JA, Cutter GR, Sørensen PS, Thompson AJ, et al. Defining the clinical course of multiple sclerosis: the 2013 revisions. Neurology. 2014;83:278–86.

73. Balcer LJ. Clinical practice. Optic neuritis. N Engl J Med. 2006;354:1273–80.

74. Abel A, McClelland C, Lee MS. Critical review: Typical and atypical optic neuritis. Surv Ophthalmol. 2019;64:770–9.

75. Cellina M, Floridi C, Rosti C, Orsi M, Panzeri M, Pirovano M, et al. MRI of acute optic neuritis (ON) at the first episode: Can we predict the visual outcome and the development of multiple sclerosis (MS)? Radiol Med (Torino). 2019;124:1296–303.

76. Optic Neuritis Study Group. Multiple sclerosis risk after optic neuritis: final optic neuritis treatment trial follow-up. Arch Neurol. 2008;65:727–32.

77. Britze J, Pihl-Jensen G, Frederiksen JL. Retinal ganglion cell analysis in multiple sclerosis and optic neuritis: a systematic review and meta-analysis. J Neurol. 2017;264:1837–53.

78. Britze J, Frederiksen JL. Optical coherence tomography in multiple sclerosis. Eye Lond Engl. 2018;32:884–8.

79. Schneider E, Zimmermann H, Oberwahrenbrock T, Kaufhold F, Kadas EM, Petzold A, et al. Optical Coherence Tomography Reveals Distinct Patterns of Retinal Damage in Neuromyelitis Optica and Multiple Sclerosis. PloS One. 2013;8:e66151.

80. Alonso R, Gonzalez-Moron D, Garcea O. Optical coherence tomography as a biomarker of neurodegeneration in multiple sclerosis: A review. Mult Scler Relat Disord. 2018;22:77–82.

81. Oberwahrenbrock T, Schippling S, Ringelstein M, Kaufhold F, Zimmermann H, Keser N, et al. Retinal damage in multiple sclerosis disease subtypes measured by high-resolution optical coherence tomography. Mult Scler Int. 2012;2012:530305.

82. Costello F, Hodge W, Pan YI, Freedman M, DeMeulemeester C. Differences in retinal nerve fiber layer atrophy between multiple sclerosis subtypes. J Neurol Sci. 2009;281:74–9.

83. Leocani L, Guerrieri S, Comi G. Visual Evoked Potentials as a Biomarker in Multiple Sclerosis and Associated Optic Neuritis. J Neuro-Ophthalmol Off J North Am Neuro-Ophthalmol Soc. 2018;38:350–7.

84. Sisto D, Trojano M, Vetrugno M, Trabucco T, Iliceto G, Sborgia C. Subclinical visual involvement in multiple sclerosis: a study by MRI, VEPs, frequency-doubling perimetry, standard perimetry, and contrast sensitivity. Invest Ophthalmol Vis Sci. 2005;46:1264–8.

85. Cordonnier C, de Seze J, Breteau G, Ferriby D, Michelin E, Stojkovic T, et al. Prospective study of patients presenting with acute partial transverse myelopathy. J Neurol. 2003;250:1447–52.

86. B B, H Z, Jc O, C C, N C, S D, et al. Long-term follow-up of acute partial transverse myelitis [Internet]. Arch. Neurol. Arch Neurol; 2012 [cited 2020 Sep 26]. Available from: https://pubmed.ncbi.nlm.nih.gov/22410443/

87. Presas-Rodríguez S, Grau-López L, Hervás-García JV, Massuet-Vilamajó A, Ramo-Tello C. Myelitis: Differences between multiple sclerosis and other aetiologies. Neurol Barc Spain. 2016;31:71–5.

88. Nij Bijvank JA, van Rijn LJ, Balk LJ, Tan HS, Uitdehaag BMJ, Petzold A. Diagnosing and quantifying a common deficit in multiple sclerosis: Internuclear ophthalmoplegia. Neurology. 2019;92:e2299–308.

89. G K, P K, Ae P, Me E, C K, N K. A neurophysiological study of facial numbness in multiple sclerosis: Integration with clinical data and imaging findings [Internet]. Mult. Scler. Relat. Disord. Mult Scler Relat Disord; 2016 [cited 2020 Sep 26]. Available from: https://pubmed.ncbi.nlm.nih.gov/27645362/

90. Zadro I, Barun B, Habek M, Brinar VV. Isolated cranial nerve palsies in multiple sclerosis. Clin Neurol Neurosurg. 2008;110:886–8.

91. Koutsis G, Evangelopoulos ME, Andreadou E, Mandellos D, Karachalios G, Potagas C, et al. The onset of multiple sclerosis in Greece: a single-center study of 1,034 consecutive patients. Eur Neurol. 2010;63:350–6.

92. L DS, P A. Symptomatic cranial neuralgias in multiple sclerosis: clinical features and treatment [Internet]. Clin. Neurol. Neurosurg. Clin Neurol Neurosurg; 2012 [cited 2020 Sep 26]. Available from: https://pubmed.ncbi.nlm.nih.gov/22130044/

93. Tornes L, Conway B, Sheremata W. Multiple sclerosis and the cerebellum. Neurol Clin. 2014;32:957–77.

94. Newsome SD, von Geldern G, Shou H, Baynes M, Marasigan RER, Calabresi PA, et al. Longitudinal assessment of hand function in individuals with multiple sclerosis. Mult Scler Relat Disord. 2019;32:107–13.

95. Osterberg A, Boivie J. Central pain in multiple sclerosis - sensory abnormalities. Eur J Pain Lond Engl. 2010;14:104–10.

96. O'Connor AB, Schwid SR, Herrmann DN, Markman JD, Dworkin RH. Pain associated with multiple sclerosis: systematic review and proposed classification. Pain. 2008;137:96–111.

97. Cruz-Almeida Y, Felix ER, Martinez-Arizala A, Widerström-Noga EG. Decreased spinothalamic and dorsal column medial lemniscus-mediated function is associated with neuropathic pain after spinal cord injury. J Neurotrauma. 2012;29:2706–15.

98. Cameron MH, Nilsagard Y. Balance, gait, and falls in multiple sclerosis. Handb Clin Neurol. 2018;159:237–50.

99. L P, E S, E R, M C, F T, M B, et al. Multiple sclerosis: white and gray matter damage associated with balance deficit detected at static posturography [Internet]. Radiology. Radiology; 2013 [cited 2020 Sep 26]. Available from: https://pubmed.ncbi.nlm.nih.gov/23533287/

100. Grzegorski T, Losy J. Cognitive impairment in multiple sclerosis - a review of current knowledge and recent research. Rev Neurosci. 2017;28:845–60.

101. Staff NP, Lucchinetti CF, Keegan BM. Multiple sclerosis with predominant, severe cognitive impairment. Arch Neurol. 2009;66:1139–43.

102. Chiaravalloti ND, DeLuca J. Cognitive impairment in multiple sclerosis. Lancet Neurol. 2008;7:1139–51.

103. L T, Ik P, M H, U S, P C. Cognitive decline in multiple sclerosis: impact of topographic lesion distribution on differential cognitive deficit patterns [Internet]. Mult. Scler. Houndmills Basingstoke Engl. Mult Scler; 2009 [cited 2020 Sep 27]. Available from: https://pubmed.ncbi.nlm.nih.gov/19667010/

104. Kalb R, Beier M, Benedict RH, Charvet L, Costello K, Feinstein A, et al. Recommendations for cognitive screening and management in multiple sclerosis care. Mult Scler Houndmills Basingstoke Engl. 2018;24:1665–80.

105. Patten SB, Beck CA, Williams JVA, Barbui C, Metz LM. Major depression in multiple sclerosis: a population-based perspective. Neurology. 2003;61:1524–7.

106. Beiske AG, Svensson E, Sandanger I, Czujko B, Pedersen ED, Aarseth JH, et al. Depression and anxiety amongst multiple sclerosis patients. Eur J Neurol. 2008;15:239–45.

107. A F, S M, Jf R, B A, C M. The link between multiple sclerosis and depression [Internet]. Nat. Rev. Neurol. Nat Rev Neurol; 2014 [cited 2020 Sep 26]. Available from: https://pubmed.ncbi.nlm.nih.gov/25112509/

108. M Z, R Z, D N, M U, A B, Ma T, et al. Depressive symptoms and MRI changes in multiple sclerosis [Internet]. Eur. J. Neurol. Eur J Neurol; 2002 [cited 2020 Sep 26]. Available from: https://pubmed.ncbi.nlm.nih.gov/12220380/

109. Endocrine and immune substrates of depressive symptoms and fatigue in multiple sclerosis patients with comorbid major depression - PubMed [Internet]. [cited 2020 Sep 26]. Available from: https://pubmed.ncbi.nlm.nih.gov/21296901/

110. Oj S, Mc W, M M. Cytokines and major depression [Internet]. Prog. Neuropsychopharmacol. Biol. Psychiatry. Prog Neuropsychopharmacol Biol Psychiatry; 2005 [cited 2020 Sep 26]. Available from: https://pubmed.ncbi.nlm.nih.gov/15694227/

111. Systemic tumor necrosis factor-alpha decreases brain stimulation reward and increases metabolites of serotonin and dopamine in the nucleus accumbens of mice - PubMed [Internet]. [cited 2020 Sep 26]. Available from: https://pubmed.ncbi.nlm.nih.gov/23896053/

112. Induruwa I, Constantinescu CS, Gran B. Fatigue in multiple sclerosis - a brief review. J Neurol Sci. 2012;323:9–15.

113. Kluger BM, Krupp LB, Enoka RM. Fatigue and fatigability in neurologic illnesses: proposal for a unified taxonomy. Neurology. 2013;80:409–16.

114. Manjaly Z-M, Harrison NA, Critchley HD, Do CT, Stefanics G, Wenderoth N, et al. Pathophysiological and cognitive mechanisms of fatigue in multiple sclerosis. J Neurol Neurosurg Psychiatry. 2019;90:642–51.

115. Phé V, Chartier-Kastler E, Panicker JN. Management of neurogenic bladder in patients with multiple sclerosis. Nat Rev Urol. 2016;13:275–88.

116. de Sèze M, Ruffion A, Denys P, Joseph P-A, Perrouin-Verbe B, GENULF. The neurogenic bladder in multiple sclerosis: review of the literature and proposal of management guidelines. Mult Scler Houndmills Basingstoke Engl. 2007;13:915–28.

117. Groen J, Pannek J, Castro Diaz D, Del Popolo G, Gross T, Hamid R, et al. Summary of European Association of Urology (EAU) Guidelines on Neuro-Urology. Eur Urol. 2016;69:324–33.

118. O Y. Latest treatment for lower urinary tract dysfunction: therapeutic agents and mechanism of action [Internet]. Int. J. Urol. Off. J. Jpn. Urol. Assoc. Int J Urol; 2013 [cited 2020 Sep 27]. Available from: https://pubmed.ncbi.nlm.nih.gov/23190275/

119. Madhuvrata P, Singh M, Hasafa Z, Abdel-Fattah M. Anticholinergic drugs for adult neurogenic detrusor overactivity: a systematic review and meta-analysis. Eur Urol. 2012;62:816–30.

120. Nitti VW, Dmochowski R, Herschorn S, Sand P, Thompson C, Nardo C, et al. OnabotulinumtoxinA for the treatment of patients with overactive bladder and urinary incontinence: results of a phase 3, randomized, placebo controlled trial. J Urol. 2013;189:2186–93.

121. Apostolidis A, Dasgupta P, Fowler CJ. Proposed mechanism for the efficacy of injected botulinum toxin in the treatment of human detrusor overactivity. Eur Urol. 2006;49:644–50.

122. Gobbi C, Digesu GA, Khullar V, El Neil S, Caccia G, Zecca C. Percutaneous posterior tibial nerve stimulation as an effective treatment of refractory lower urinary tract symptoms in patients with multiple sclerosis: preliminary data from a multicentre, prospective, open label trial. Mult Scler Houndmills Basingstoke Engl. 2011;17:1514–9.

123. Sm A, O L, J C. Treatment of lower urinary tract symptoms in multiple sclerosis patients: Review of the literature and current guidelines [Internet]. Can. Urol. Assoc. J. J. Assoc. Urol. Can. Can Urol Assoc J; 2017 [cited 2020 Sep 27]. Available from: https://pubmed.ncbi.nlm.nih.gov/28360957/

124. Nakipoglu GF, Kaya AZ, Orhan G, Tezen O, Tunc H, Ozgirgin N, et al. Urinary dysfunction in multiple sclerosis. J Clin Neurosci Off J Neurosurg Soc Australas. 2009;16:1321–4.

125. Gupta A, Kumar SN, Taly AB. Urodynamic profile in acute transverse myelitis patients: Its correlation with neurological outcome. J Neurosci Rural Pract. 2017;8:44–8.

126. Donzé C, Hautecoeur P. [Urinary, sexual, and bowel disorders in early-stage multiple sclerosis]. Rev Neurol (Paris). 2009;165 Suppl 4:S148-155.

127. Lew-Starowicz M, Rola R. Correlates of sexual function in male and female patients with multiple sclerosis. J Sex Med. 2014;11:2172–80.

128. M D, Y S, S U, D Y, K A. Multiple sclerosis patients with and without sexual dysfunction: are there any differences? [Internet]. Mult. Scler. Houndmills Basingstoke Engl. Mult Scler; 2006 [cited 2020 Sep 27]. Available from: https://pubmed.ncbi.nlm.nih.gov/16629425/

129. Rae-Grant AD. Unusual symptoms and syndromes in multiple sclerosis. Contin Minneap Minn. 2013;19:992–1006.

130. Hufschmidt A, Shabarin V, Rauer S, Zimmer T. Neurological symptoms accompanying urinary tract infections. Eur Neurol. 2010;63:180–3.

131. Rakusa M, Murphy O, McIntyre L, Porter B, Panicker J, Fowler C, et al. Testing for urinary tract colonization before high-dose corticosteroid treatment in acute multiple sclerosis relapses: prospective algorithm validation. Eur J Neurol. 2013;20:448–52.

132. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology. 1983;33:1444–52.

133. Meyer-Moock S, Feng Y-S, Maeurer M, Dippel F-W, Kohlmann T. Systematic literature review and validity evaluation of the Expanded Disability Status Scale (EDSS) and the Multiple Sclerosis Functional Composite (MSFC) in patients with multiple sclerosis. BMC Neurol. 2014;14:58.

134. Fischer JS, Rudick RA, Cutter GR, Reingold SC. The Multiple Sclerosis Functional Composite Measure (MSFC): an integrated approach to MS clinical outcome assessment. National MS Society Clinical Outcomes Assessment Task Force. Mult Scler Houndmills Basingstoke Engl. 1999;5:244–50.

135. Roxburgh RHSR, Seaman SR, Masterman T, Hensiek AE, Sawcer SJ, Vukusic S, et al. Multiple Sclerosis Severity Score: using disability and disease duration to rate disease severity. Neurology. 2005;64:1144–51.

136. Pachner AR, Steiner I. The multiple sclerosis severity score (MSSS) predicts disease severity over time. J Neurol Sci. 2009;278:66–70.

137. Hauser SL, Dawson DM, Lehrich JR, Beal MF, Kevy SV, Propper RD, et al. Intensive immunosuppression in progressive multiple sclerosis. A randomized, three-arm study of high-dose intravenous cyclophosphamide, plasma exchange, and ACTH. N Engl J Med. 1983;308:173–80.

138. Sipe JC, Knobler RL, Braheny SL, Rice GP, Panitch HS, Oldstone MB. A neurologic rating scale (NRS) for use in multiple sclerosis. Neurology. 1984;34:1368–72.

139. Mickey MR, Ellison GW, Myers LW. An illness severity score for multiple sclerosis. Neurology. 1984;34:1343–7.

140. Dilorenzo T, Halper J, Picone MA. Reliability and validity of the multiple sclerosis quality of life inventory in older individuals. Disabil Rehabil. 2003;25:891–7.

141. Lublin FD, Reingold SC. Defining the clinical course of multiple sclerosis: results of an international survey. National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis. Neurology. 1996;46:907–11.

142. Dt O, A S, O K, M I, I K, M T, et al. Radiologically isolated syndrome: 5-year risk for an initial clinical event [Internet]. PloS One. PLoS One; 2014 [cited 2020 Sep 25]. Available from: https://pubmed.ncbi.nlm.nih.gov/24598783/

143. Miller DH, Chard DT, Ciccarelli O. Clinically isolated syndromes. Lancet Neurol. 2012;11:157–69.

144. Miller D, Barkhof F, Montalban X, Thompson A, Filippi M. Clinically isolated syndromes suggestive of multiple sclerosis, part I: natural history, pathogenesis, diagnosis, and prognosis. Lancet Neurol. 2005;4:281–8.

145. De Stefano N, Giorgio A, Battaglini M, Rovaris M, Sormani MP, Barkhof F, et al. Assessing brain atrophy rates in a large population of untreated multiple sclerosis subtypes. Neurology. 2010;74:1868–76.

146. Kidd D, Thorpe JW, Kendall BE, Barker GJ, Miller DH, McDonald WI, et al. MRI dynamics of brain and spinal cord in progressive multiple sclerosis. J Neurol Neurosurg Psychiatry. 1996;60:15–9.

147. Novakova L, Zetterberg H, Sundström P, Axelsson M, Khademi M, Gunnarsson M, et al. Monitoring disease activity in multiple sclerosis using serum neurofilament light protein. Neurology. 2017;89:2230–7.

148. Moccia M, de Stefano N, Barkhof F. Imaging outcome measures for progressive multiple sclerosis trials. Mult Scler Houndmills Basingstoke Engl. 2017;23:1614–26.

149. Rovaris M, Confavreux C, Furlan R, Kappos L, Comi G, Filippi M. Secondary progressive multiple sclerosis: current knowledge and future challenges. Lancet Neurol. 2006;5:343–54.

150. Menon S, Zhu F, Shirani A, Oger J, Freedman MS, Tremlett H. Disability progression in aggressive multiple sclerosis. Mult Scler Houndmills Basingstoke Engl. 2017;23:456–63.

151. Scalfari A, Neuhaus A, Daumer M, Muraro PA, Ebers GC. Onset of secondary progressive phase and long-term evolution of multiple sclerosis. J Neurol Neurosurg Psychiatry. 2014;85:67–75.

152. Confavreux C, Vukusic S, Moreau T, Adeleine P. Relapses and progression of disability in multiple sclerosis. N Engl J Med. 2000;343:1430–8.

153. Tutuncu M, Tang J, Zeid NA, Kale N, Crusan DJ, Atkinson EJ, et al. Onset of progressive phase is an age-dependent clinical milestone in multiple sclerosis. Mult Scler Houndmills Basingstoke Engl. 2013;19:188–98.

154. Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol. 2011;69:292–302.

155. Confavreux C, Vukusic S. Natural history of multiple sclerosis: a unifying concept. Brain J Neurol. 2006;129:606–16.

156. McDonald WI, Compston A, Edan G, Goodkin D, Hartung HP, Lublin FD, et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. Ann Neurol. 2001;50:121–7.

157. Polman CH, Reingold SC, Edan G, Filippi M, Hartung H-P, Kappos L, et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the 'McDonald Criteria'. Ann Neurol. 2005;58:840–6.

158. Thompson AJ, Banwell BL, Barkhof F, Carroll WM, Coetzee T, Comi G, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol. 2018;17:162–73.

159. Pelayo R, Montalban X, Minoves T, Moncho D, Rio J, Nos C, et al. Do multimodal evoked potentials add information to MRI in clinically isolated syndromes? Mult Scler Houndmills Basingstoke Engl. 2010;16:55–61.

160. Zipoli V, Hakiki B, Portaccio E, Lolli F, Siracusa G, Giannini M, et al. The contribution of cerebrospinal fluid oligoclonal bands to the early diagnosis of multiple sclerosis. Mult Scler Houndmills Basingstoke Engl. 2009;15:472–8.

161. Link H, Huang Y-M. Oligoclonal bands in multiple sclerosis cerebrospinal fluid: an update on methodology and clinical usefulness. J Neuroimmunol. 2006;180:17–28.

162. Masjuan J, Alvarez-Cermeño JC, García-Barragán N, Díaz-Sánchez M, Espiño M, Sádaba MC, et al. Clinically isolated syndromes: a new oligoclonal band test accurately predicts conversion to MS. Neurology. 2006;66:576–8.

163. Trojano M, Tintore M, Montalban X, Hillert J, Kalincik T, Iaffaldano P, et al. Treatment decisions in multiple sclerosis — insights from real-world observational studies. Nat Rev Neurol. 2017;13:105–18.

164. Trapp BD, Nave K-A. Multiple sclerosis: an immune or neurodegenerative disorder? Annu Rev Neurosci. 2008;31:247–69.

165. Berger T, Rubner P, Schautzer F, Egg R, Ulmer H, Mayringer I, et al. Antimyelin antibodies as a predictor of clinically definite multiple sclerosis after a first demyelinating event. N Engl J Med. 2003;349:139–45.

166. Gresle MM, Butzkueven H, Shaw G. Neurofilament proteins as body fluid biomarkers of neurodegeneration in multiple sclerosis. Mult Scler Int. 2011;2011:315406.

167. Disanto G, Barro C, Benkert P, Naegelin Y, Schädelin S, Giardiello A, et al. Serum Neurofilament light: A biomarker of neuronal damage in multiple sclerosis. Ann Neurol. 2017;81:857–70.

168. Miller DH, Weinshenker BG, Filippi M, Banwell BL, Cohen JA, Freedman MS, et al. Differential diagnosis of suspected multiple sclerosis: a consensus approach. Mult Scler Houndmills Basingstoke Engl. 2008;14:1157–74.

169. Wingerchuk DM, Banwell B, Bennett JL, Cabre P, Carroll W, Chitnis T, et al. International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. Neurology. 2015;85:177–89.

170. Cole J, Evans E, Mwangi M, Mar S. Acute Disseminated Encephalomyelitis in Children: An Updated Review Based on Current Diagnostic Criteria. Pediatr Neurol. 2019;100:26–34.

171. Kesselring J, Miller DH, Robb SA, Kendall BE, Moseley IF, Kingsley D, et al. Acute disseminated encephalomyelitis. MRI findings and the distinction from multiple sclerosis. Brain J Neurol. 1990;113 (Pt 2):291–302.

172. de Seze J, Debouverie M, Zephir H, Lebrun C, Blanc F, Bourg V, et al. Acute fulminant demyelinating disease: a descriptive study of 60 patients. Arch Neurol. 2007;64:1426–32.

173. Hauser SL, Cree BAC. Treatment of Multiple Sclerosis: A Review. Am J Med. 2020;

174. Le Page E, Veillard D, Laplaud DA, Hamonic S, Wardi R, Lebrun C, et al. Oral versus intravenous high-dose methylprednisolone for treatment of relapses in patients with multiple sclerosis (COPOUSEP): a randomised, controlled, double-blind, non-inferiority trial. Lancet Lond Engl. 2015;386:974–81.

175. Henze T, Rieckmann P, Toyka KV, Multiple Sclerosis Therapy Consensus Group of the German Multiple Sclerosis Society. Symptomatic treatment of multiple sclerosis. Multiple Sclerosis Therapy Consensus Group (MSTCG) of the German Multiple Sclerosis Society. Eur Neurol. 2006;56:78–105.

176. Izquierdo G. Multiple sclerosis symptoms and spasticity management: new data. Neurodegener Dis Manag. 2017;7:7–11.

177. Collin C, Ehler E, Waberzinek G, Alsindi Z, Davies P, Powell K, et al. A double-blind, randomized, placebo-controlled, parallel-group study of Sativex, in subjects with symptoms of spasticity due to multiple sclerosis. Neurol Res. 2010;32:451–9.

178. Novotna A, Mares J, Ratcliffe S, Novakova I, Vachova M, Zapletalova O, et al. A randomized, double-blind, placebo-controlled, parallel-group, enriched-design study of nabiximols* (Sativex(®)), as add-on therapy, in subjects with refractory spasticity caused by multiple sclerosis. Eur J Neurol. 2011;18:1122–31.

179. Chang E, Ghosh N, Yanni D, Lee S, Alexandru D, Mozaffar T. A Review of Spasticity Treatments: Pharmacological and Interventional Approaches. Crit Rev Phys Rehabil Med. 2013;25:11–22.

180. Dhib-Jalbut S, Marks S. Interferon-beta mechanisms of action in multiple sclerosis. Neurology. 2010;74 Suppl 1:S17-24.

181. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. I. Clinical results of a multicenter, randomized, double-blind, placebo-controlled trial. The IFNB Multiple Sclerosis Study Group. Neurology. 1993;43:655–61.

182. PRISMS Study Group and the University of British Columbia MS/MRI Analysis Group. PRISMS-4: Long-term efficacy of interferon-beta-1a in relapsing MS. Neurology. 2001;56:1628–36.

183. Sleijfer S, Bannink M, Van Gool AR, Kruit WHJ, Stoter G. Side effects of interferon-alpha therapy. Pharm World Sci PWS. 2005;27:423–31.

184. Montero JL, Cerezo A, Fraga E, Barrera P, López-Cillero P, Costán G, et al. Acute liver failure in a patient with multiple sclerosis treated with interferon-beta. Mult Scler Houndmills Basingstoke Engl. 2007;13:820.

185. Sørensen PS, Deisenhammer F, Duda P, Hohlfeld R, Myhr K-M, Palace J, et al. Guidelines on use of anti-IFN-beta antibody measurements in multiple sclerosis: report of an EFNS Task Force on IFN-beta antibodies in multiple sclerosis. Eur J Neurol. 2005;12:817–27.

186. Blanchette F, Neuhaus O. Glatiramer acetate: evidence for a dual mechanism of action. J Neurol. 2008;255 Suppl 1:26–36.

187. Comi G, Filippi M, Wolinsky JS. European/Canadian multicenter, double-blind, randomized, placebo-controlled study of the effects of glatiramer acetate on magnetic resonance imaging--measured disease activity and burden in patients with relapsing multiple sclerosis. European/Canadian Glatiramer Acetate Study Group. Ann Neurol. 2001;49:290–7.

188. Comi G, Martinelli V, Rodegher M, Moiola L, Bajenaru O, Carra A, et al. Effect of glatiramer acetate on conversion to clinically definite multiple sclerosis in patients with clinically isolated syndrome (PreCISe study): a randomised, double-blind, placebo-controlled trial. Lancet Lond Engl. 2009;374:1503–11.

189. Ball NJ, Cowan BJ, Moore GRW, Hashimoto SA. Lobular panniculitis at the site of glatiramer acetate injections for the treatment of relapsing-remitting multiple sclerosis. A report of two cases. J Cutan Pathol. 2008;35:407–10.

190. Bar-Or A, Pachner A, Menguy-Vacheron F, Kaplan J, Wiendl H. Teriflunomide and its mechanism of action in multiple sclerosis. Drugs. 2014;74:659–74.

191. Freedman MS, Wolinsky JS, Comi G, Kappos L, Olsson TP, Miller AE, et al. The efficacy of teriflunomide in patients who received prior disease-modifying treatments: Subgroup analyses of the teriflunomide phase 3 TEMSO and TOWER studies. Mult Scler Houndmills Basingstoke Engl. 2018;24:535–9.

192. Vermersch P, Czlonkowska A, Grimaldi LME, Confavreux C, Comi G, Kappos L, et al. Teriflunomide versus subcutaneous interferon beta-1a in patients with relapsing multiple sclerosis: a randomised, controlled phase 3 trial. Mult Scler Houndmills Basingstoke Engl. 2014;20:705–16.

193. Miller AE. Oral teriflunomide in the treatment of relapsing forms of multiple sclerosis: clinical evidence and long-term experience. Ther Adv Neurol Disord. 2017;10:381–96.

194. Garnock-Jones KP. Teriflunomide: a review of its use in relapsing multiple sclerosis. CNS Drugs. 2013;27:1103–23.

195. Yadav SK, Soin D, Ito K, Dhib-Jalbut S. Insight into the mechanism of action of dimethyl fumarate in multiple sclerosis. J Mol Med Berl Ger. 2019;97:463–72.

196. Bar-Or A, Gold R, Kappos L, Arnold DL, Giovannoni G, Selmaj K, et al. Clinical efficacy of BG-12 (dimethyl fumarate) in patients with relapsing-remitting multiple sclerosis: subgroup analyses of the DEFINE study. J Neurol. 2013;260:2297–305.

197. Hutchinson M, Fox RJ, Miller DH, Phillips JT, Kita M, Havrdova E, et al. Clinical efficacy of BG-12 (dimethyl fumarate) in patients with relapsing-remitting multiple sclerosis: subgroup analyses of the CONFIRM study. J Neurol. 2013;260:2286–96.

198. Gold R, Arnold DL, Bar-Or A, Hutchinson M, Kappos L, Havrdova E, et al. Long-term effects of delayed-release dimethyl fumarate in multiple sclerosis: Interim analysis of ENDORSE, a randomized extension study. Mult Scler Houndmills Basingstoke Engl. 2017;23:253–65.

199. Blair HA. Dimethyl Fumarate: A Review in Relapsing-Remitting MS. Drugs. 2019;79:1965–76.

200. Montalban X, Gold R, Thompson AJ, Otero-Romero S, Amato MP, Chandraratna D, et al. ECTRIMS/EAN Guideline on the pharmacological treatment of people with multiple sclerosis. Mult Scler Houndmills Basingstoke Engl. 2018;24:96–120.

201. Hunter SF, Bowen JD, Reder AT. The Direct Effects of Fingolimod in the Central Nervous System: Implications for Relapsing Multiple Sclerosis. CNS Drugs. 2016;30:135–47.

202. Noda H, Takeuchi H, Mizuno T, Suzumura A. Fingolimod phosphate promotes the neuroprotective effects of microglia. J Neuroimmunol. 2013;256:13–8.

203. Brinkmann V, Billich A, Baumruker T, Heining P, Schmouder R, Francis G, et al. Fingolimod (FTY720): discovery and development of an oral drug to treat multiple sclerosis. Nat Rev Drug Discov. 2010;9:883–97.

204. Cohen JA, Barkhof F, Comi G, Hartung H-P, Khatri BO, Montalban X, et al. Oral fingolimod or intramuscular interferon for relapsing multiple sclerosis. N Engl J Med. 2010;362:402–15.

205. Kappos L, Radue E-W, O'Connor P, Polman C, Hohlfeld R, Calabresi P, et al. A placebo-controlled trial of oral fingolimod in relapsing multiple sclerosis. N Engl J Med. 2010;362:387–401.

206. Comi G, Patti F, Rocca MA, Mattioli FC, Amato MP, Gallo P, et al. Efficacy of fingolimod and interferon beta-1b on cognitive, MRI, and clinical outcomes in relapsing-remitting multiple sclerosis: an 18-month, open-label, rater-blinded, randomised, multicentre study (the GOLDEN study). J Neurol. 2017;264:2436–49.

207. Calabresi PA, Radue E-W, Goodin D, Jeffery D, Rammohan KW, Reder AT, et al. Safety and efficacy of fingolimod in patients with relapsing-remitting multiple sclerosis (FREEDOMS II): a double-blind, randomised, placebo-controlled, phase 3 trial. Lancet Neurol. 2014;13:545–56.

208. Selmaj K, Li DKB, Hartung H-P, Hemmer B, Kappos L, Freedman MS, et al. Siponimod for patients with relapsing-remitting multiple sclerosis (BOLD): an adaptive, dose-ranging, randomised, phase 2 study. Lancet Neurol. 2013;12:756–67.

209. Kappos L, Bar-Or A, Cree BAC, Fox RJ, Giovannoni G, Gold R, et al. Siponimod versus placebo in secondary progressive multiple sclerosis (EXPAND): a double-blind, randomised, phase 3 study. Lancet Lond Engl. 2018;391:1263–73.

210. Yednock TA, Cannon C, Fritz LC, Sanchez-Madrid F, Steinman L, Karin N. Prevention of experimental autoimmune encephalomyelitis by antibodies against alpha 4 beta 1 integrin. Nature. 1992;356:63–6.

211. Polman CH, O'Connor PW, Havrdova E, Hutchinson M, Kappos L, Miller DH, et al. A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. N Engl J Med. 2006;354:899–910.

212. Rudick RA, Stuart WH, Calabresi PA, Confavreux C, Galetta SL, Radue E-W, et al. Natalizumab plus interferon beta-1a for relapsing multiple sclerosis. N Engl J Med. 2006;354:911–23.

213. Langer-Gould A, Atlas SW, Green AJ, Bollen AW, Pelletier D. Progressive multifocal leukoencephalopathy in a patient treated with natalizumab. N Engl J Med. 2005;353:375–81.

214. Van Assche G, Van Ranst M, Sciot R, Dubois B, Vermeire S, Noman M, et al. Progressive multifocal leukoencephalopathy after natalizumab therapy for Crohn's disease. N Engl J Med. 2005;353:362–8.

215. J A. The TOUCH program and natalizumab: Fundamental flaw in patient protection [Internet]. F1000Research. F1000Res; 2015 [cited 2020 Sep 18]. Available from: https://pubmed.ncbi.nlm.nih.gov/27134725/

216. Rudick RA, Panzara MA. Natalizumab for the treatment of relapsing multiple sclerosis. Biol Targets Ther. 2008;2:189–99.

217. Goodin DS, Cohen BA, O'Connor P, Kappos L, Stevens JC, Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. Assessment: the use of natalizumab (Tysabri) for the treatment of multiple sclerosis (an evidence-based review): report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. Neurology. 2008;71:766–73.

218. Goodman AD, Rossman H, Bar-Or A, Miller A, Miller DH, Schmierer K, et al. GLANCE: results of a phase 2, randomized, double-blind, placebo-controlled study. Neurology. 2009;72:806–12.

219. Sangalli F, Moiola L, Bucello S, Annovazzi P, Rizzo A, Radaelli M, et al. Efficacy and tolerability of natalizumab in relapsing-remitting multiple sclerosis patients: a post-marketing observational study. Neurol Sci Off J Ital Neurol Soc Ital Soc Clin Neurophysiol. 2011;31 Suppl 3:299–302.

220. Piehl F, Holmén C, Hillert J, Olsson T. Swedish natalizumab (Tysabri) multiple sclerosis surveillance study. Neurol Sci Off J Ital Neurol Soc Ital Soc Clin Neurophysiol. 2011;31 Suppl 3:289–93.

221. Putzki N, Yaldizli O, Bühler R, Schwegler G, Curtius D, Tettenborn B. Natalizumab reduces clinical and MRI activity in multiple sclerosis patients with high disease activity: results from a multicenter study in Switzerland. Eur Neurol. 2010;63:101–6.

222. Hutchinson M, Kappos L, Calabresi PA, Confavreux C, Giovannoni G, Galetta SL, et al. The efficacy of natalizumab in patients with relapsing multiple sclerosis: subgroup analyses of AFFIRM and SENTINEL. J Neurol. 2009;256:405–15.

223. Rae-Grant A, Day GS, Marrie RA, Rabinstein A, Cree BAC, Gronseth GS, et al. Comprehensive systematic review summary: Disease-modifying therapies for adults with multiple sclerosis: Report of the Guideline Development, Dissemination, and Implementation Subcommittee of the American Academy of Neurology. Neurology. 2018;90:789–800.

224. Ruck T, Bittner S, Wiendl H, Meuth SG. Alemtuzumab in Multiple Sclerosis: Mechanism of Action and Beyond. Int J Mol Sci. 2015;16:16414–39.

225. Havrdova E, Arnold DL, Cohen JA, Hartung H-P, Fox EJ, Giovannoni G, et al. Alemtuzumab CARE-MS I 5-year follow-up: Durable efficacy in the absence of continuous MS therapy. Neurology. 2017;89:1107–16.

226. Giovannoni G, Cohen JA, Coles AJ, Hartung H-P, Havrdova E, Selmaj KW, et al. Alemtuzumab improves preexisting disability in active relapsing-remitting MS patients. Neurology. 2016;87:1985–92.

227. Van Wijmeersch B, Singer BA, Boster A, Broadley S, Fernández Ó, Freedman MS, et al. Efficacy of alemtuzumab over 6 years in relapsing-remitting multiple sclerosis patients who relapsed between courses 1 and 2: Post hoc analysis of the CARE-MS studies. Mult Scler Houndmills Basingstoke Engl. 2019;1352458519881759.

228. Coles AJ, Cohen JA, Fox EJ, Giovannoni G, Hartung H-P, Havrdova E, et al. Alemtuzumab CARE-MS II 5-year follow-up: Efficacy and safety findings. Neurology. 2017;89:1117–26.

229. Li Z, Richards S, Surks HK, Jacobs A, Panzara MA. Clinical pharmacology of alemtuzumab, an anti-CD52 immunomodulator, in multiple sclerosis. Clin Exp Immunol. 2018;194:295–314.

230. Menge T, Stüve O, Kieseier BC, Hartung H-P. Alemtuzumab: the advantages and challenges of a novel therapy in MS. Neurology. 2014;83:87–97.

231. Berger T, Elovaara I, Fredrikson S, McGuigan C, Moiola L, Myhr K-M, et al. Alemtuzumab Use in Clinical Practice: Recommendations from European Multiple Sclerosis Experts. CNS Drugs. 2017;31:33–50.

232. Li R, Rezk A, Miyazaki Y, Hilgenberg E, Touil H, Shen P, et al. Proinflammatory GM-CSF-producing B cells in multiple sclerosis and B cell depletion therapy. Sci Transl Med. 2015;7:310ra166.

233. Graves J, Vinayagasundaram U, Mowry EM, Matthews IR, Marino JA, Cheng J, et al. Effects of rituximab on lymphocytes in multiple sclerosis and neuromyelitis optica. Mult Scler Relat Disord. 2014;3:244–52.

234. Hauser SL, Waubant E, Arnold DL, Vollmer T, Antel J, Fox RJ, et al. B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. N Engl J Med. 2008;358:676–88.

235. Hawker K, O'Connor P, Freedman MS, Calabresi PA, Antel J, Simon J, et al. Rituximab in patients with primary progressive multiple sclerosis: results of a randomized double-blind placebo-controlled multicenter trial. Ann Neurol. 2009;66:460–71.

236. Dunn N, Juto A, Ryner M, Manouchehrinia A, Piccoli L, Fink K, et al. Rituximab in multiple sclerosis: Frequency and clinical relevance of anti-drug antibodies. Mult Scler Houndmills Basingstoke Engl. 2018;24:1224–33.

237. DiLillo DJ, Hamaguchi Y, Ueda Y, Yang K, Uchida J, Haas KM, et al. Maintenance of long-lived plasma cells and serological memory despite mature and memory B cell depletion during CD20 immunotherapy in mice. J Immunol Baltim Md 1950. 2008;180:361–71.

238. Hauser SL, Bar-Or A, Comi G, Giovannoni G, Hartung H-P, Hemmer B, et al. Ocrelizumab versus Interferon Beta-1a in Relapsing Multiple Sclerosis. N Engl J Med. 2017;376:221–34.

239. Montalban X, Hauser SL, Kappos L, Arnold DL, Bar-Or A, Comi G, et al. Ocrelizumab versus Placebo in Primary Progressive Multiple Sclerosis. N Engl J Med. 2017;376:209–20.

240. Jacobs BM, Ammoscato F, Giovannoni G, Baker D, Schmierer K. Cladribine: mechanisms and mysteries in multiple sclerosis. J Neurol Neurosurg Psychiatry. 2018;89:1266–71.

241. Baker D, Herrod SS, Alvarez-Gonzalez C, Zalewski L, Albor C, Schmierer K. Both cladribine and alemtuzumab may effect MS via B-cell depletion. Neurol Neuroimmunol Neuroinflammation. 2017;4:e360.

242. Pakpoor J, Disanto G, Altmann DR, Pavitt S, Turner BP, Marta M, et al. No evidence for higher risk of cancer in patients with multiple sclerosis taking cladribine. Neurol Neuroimmunol Neuroinflammation. 2015;2:e158.

243. Cook S, Vermersch P, Comi G, Giovannoni G, Rammohan K, Rieckmann P, et al. Safety and tolerability of cladribine tablets in multiple sclerosis: the CLARITY (CLAdRIbine Tablets treating multiple sclerosis orallY) study. Mult Scler Houndmills Basingstoke Engl. 2011;17:578–93.

244. Leist TP, Comi G, Cree BAC, Coyle PK, Freedman MS, Hartung H-P, et al. Effect of oral cladribine on time to conversion to clinically definite multiple sclerosis in patients with a first demyelinating event (ORACLE MS): a phase 3 randomised trial. Lancet Neurol. 2014;13:257–67.

245. Bielekova B. Daclizumab therapy for multiple sclerosis. Neurother J Am Soc Exp Neurother. 2013;10:55–67.

246. Gold R, Radue E-W, Giovannoni G, Selmaj K, Havrdova E, Stefoski D, et al. Safety and efficacy of daclizumab in relapsing-remitting multiple sclerosis: 3-year results from the SELECTED open-label extension study. BMC Neurol. 2016;16:117.

247. Varrin-Doyer M, Zamvil SS, Schulze-Topphoff U. Laquinimod, an up-and-coming immunomodulatory agent for treatment of multiple sclerosis. Exp Neurol. 2014;262 Pt A:66–71.

248. Derfuss T, Mehling M, Papadopoulou A, Bar-Or A, Cohen JA, Kappos L. Advances in oral immunomodulating therapies in relapsing multiple sclerosis. Lancet Neurol. 2020;19:336–47.

249. Bk K-D, Kl T. Progressive multifocal leukoencephalopathy complicating treatment with natalizumab and interferon beta-1a for multiple sclerosis [Internet]. N. Engl. J. Med. N Engl J Med; 2005 [cited 2020 Sep 21]. Available from: https://pubmed.ncbi.nlm.nih.gov/15947079/

250. A L-G, Sw A, Aj G, Aw B, D P. Progressive multifocal leukoencephalopathy in a patient treated with natalizumab [Internet]. N. Engl. J. Med. N Engl J Med; 2005 [cited 2020 Sep 21]. Available from: https://pubmed.ncbi.nlm.nih.gov/15947078/

251. Baldwin KJ, Hogg JP. Progressive multifocal leukoencephalopathy in patients with multiple sclerosis. Curr Opin Neurol. 2013;26:318–23.

252. Ps S, A B, G E, G G, R G, E H, et al. Risk stratification for progressive multifocal leukoencephalopathy in patients treated with natalizumab [Internet]. Mult. Scler. Houndmills Basingstoke Engl. Mult Scler; 2012 [cited 2020 Sep 18]. Available from: https://pubmed.ncbi.nlm.nih.gov/22312009/

253. Major EO, Yousry TA, Clifford DB. Pathogenesis of progressive multifocal leukoencephalopathy and risks associated with treatments for multiple sclerosis: a decade of lessons learned. Lancet Neurol. 2018;17:467–80.

254. Zurhein G, Chou SM. PARTICLES RESEMBLING PAPOVA VIRUSES IN HUMAN CEREBRAL DEMYELINATING DISEASE. Science. 1965;148:1477–9.

255. R C-S, L V, E O-Z, M M, L L, M N, et al. Detection and typing of JC virus in autopsy brains and extraneural organs of AIDS patients and non-immunocompromised individuals [Internet]. J. Neurovirol. J Neurovirol; 1999 [cited 2020 Sep 20]. Available from: https://pubmed.ncbi.nlm.nih.gov/10321976/

256. Pn J, Eo M. Viral variant nucleotide sequences help expose leukocytic positioning in the JC virus pathway to the CNS [Internet]. J. Leukoc. Biol. J Leukoc Biol; 1999 [cited 2020 Sep 20]. Available from: https://pubmed.ncbi.nlm.nih.gov/10204570/

257. Ferenczy MW, Marshall LJ, Nelson CDS, Atwood WJ, Nath A, Khalili K, et al. Molecular biology, epidemiology, and pathogenesis of progressive multifocal leukoencephalopathy, the JC virus-induced demyelinating disease of the human brain. Clin Microbiol Rev. 2012;25:471–506.

258. Tan CS, Koralnik IJ. Progressive multifocal leukoencephalopathy and other disorders caused by JC virus: clinical features and pathogenesis. Lancet Neurol. 2010;9:425–37.

259. Stüve O, Marra CM, Jerome KR, Cook L, Cravens PD, Cepok S, et al. Immune surveillance in multiple sclerosis patients treated with natalizumab. Ann Neurol. 2006;59:743–7.

260. Bohra C, Sokol L, Dalia S. Progressive Multifocal Leukoencephalopathy and Monoclonal Antibodies: A Review. Cancer Control J Moffitt Cancer Cent. 2017;24:1073274817729901.

261. Sabath BF, Major EO. Traffic of JC virus from sites of initial infection to the brain: the path to progressive multifocal leukoencephalopathy. J Infect Dis. 2002;186 Suppl 2:S180-186.

262. Alstadhaug KB, Myhr K-M, Rinaldo CH. Progressive multifocal leukoencephalopathy. Tidsskr Den Nor Laegeforening Tidsskr Prakt Med Ny Raekke. 2017;137.

263. Assetta B, Atwood WJ. The biology of JC polyomavirus. Biol Chem. 2017;398:839–55.

264. Astrom KE, Mancall EL, Richardson EP. Progressive multifocal leuko-encephalopathy; a hitherto unrecognized complication of chronic lymphatic leukaemia and Hodgkin's disease. Brain J Neurol. 1958;81:93–111.

265. Du Pasquier RA, Corey S, Margolin DH, Williams K, Pfister L-A, De Girolami U, et al. Productive infection of cerebellar granule cell neurons by JC virus in an HIV+ individual. Neurology. 2003;61:775–82.

266. Engsig FN, Hansen A-BE, Omland LH, Kronborg G, Gerstoft J, Laursen AL, et al. Incidence, clinical presentation, and outcome of progressive multifocal leukoencephalopathy in HIV-infected patients during the highly active antiretroviral therapy era: a nationwide cohort study. J Infect Dis. 2009;199:77–83.

267. Khanna N, Elzi L, Mueller NJ, Garzoni C, Cavassini M, Fux CA, et al. Incidence and outcome of progressive multifocal leukoencephalopathy over 20 years of the Swiss HIV Cohort Study. Clin Infect Dis Off Publ Infect Dis Soc Am. 2009;48:1459–66.

268. Jelcic I, Jelcic I, Kempf C, Largey F, Planas R, Schippling S, et al. Mechanisms of immune escape in central nervous system infection with neurotropic JC virus variant. Ann Neurol. 2016;79:404–18.

269. Jr B, B K, Mj P, G D. Progressive multifocal leukoencephalopathy associated with human immunodeficiency virus infection. A review of the literature with a report of sixteen cases [Internet]. Ann. Intern. Med. Ann Intern Med; 1987 [cited 2020 Sep 20]. Available from: https://pubmed.ncbi.nlm.nih.gov/3296901/

270. Parr J, Horoupian DS, Winkelman AC. Cerebellar form of progressive multifocal leukoencephalopathy (PML). Can J Neurol Sci J Can Sci Neurol. 1979;6:123–8.

271. Hou J, Major E. Management of infections by the human polyomavirus JC: past, present and future. Expert Rev Anti Infect Ther. 2005;3:629–40.

272. Yaldizli O, Putzki N. Natalizumab in the treatment of multiple sclerosis. Ther Adv Neurol Disord. 2009;2:115–28.

273. Berger JR. Classifying PML risk with disease modifying therapies. Mult Scler Relat Disord. 2017;12:59–63.

274. Longbrake EE, Ramsbottom MJ, Cantoni C, Ghezzi L, Cross AH, Piccio L. Dimethyl fumarate selectively reduces memory T cells in multiple sclerosis patients. Mult Scler Houndmills Basingstoke Engl. 2016;22:1061–70.

275. Lorefice L, Fenu G, Gerevini S, Frau J, Coghe G, Barracciu MA, et al. PML in a person with multiple sclerosis: Is teriflunomide the felon? Neurology. 2018;90:83–5.

276. Scarpazza C, De Rossi N, Tabiadon G, Turrini MV, Gerevini S, Capra R. Four cases of natalizumab-related PML: a less severe course in extended interval dosing? Neurol Sci Off J Ital Neurol Soc Ital Soc Clin Neurophysiol. 2019;

277. Steiner I, Berger JR. Update on progressive multifocal leukoencephalopathy. Curr Neurol Neurosci Rep. 2012;12:680–6.

278. Berghoff M, Schänzer A, Hildebrandt GC, Dassinger B, Klappstein G, Kaps M, et al. Development of progressive multifocal leukoencephalopathy in a patient with non-Hodgkin lymphoma 13 years after treatment with cladribine. Leuk Lymphoma. 2013;54:1340–2.

279. Alstadhaug KB, Fykse Halstensen R, Odeh F. Progressive multifocal leukoencephalopathy in a patient with systemic mastocytosis treated with cladribine. J Clin Virol Off Publ Pan Am Soc Clin Virol. 2017;88:17–20.

280. Wattjes MP, Wijburg MT, van Eijk J, Frequin S, Uitdehaag BMJ, Barkhof F, et al. Inflammatory natalizumab-associated PML: baseline characteristics, lesion evolution and relation with PML-IRIS. J Neurol Neurosurg Psychiatry. 2018;89:535–41.

281. Wattjes MP, Vennegoor A, Steenwijk MD, de Vos M, Killestein J, van Oosten BW, et al. MRI pattern in asymptomatic natalizumab-associated PML. J Neurol Neurosurg Psychiatry. 2015;86:793–8.

282. Yousry TA, Pelletier D, Cadavid D, Gass A, Richert ND, Radue E-W, et al. Magnetic resonance imaging pattern in natalizumab-associated progressive multifocal leukoencephalopathy. Ann Neurol. 2012;72:779–87.

283. Progressive multifocal leukoencephalopathy: a review of the neuroimaging features and differential diagnosis - PubMed [Internet]. [cited 2020 Sep 20]. Available from: https://pubmed.ncbi.nlm.nih.gov/22136455/

284. Berger JR, Aksamit AJ, Clifford DB, Davis L, Koralnik IJ, Sejvar JJ, et al. PML diagnostic criteria: consensus statement from the AAN Neuroinfectious Disease Section. Neurology. 2013;80:1430–8.

285. Mentzer D, Prestel J, Adams O, Gold R, Hartung H-P, Hengel H, et al. Case definition for progressive multifocal leukoencephalopathy following treatment with monoclonal antibodies. J Neurol Neurosurg Psychiatry. 2012;83:927–33.

286. Bloomgren G, Richman S, Hotermans C, Subramanyam M, Goelz S, Natarajan A, et al. Risk of natalizumab-associated progressive multifocal leukoencephalopathy. N Engl J Med. 2012;366:1870–80.

287. Ho P-R, Koendgen H, Campbell N, Haddock B, Richman S, Chang I. Risk of natalizumab-associated progressive multifocal leukoencephalopathy in patients with multiple sclerosis: a retrospective analysis of data from four clinical studies. Lancet Neurol. 2017;16:925–33.

288. Plavina T, Subramanyam M, Bloomgren G, Richman S, Pace A, Lee S, et al. Anti-JC virus antibody levels in serum or plasma further define risk of natalizumab-associated progressive multifocal leukoencephalopathy. Ann Neurol. 2014;76:802–12.

289. Clifford DB, De Luca A, DeLuca A, Simpson DM, Arendt G, Giovannoni G, et al. Natalizumab-associated progressive multifocal leukoencephalopathy in patients with multiple sclerosis: lessons from 28 cases. Lancet Neurol. 2010;9:438–46.

290. Tan IL, McArthur JC, Clifford DB, Major EO, Nath A. Immune reconstitution inflammatory syndrome in natalizumab-associated PML. Neurology. 2011;77:1061–7.

291. Gorelik L, Lerner M, Bixler S, Crossman M, Schlain B, Simon K, et al. Anti-JC virus antibodies: implications for PML risk stratification. Ann Neurol. 2010;68:295–303.

292. Lee P, Plavina T, Castro A, Berman M, Jaiswal D, Rivas S, et al. A second-generation ELISA (STRATIFY JCV™ DxSelect™) for detection of JC virus antibodies in human serum and plasma to support progressive multifocal leukoencephalopathy risk stratification. J Clin Virol Off Publ Pan Am Soc Clin Virol. 2013;57:141–6.

293. Schwab N, Schneider-Hohendorf T, Pignolet B, Breuer J, Gross CC, Göbel K, et al. Therapy with natalizumab is associated with high JCV seroconversion and rising JCV index values. Neurol Neuroimmunol Neuroinflammation. 2016;3:e195.

294. McGuigan C, Craner M, Guadagno J, Kapoor R, Mazibrada G, Molyneux P, et al. Stratification and monitoring of natalizumab-associated progressive multifocal leukoencephalopathy risk: recommendations from an expert group. J Neurol Neurosurg Psychiatry. 2016;87:117–25.

295. Sangalli F, Moiola L, Ferrè L, Radaelli M, Barcella V, Rodegher M, et al. Long-term management of natalizumab discontinuation in a large monocentric cohort of multiple sclerosis patients. Mult Scler Relat Disord. 2014;3:520–6.

296. O'Connor PW, Goodman A, Kappos L, Lublin FD, Miller DH, Polman C, et al. Disease activity return during natalizumab treatment interruption in patients with multiple sclerosis. Neurology. 2011;76:1858–65.

297. Berger JR, Centonze D, Comi G, Confavreux C, Cutter G, Giovannoni G, et al. Considerations on discontinuing natalizumab for the treatment of multiple sclerosis. Ann Neurol. 2010;68:409–11.

298. Iaffaldano P, Viterbo RG, Trojano M. Natalizumab discontinuation is associated with a rebound of cognitive impairment in multiple sclerosis patients. J Neurol. 2016;263:1620–5.

299. Evangelopoulos ME, Koutoulidis V, Andreadou E, Evangelopoulos DS, Kilidireas C. Pulsed corticosteroid treatment in MS patients stabilizes disease activity following natalizumab withdrawal prior to switching to fingolimod. Int J Neurosci. 2016;126:1097–102.

300. Weinstock-Guttman B, Hagemeier J, Kavak KS, Saini V, Patrick K, Ramasamy DP, et al. Randomised natalizumab discontinuation study: taper protocol may prevent disease reactivation. J Neurol Neurosurg Psychiatry. 2016;87:937–43.

301. Hegen H, Walde J, Bsteh G, Auer M, Wurth S, Zinganell A, et al. Impact of Disease-Modifying Treatments on the Longitudinal Evolution of Anti-JCV Antibody Index in Multiple Sclerosis. Front Immunol. 2018;9:2435.

302. van Kempen ZLE, Leurs CE, de Vries A, Vennegoor A, Rispens T, Wattjes MP, et al. John Cunningham virus conversion in relation to natalizumab concentration in multiple sclerosis patients. Eur J Neurol. 2017;24:1196–9.

303. Peters J, Williamson E. Natalizumab therapy is associated with changes in serum JC virus antibody indices over time. J Neurol. 2017;264:2409–12.

304. Outteryck O, Zéphir H, Salleron J, Ongagna J-C, Etxeberria A, Collongues N, et al. JC-virus seroconversion in multiple sclerosis patients receiving natalizumab. Mult Scler Houndmills Basingstoke Engl. 2014;20:822–9.

305. Raffel J, Gafson AR, Malik O, Nicholas R. Anti-JC virus antibody titres increase over time with natalizumab treatment. Mult Scler Houndmills Basingstoke Engl. 2015;21:1833–8.

306. Correia I, Jesus-Ribeiro J, Batista S, Martins AI, Nunes C, Macário MC, et al. Anti-JCV antibody serostatus and longitudinal evaluation in a Portuguese Multiple Sclerosis population. J Clin Neurosci Off J Neurosurg Soc Australas. 2017;45:257–60.

307. Paz SPC, Branco L, Pereira MA de C, Spessotto C, Fragoso YD. Systematic review of the published data on the worldwide prevalence of John Cunningham virus in patients with multiple sclerosis and neuromyelitis optica. Epidemiol Health. 2018;40:e2018001.

308. Bellizzi A, Anzivino E, Rodio DM, Palamara AT, Nencioni L, Pietropaolo V. New insights on human polyomavirus JC and pathogenesis of progressive multifocal leukoencephalopathy. Clin Dev Immunol. 2013;2013:839719.

309. Vennegoor A, van Rossum JA, Leurs C, Wattjes MP, Rispens T, Murk JL a. N, et al. High cumulative JC virus seroconversion rate during long-term use of natalizumab. Eur J Neurol. 2016;23:1079–85.

310. Schwab N, Schneider-Hohendorf T, Hoyt T, Gross CC, Meuth SG, Klotz L, et al. Anti-JCV serology during natalizumab treatment: Review and meta-analysis of 17 independent patient cohorts analyzing anti-John Cunningham polyoma virus sero-conversion rates under natalizumab treatment and differences between technical and biological sero-converters. Mult Scler Houndmills Basingstoke Engl. 2018;24:563–73.

311. Cambron M, Hadhoum N, Duhin E, Lacour A, Chouraki A, Vermersch P. JCV serology in time: 3 years of follow-up. Acta Neurol Scand. 2017;136:54–8.

312. Clerico M, Artusi CA, Di Liberto A, Rolla S, Bardina V, Barbero P, et al. Natalizumab in Multiple Sclerosis: Long-Term Management. Int J Mol Sci [Internet]. 2017 [cited 2019 Jan 16];18. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5454853/

313. Aoyama S, Mori M, Uzawa A, Uchida T, Masuda H, Ohtani R, et al. Serum anti-JCV antibody indexes in Japanese patients with multiple sclerosis: elevations along with fingolimod treatment duration. J Neurol. 2018;265:1145–50.

314. Blumenfeld-Kan S, Staun-Ram E, Miller A. Fingolimod reduces CXCR4-mediated B cell migration and induces regulatory B cells-mediated anti-inflammatory immune repertoire. Mult Scler Relat Disord. 2019;34:29–37.

315. Duddy M, Niino M, Adatia F, Hebert S, Freedman M, Atkins H, et al. Distinct effector cytokine profiles of memory and naive human B cell subsets and implication in multiple sclerosis. J Immunol Baltim Md 1950. 2007;178:6092–9.

316. Harp CT, Ireland S, Davis LS, Remington G, Cassidy B, Cravens PD, et al. Memory B cells from a subset of treatment-naïve relapsing-remitting multiple sclerosis patients elicit CD4(+) T-cell proliferation and IFN-γ production in response to myelin basic protein and myelin oligodendrocyte glycoprotein. Eur J Immunol. 2010;40:2942–56.

317. Baker D, Marta M, Pryce G, Giovannoni G, Schmierer K. Memory B Cells are Major Targets for Effective Immunotherapy in Relapsing Multiple Sclerosis. EBioMedicine. 2017;16:41–50.

318. McCarthy CL, Tuohy O, Compston DAS, Kumararatne DS, Coles AJ, Jones JL. Immune competence after alemtuzumab treatment of multiple sclerosis. Neurology. 2013;81:872–6.

319. Palanichamy A, Jahn S, Nickles D, Derstine M, Abounasr A, Hauser SL, et al. Rituximab efficiently depletes increased CD20-expressing T cells in multiple sclerosis patients. J Immunol Baltim Md 1950. 2014;193:580–6.

320. Borchardt J, Berger JR. Re-evaluating the incidence of natalizumab-associated progressive multifocal leukoencephalopathy. Mult Scler Relat Disord. 2016;8:145–50.

321. Schwab N, Schneider-Hohendorf T, Pignolet B, Spadaro M, Görlich D, Meinl I, et al. PML risk stratification using anti-JCV antibody index and L-selectin. Mult Scler Houndmills Basingstoke Engl. 2016;22:1048–60.

322. Delgado-García M, Matesanz F, Alcina A, Fedetz M, García-Sánchez MI, Ruiz-Peña JL, et al. A new risk variant for multiple sclerosis at the immunoglobulin heavy chain locus associates with intrathecal IgG, IgM index and oligoclonal bands. Mult Scler Houndmills Basingstoke Engl. 2015;21:1104–11.

323. Ryerson LZ, Foley J, Chang I, Kister I, Cutter G, Metzger RR, et al. Risk of natalizumab-associated PML in patients with MS is reduced with extended interval dosing. Neurology. 2019;93:e1452–62.

324. Chang I, Muralidharan KK, Campbell N, Ho P-R. Modeling the Efficacy of Natalizumab in Multiple Sclerosis Patients Who Switch From Every-4-Week Dosing to Extended-Interval Dosing. J Clin Pharmacol. 2020;