Multiple Sclerosis: Literature review

Graduate Program in Neuroscience

Master Thesis

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1. Epidemiology

Multiple Sclerosis (MS) is a chronic autoimmune, inflammatory neurological disease of the CNS [Hauser *et al.*, 2008]. It is one of the most common neurological diseases and the leading cause of non-traumatic disability in young adults, with mean onset between 20 and 45 years of age [Goldenberg, 2012]. Initial symptoms rarely occur before age 10 years and after age 60 years [Tullman, 2013]. The prevalence of MS has increased the past years with 33/100000 diagnosed in 2013 globally and it varies according to geographic location. It is debatable whether the increase in prevalence is attributed to improved diagnostic tools and reporting, or changes in current lifestyle (e.g. increase in smoking, vitamin D deficiency) [Compston and Coles, 2002], but it is mostly a result of all factors.

In accordance to other autoimmune diseases [Ahlgren *et al.*, 2011], MS is universally found to be more prevalent in women than in men [Bove *et al.*, 2013], with the exception of the primary-progressive form of the disease, where there is no gender preponderance [Tullman, 2013]. Although the exact mechanism accounting for this gender difference is unclear, it has generated extensive studies for possible differences in the immune system or the nervous system between women and men, which could be attributed to effects of gonadal hormones, genetic differences, different environmental exposures and lifestyle in men and women [Greer and McCombe, 2011]. The estimated MS prevalence ratio of women to men has markedly increased during the last decades (2.3-3.5:1) [Orton *et al.*, 2010; Wallin *et al.*, 2012], which has been validated by multiple epidemiology studies in different cohorts.

2. Etiology

The exact etiology of MS is unknown. However, it seems that a combination of genetic and environmental factors contributes to MS pathogenesis.

2.1.Genetic factors

The current hypothesis is that of complex interactions between genetic susceptibility and a nongenetic trigger, leading to an aberrant immune response and consequent damage to the myelin sheath. Within the years, a number of environmental and genetic factors have been linked to increased risk for MS, through a number of

studies. The strongest known and widely studied genetic factor influencing MS susceptibility is the Major Histocompatibility Complex (MHC), or human leucocyte antigen (HLA). Many studies have associated HLA-DR15 haplotype and MS in European and non-European populations with MS [Schmidt *et al.*, 2007]. The association between MS and HLA was first described in the 1970s [Naito *et al.*, 1972]. However, it only increases the risk by 2- to 4-fold when present, and it is present in 20-30% of healthy individuals, indicating that it is not essential for the development of the disease. The past years other susceptibility loci have been identified through GWAS and meta-analysis studies, with more than 50 confirmed associated genetic loci in larger scale studies [Sawcer *et al.*, 2011; Patsopoulos *et al.*, 2011], including genes involved in vitamin D metabolism.

Additional evidence for genetic predisposition was given through epidemiologic studies in monozygotic and dizygotic twins and first-degree relatives of MS patients, indicating a 20- to 40-fold increased risk in first-degree relatives and a 25% to 30% concordance in monozygotic twins, as opposed to dizygotic twins (only 5%). Since the remaining 70% of identical twins are discordant for MS, it can be concluded that other factors also contribute to disease susceptibility [Sadovnick *et al.*, 1996; Hemmer *et al.* 2006; Koch-Henriksen *et al.*, 2010]. On the other hand, studies of half-siblings, adoptees and step-siblings have found no effect of the family microenvironment on MS risk, further enhancing the role of environmental factors at a population level [Sadovnick *et al.*, 1996, Ebers *et al.*, 2004; Dyment *et al.*, 2006].

2.2.Environmental factors

2.2.1 Latitude

There seems to be a variation in MS prevalence with latitude, exhibiting higher levels in North America and Europe (>100/ 100000 inhabitants) to low rates in Eastern Asia and sub-Saharan Africa (2/ 100000 inhabitants) [Leray *et al.*, 2016; Kampman *et al.*, 2013; Trojano *et al.*, 2012]. Countries closer to the equator seem to have lower prevalence, and an increase is marked as the distance grows longer, (e.g. Scandinavian countries). Latitudinal gradients have been identified through multiple studies across the world, including Europe, North America, Australia and New Zealand [Vukusic *et al.*, 2007; Kurtzke *et al.*, 2008, Hammond *et al.*, 1988; Taylor *et al.*, 2010]. As an exception to the latitude gradient-based theory, a discrete focus of

higher MS prevalence in Sardinia has been repeatedly highlighted through multiple studies, thus disproving the latitude gradient-based theory and supporting the hypothesis of an MS focus due to genetical isolation and founder effect [Puqliatti *et al.*, 2001]. The first indications of latitudinal influences on MS prevalence were detected in a 1922 study among men of the US army, where a higher prevalence among certain ethnic groups (Finns, Scandinavians) was noted [Davenport, 1922].

Vitamin D deficiency has been proposed as a potential etiologic factor for the higher prevalence at the highest latitudes, since the primary source of the vitamin D in humans is UVB radiation (direct sunlight) of the skin [Holick, 2004]. Since the intensity of UVB wavelengths varies according to latitude and season, one could safely assume that habitants of areas at higher latitudes, such as Scandinavian countries have lower UVB exposure, thus decreased vitamin D composition. However, in spite of considerable evidence for the role of vitamin D in MS pathogenesis, recent studies have proposed that it may not be the only mediator in the latitude effect related to UV radiation [Lucas *et al.*, 2011].

2.2.2 Vitamin D

The active form ($1,25(OH)_2D$) has been proved to have wide-ranging effects in the human body, mediated by gene expression at the nuclear level. The most studied and recognized role is that in calcium homeostasis, where, in combination with parathyroid hormone (PTH), it acts to maintain stable calcium levels through action on the bone, calcium absorption and renal calcium excretion. Recent studies prove further evidence for the role of $1,25(OH)_2D$ in brain development and function, as well as musculoskeletal and cardiovascular health [Norman, 2006].

Two main forms of vitamin D are recognized: ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3), the latter being the primary precursor in the human body, and the only form that can be produced in the epidermis, through the act of UVB wavelengths on the β ring of the precursor pre-vitamin D_3, which forms pre-vitamin D, that spontaneously isomerizes to vitamin D_3. Both can be derived from dietary sources, but vitamin D_2 is less efficiently absorbed and less biologically active. Once it enters the circulation, vitamin D (both D_2 and D_3) is transported to the liver, where it is hydroxylated to the major circulating form, 25(OH)D. It then bounds

to the vitamin D binding protein (VDBP) and transported to the kidneys and other target organs, so as to be converted to its biologically active form ($1,25(OH)_2D$).

The active form can be produced by the kidneys (endocrine), by the action of the 1α hydroxylase enzyme encoded by the *CYP27B1* gene, as well as other local cells (paracrine) or the cell itself (autocrine). It can then act upon target cells by binding to membrane based vitamin D receptors (VDRs) or by crossing the cell membrane and exert genomic or non-genomic effects. Further studies on $1,25(OH)_2D$ have proved *in-vitro* its ability to modify the immune response, by either depressing or inhibiting the production of pro-inflammatory Th1 cytokines, while at the same time T_h2 and T_{regg} are stimulated [May *et al.*, 2004], further providing a hint on the role of vitamin D in MS immunopathology. Apart from the known role of vitamin D in neuronal and neurotransmitter function [Tenenhouse *et al.*, 1991], it has also been proved to regulate demyelination and remyelination, pause oligodendrocyte apoptosis and enhance the differentiation of oligodendrocyte precursors into mature cells [Goudarzvand *et al.*, 2010]. Lastly, a recent study provided proof that the HLA-DBR1*1501 allele of HLA-DR gene is in fact upregulated by $1,25(OH)_2D$, via a highly conservative vitamin D responsive element (VDRE) [Ramagopalan *et al.*, 2009].

2.2.2.1 Epidemiological and genetic evidence of the vitamin D role in MS pathophysiology

Two prospective epidemiologic studies have shown a link between low vitamin D levels and increased risk for MS development [Munger *et al.*, 2004; Munger *et al.*, 2006]. Moreover, serum 25(OH)D levels appear to be lower in MS patients in general [Nieves *et al.*, 1994; Ozgocmen *et al.*, 2005], as well as at the time of relapse [Soilu-Hanninen *et al.*, 2005]. There have been some studies that associate polymorphisms of VDR and *CYP27B1* genes with increased risk for developing MS [Sawcer *et al.*, 2011; Sundqvist *et al.*, 2010; Tajouri *et al.*, 2005; Partridge *et al.*, 2004; Mamutse *et al.*, 2008], although others find no association. Additionally, interactions between some VDR polymorphisms and HLA-DR15 or exposure to sunlight have been observed, but remain to be confirmed [Dickinson *et al.*, 2009; Niino *et al.*, 2000]. A recent genetic analysis in multiplex Canadian families led to the discovery of the first

rare variants within the *CYP27B1* gene that were associated with an increased risk for MS onset [Ramagopalan *et al.*, 2011].

2.2.3 Epstein-Barr Virus (EBV)

Several infections have been linked to MS pathogenesis, with the more consistent findings concerning past infection with EBV. EBV is a herpes virus that is asymptomatic in childhood, but causes infectious mononucleosis (IM) in adolescence and adulthood. There is a high incidence in under-developed countries, with more than 90% of children becoming seropositive within the first ten years of life [Pancharoen *et al.*, 2001]. In developing countries that percentage drops almost in half (50%) for the age 5-9 years, whereas as soon as a person reaches adulthood, there is 80% chance that he will be seropositive [Takeuchi *et al.*, 2006]. Several studies have suggested an increased risk for MS associated with past history of IM or high antibodies for EBV [Lucas *et al.*, 2011] and decreased risk with seronegativity, one study reporting a two-fold increased risk [Handel *et al.*, 2010]. It seems that the increased risk is associated with higher IgG antibodies titres to Epstein-Barr nuclear antigens (EBNA), and not antibodies to other EBV antigens or other viral infections such as HSV, VZV, CMV or measles [Levin *et al.*, 2005; Ascherio *et al.*, 2001; Sundstrom *et al.*, 2004], thus, indicating a specific immune response to EBV, rather than a non-specific immune dysregulation.

The environmental and genetic factors that may influence EBV infection and MS risk have been a subject to many studies. As mentioned previously, many studies have focused on the role of vitamin D deficiency in MS pathogenesis, through its immunomodulatory effects, and there was a suggestion that it could affect the risk, severity or persistence of EBV infection. One study has highlighted an inverse correlation between serum vitamin D levels and EBV DNA load [Lucas *et al.*, 2011], but there is yet no solid evidence for a significant interaction between EBV seropositivity and vitamin D deficiency with increased MS risk. Some studies also focused on the interaction between smoking and EBV infection with increased MS risk, but further outcomes were inconclusive [Sundqvist *et el.*, 2012; Riise *et al.*, 2011]. Moreover, there is some evidence that previous viral infections or EBV co-infection with different viruses or different EBV types may have a role in MS risk. Two studies reported an increased MS risk in EBV infections without an earlier

infection with another virus, e.g. herpes virus simplex (HSV). There are some proposed theories concerning the causal pathways of EBV infection and MS pathogenesis. The hypothesis of immunological memory proposes that exposure to EBV produces a broadened specificity in T cell response to EBNA-1 and EBNA-1 specific T cells were cross-reactive with myelin antigens [Lunemann *et al.*, 2006; Lunemann *et al.*, 2008]. There is a suggestion that EBV shares sequence homology with antigenic structures in the CNS [Quandt *et al.*, 2004], especially MBP, thus, causing dysregulation of the immune system through the hypothesis of molecular mimicry [Sospedra *et al.*, 2006].

2.2.4 Smoking

There is strong evidence from epidemiological studies that cigarette smoking is an important risk factor for MS. In a large North American cohort, more than 50% of patients with MS were either smokers or ex-smokers [Marrie *et al.*, 2009], and it seemed that MS patients were heavier smokers than the general population [Nortvedt *et al.*, 2005; Friend *et al.*, 2006] and they chose not to quit smoking after diagnosis [Turner *et al.*, 2007]. Moreover, smoking is associated with an increased risk for conversion to secondary progressive disease [Hernan *et al.*, 2005; Sundstrom *et al.*, 2008; Healy *et al.*, 2009, Pittas *et al.*, 2009], along with early conversion of clinically associated syndrome to definite MS [Di Pauli *et al.*, 2008]. It was also linked to increased burden of active inflammation and brain atrophy on imaging studies, with consequent greater disability on long-term follow-up [Zivadinov *et al.*, 2009; Bakshi *et al.*, 2008]. A study proposed that the smoking- associated increased MS risk can diminish after five years of abstinence [Hedstrom *at al.*, 2009].

Apart from MS, cigarette smoking has been linked to many other autoimmune diseases, such as rheumatoid arthritis and systemic lupus erythematosus [Vessey *et al.*, 1987; Nagata *et al.*, 1995]. Smoke contains thousands of components with known effects such as pro-inflammatory actions, apoptosis and direct tissue damage and immunosuppressant effects. Smokers have higher levels of fibrinogen, CRP and pro-inflammatory cytokines. It seems that smoking causes a prolonged pro-inflammatory state that predisposes to autoimmunity. In the case of MS, a disease assumed to be driven by T or B cells autosensitized to myelin components, alteration of cellular and humoral immunity smoke seems to play an important role. One theory suggests that

direct tissue damage by neurotoxic compounds of smoke leads to accumulation of cellular debris that could “overwhelm” apoptotic mechanisms, leading to an increased local immune reaction and consequent sensitization to self-antigens. A study found that smokers tend to have increased expression of first apoptosis signal receptor (Fas/ CD95), a cell surface molecule on CD4 T cells and B cells, that makes them more susceptible to apoptosis, creating increased burden of apoptosed material [Bijil et al., 2001]. Another theory is based on the fact that cigarette smoking can cause direct damage to cells or DNA, due to high concentration in free radicals, causing mutation and gene activation enhancing autoimmunity [Pryor and Stone, 1997]. Since neurons and oligodendrocytes have higher oxygen utilization, they are the ones more vulnerable to oxidative stress [Blomgren and Hagberg, 2006].

In animal models, nicotine seems to act directly upon the blood-brain barrier (BBB), increasing influx of permeable solutes and changing blood flow to brain structures [Chen *et al.*, 1995; Grunwald *et al.*, 1991]. Apart from nicotine, cigarette smoke contains three biproducts that are involved in oxidative stress to neural tissue. Many animal experiments have produced demyelinating lesions in the CNS after exposure to cyanide, a smoke byproduct, either in large doses or after chronic low dose exposure. Cigarette smoking is the major source of exogenous source of nitric oxide (NO), which can also be induced by nicotine in the CNS [Smith *et al.*, 1998; Suemaru *et al.*, 1997; Tonnessen *et al.*, 2000]. The actions of NO can be devastating for the CNS, including axonal degeneration or nerve block conduction, with the active and demyelinated axons being more susceptible [Smith *et al.*, 2001; Redford *et al.*, 1997]. NO also induces targeted oligodendrocyte necrosis, leaving astrocytes and microglia unharmed. If NO or NO metabolites are measured in the CSF during an acute relapse, they will be found to be elevated, and persistently elevated levels of NO metabolites are linked to clinical progression of the disease [Rejdak et al., 2004].

3. Pathophysiology

MS involves two main steps: (i) myelin sheath damage that results in the lesions of the CNS and (ii) inflammation, both resulting in further destruction of the neural tissue [Dolati *et al.*, 2017; Koriem, 2016]. Damage of oligodendrocytes and destruction of the myelin sheath leads to breakdown of the axon and loss of neuronal function. The axon is the long, slender projection of the neuron that typically conducts

electrical impulses, known as action potentials, away from the nerve cell body. For the axon to function, though, a protective sheath is necessary. The CNS myelin sheath is a lipid-rich multilayered structure, composed of oligodendritic plasma membranes, wrapping around the CNS axons [Aggarwal *et al.*, 2001], insulating them. The myelin membrane is highly compacted, stable and continuous, only interrupted by rich in voltage-gated sodium channels areas, the nodes of Ranvier. The action potential that is generated at the action potential initiation zone region of the soma, and propagated along the axon, jumps rapidly, in a saltatory way, from one node to another, with a high safety factor for transmission, ensuring the fast processing of the information. In the nodes of Ranvier, the sodium channels open, resulting in a sodium influx and current generation. The current, then, moves to the next node, and does not flow out, due to the protective action of the myelin sheath in the internodal segments. The electrical flow is terminated by opening of the potassium channels and subsequent repolarization [Lublin *et al.*, 2008]. The Na+/K+ adenosine triphosphatase (ATPase) in the axon membrane is responsible for ionic balance after high-frequency firing. When myelin is damaged, the axon suffers a range of physiological changes, such as loss of saltatory properties of electrical conduction, reduction in conduction velocity and a predisposition to conduction block [Smith *et al.*, 2006]. Demyelination interrupts the current flow and generation, since the insulator of the internodal current flow is removed, and current cannot flow by the necessary continuous propagation. Due to the naturally low density of internodal Na+ channels, impulse propagation is inhibited. If conduction occurs at a demyelinated axon, it is of reduced speed (5%-10% of normal) and refractory period is prolonged. All these changes that happen to a demyelinated axon are responsible for some observed clinical signs and symptoms of MS. Persistent neurological deficits are on the grounds of large plaques of demyelination, causing conduction block. Transient function deterioration, as observed with increased body temperature in Uhthoff phenomenon, reflects a decrease of the safety threshold for conduction, due to the physiological changes of the partially demyelinated axon [Opara *et al.*, 2016]. Lhermitte phenomenon, that is, the sensation of electric shock down the spine precipitated by neck flexion, can be attributed to generation of de novo action potentials by mechanical stimulation of the axon. Some paroxysmal signs of MS, such as myokymia and trigeminal neuralgia could be explained by spontaneous action potentials from demyelinated axons. Apart from structural changes due to disruption of the insulating membrane, functional

impairment also happens, due to edema and/ or toxic compounds secreted by immunocompetent cells.

4. Immunopathology

One of the crucial stages in MS pathogenesis is the disruption of the BBB. This leads to migration of macrophages, T and B cells, and secretion of pro-inflammatory cytokines and chemokines, inducing inflammation, demyelination and degeneration. The major components of the myelin sheath that are targets of autoreactive T cells and antibodies, are myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG) and proteolipid protein (PLP).

Brain is considered a highly immune-advantaged organ, although a number of studies have challenged this thesis [Hemmer *et al.*, 2006]. Recent research suggests that MS is not only a disease of the immune system, but factors of the CNS are equally involved [Jiang and Kelly, 2011; Bianchini *et al.*, 2017]. That means that microglial cells and macrophages that reside in the CNS are activated after CNS damage. They upregulate MCH I and II molecules and secrete cytokines and chemokines, allowing the entry of CD4 and CD8 T cells, B cells, monocytes, macrophages and dendritic-like (DC) cells into the lesions. These infiltrating cells proceed further in secreting pro-inflammatory cytokines, NO and metalloproteinases, leading to the further destruction of the myelin sheath and consequent axonal damage [Tabarkiewicz *et al.*, 2015; Van Hamburg *et al.*, 2011].

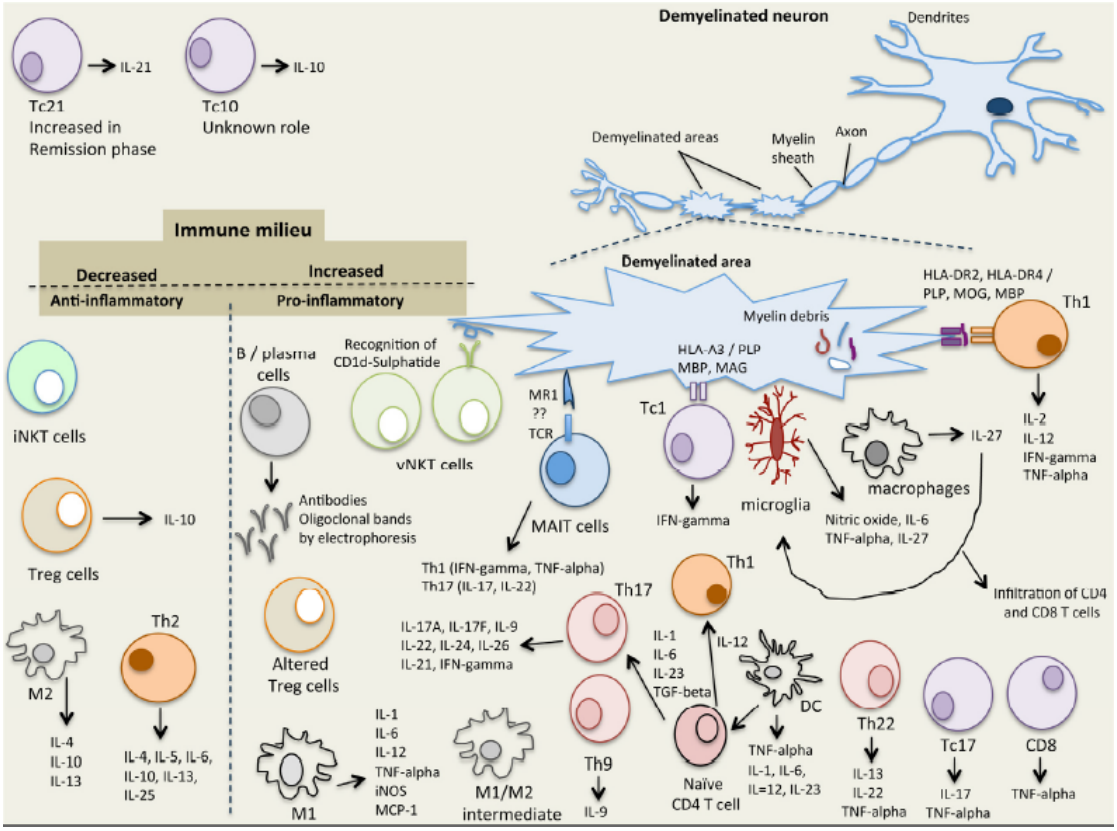

Figure 1. The immune/ cytokine network in Multiple Sclerosis (adapted from Dargahi et al., Multiple Sclerosis: Immunopathology and treatment update, *Brain Sci.* 2017, 7, 78).

4.1 Natural Killer T cells

Natural killer T (NKT) cells is a heterogenous group of T cells, that share properties of both T cells and NK cells. Many of these recognize the non-polymorphic CD1d molecule, an MHC class I-like antigen-presenting molecule that binds self and foreign lipids and glycolipids. Three subsets of NKT cells have been identified (type I, invariant NKT (iNKT) cells, type II, variant NKT (vNKT) cells) and NKT-like cells, with the first two been implicated in the pathogenesis of MS in humans and in animal models of MS (experimental autoimmune encephalomyelitis- EME). iNKT cells express an invariant T-cell receptor (TCR) α chain, forming cell surface markers characteristic of activated or memory T cells (CD25, CD44, CD69) with the majority being CD4+, as well as markers characteristic of NK cells (NK1.1 or CD161, Ly49). iNKT cells are activated via binding to α- Galactosylceramide (αGalCer) -CD1d complex and can impact the type and strength of an immune response, through an array of cytokines that are associated with both pro- and anti-inflammatory immune responses and play a role in both innate and acquired immunity. iNKT cells secrete

interleukin (IL)-4 and IL-13 which stimulate CD4+ T cells to differentiate into anti-inflammatory Th2 cells (IL-4, IL-10 producers) which then inhibit Th17, Th1, CD8+ T cells in the CNS. They also secrete IL-2 and tumor growth factor (TGF)-beta which stimulate the production of T regulatory (Treg) cells (IL-10, TGF-beta producers) which inhibit Th17, Th1 and CD8+ T cells in the CNS. Lastly, they secrete IL-4, IL-10, IL-13, interferon (IFN)-gamma and GM-CSF which activate suppressive myeloid derived suppressor cells (MDCs), DC and macrophages which in turn secrete IL-10 to activate Treg cells and suppress Th17, Th1 and CD8+ T cells in the CNS [Van Kaer *et al.*, 2015]. The pleiotropic properties of iNKT cells, allow them to protect the host against pathogens, tumors, autoimmunity; deficiency or dysfunction of iNKT cells is a risk factor for the development of autoimmune diseases. For proof of that hypothesis, it seems that iNKT cell numbers are decreased in patients with MS and restored when patients enter remission [Gigli *et al.*, 2015]. In the EAE mouse model, high levels of iNKT cells lead to protection for EAE [Mars *et al.*, 2002]. Injections of α-GalCer and analogues have protective action on the mice against autoimmune disorders. These data provide an insight to future iNKT cell based modulating therapies for MS [Van Kaer, 2005, Van Kaer *et al.*, 2011].

vNKT cells recognize α-linked glycolipid antigens in complex with CD1d. They are less common in mice compared to iNKT cells but are more abundant in humans. vNKT cells recognize sulphatide, a self-glycolipid which is expressed within the myelin sheath suggesting a role in MS although not yet established. In animal models with EAE, vNKT cells recognizing sulphatide self-myelin ligand are present in high levels, suggesting their role in disease progression [Jahng *et al.*, 2004].

4.2. Mucosal-Associated Invariant T (MAIT) Cells

MAIT cells are a subset of T cells of the immune system that display innate qualities. They are found in the liver, lungs, mucosa and blood and make up to 25% of CD8 T cells in healthy individuals, defending against microbial activity and infection; they also support adaptive immune responses in that they have a memory like phenotype [Napier *et al.*, 2015]. The MHC class I-like protein, MRI, is responsible for presenting microbial antigens and bacterially-induced vitamin B metabolites to MAIT cells, leading to their activation [Kjer-Nielsen *et al.*, 2012]. MAIT cells can also be activated through MR1 independent signaling. After the presentation of the antigen by

MR1, MAIT cells secrete pro-inflammatory cytokines and they are capable of lysing bacterially-infected cells. However, MAIT cells have also been found at the site of autoimmune attack in diseases such as MS, inflammatory bowel disease and rheumatoid arthritis. It was recently reported that in MS, MAIT cells are highly present at the plaques of demyelination, where they secrete the major cytokines in the pathogenesis of chronic inflammation and autoimmunity (IFN-gamma and TNF-alpha), inducing activation of Th17 cells (IL-17 and IL-22 cytokines) [Bianchini *et al.*, 2017; Abrahamsson *et al.*, 2013]. However, it has been reported that MAIT cells are decreased in blood of patients with RRMS [Miyazaki *et al.*, 2011]. It is yet to be understood whether MAIT cells play a protective or a non-protective role in MS pathogenesis, so as to guide new treatment options.

4.3 Regulatory T cells (Tregs)

Tregs (formerly known as suppressor T cells) are a subpopulation of T cells that modulate the immune system, maintain tolerance to self-antigens and prevent autoimmunity. They are immunosuppressive and generally suppress or downregulate induction and proliferation of effector T cells. Tregs are primarily characterized as Foxp3+CD25+CD4+, they secrete IL-10, and they are thought to be derived from the same lineage as naïve CD4 cells. Because they express CD4 and CD25, they are very difficult to be distinguished from effector CD4+, making them difficult to be studied. The role of Treg in MS regulation was first studied in mouse models with EAE. Adoptive transfer of Treg cells from control mice into MOG or PLP induced EAE mice prevented the onset and progression of EAE [Kohm *et al.*, 2002; Zhang *et al.*, 2004]. When Treg cells from mice recovering from EAE were transferred into MOG-induced active EAE mice, resolution of EAE was achieved [McGeachy *et al.*, 2005]. In MOG-EAE, induction of Treg cells by estradiol or by monocytes under glatiramer acetate treatment reduced clinical signs [Matejuk *et al.*, 2004; Weber *et al.*, 2007]. In patients with MS, the frequency of Foxp3+CD25+CD4+ Treg cells does not seem to differ to those in healthy individuals, however, maturation and migration of these cells is impaired [Zozulya and Wiendl, 2008]. Impaired functionality of Treg cells is primarily observed in the early stages of MS but not in their chronic stage, suggesting a role in the pathogenesis of the disease [Diebold and Derfuss, 2016]. However, it is not clear whether the impaired function of Treg cells is a direct cause of MS or whether such impairment is a common feature of all autoimmune disorders.

4.4 Macrophages and microglia

Macrophages are a type of white blood cell. They engulf and digest cellular debris, foreign substances, microbes, cancer cells, through phagocytosis. They take various forms throughout the body (e.g. Kupffer cells, alveolar macrophages, microglia), but they are all part of the mononuclear phagocyte system. Besides phagocytosis, they play a critical role in innate immunity and they help initiate specific defense mechanisms (adaptive immunity). Macrophages are divided into M1 or M2 based on their pro- or anti-inflammatory cytokine secretion phenotype [Mosser and Edwards, 2008]. M1 macrophage phenotype (CD40+CD86+CD64+CD32+) is induced in the presence of interferon (IFN)-gamma and/or toll-like receptor (TLR) ligands such as lipopolysaccharide (LPS). They are pro-inflammatory and primarily secrete IL-1, IL-6, IL-12, TNF-alpha, iNOS and MCP-1, stimulating adaptive immune responses. The M2 macrophage phenotype (CD163+CD206+) is induced in the presence of IL-4, IL-10, IL-13 and Arg1 that blocks iNOS activity. They are anti-inflammatory and primarily secrete IL-1 receptor antagonist, IL-4, IL-10, transforming growth factor (TGF)-beta1. Macrophages are highly present in active demyelinating and early re-myelinating lesions, compared to inactive, demyelinated or late re-myelinated lesions [Bruck *et al.*, 1996].

Like macrophages, microglia cells are divided into M1- and M2-polarized microglia cells. M1 microglia cells are pro-inflammatory (CD40, CD74, CD86, CCR7), whereas, M2 microglia cells are anti-inflammatory and express mannose receptor (CD206) and CCL22. In an EAE model, suppression of CCL22 lead to reduction of M1 macrophage accumulation in the CNS, thus therapies targeted to suppress CCL22 could potentially decrease demyelination and progression of disease [Miron *et al.*, 2013].

4.5 T helper cells

T helper cells (Th cells) are a type of T cell playing an important role in the adaptive immune system. They help the activity of other immune cells by releasing T cell cytokines, suppressing and regulating immune responses. They are essential in B cell antibody class switching, activation and growth of cytotoxic T cells and in maximizing bactericidal activity of phagocytes (e.g. macrophages). Mature Th cells

express the surface protein CD4 and are referred to as CD4+ T cells. The variable region of the T cell receptor (TCR) they express, has an affinity for Class II MHC. Proteins of Class II MHC are found on the surface of antigen-presenting cells (APC). APC are primarily dendritic cells, macrophages and B cells. The antigens that bind to MHC proteins are short 9–17 amino acid peptides. Proliferating helper T cells that develop into effector T cells differentiate into two major subtypes, known as Th1 and Th2, depending on the cytokine secretion profiles [Apostolopoulos *et al.*, 2016]. Th1 cells are pro-inflammatory, producing high levels of IL-2, IL-12, TNF-alpha and IFN-gamma. Th2 cells are anti-inflammatory and secrete IL-4, IL-5, IL-6, IL-10, IL-13, IL-25. Th17 cells are a subset of T helper cells developmentally distinct from Th1 and Th2 lineages. They are pro-inflammatory cells and secrete high levels of IL-17A, IL-17F, IL-21, IL-22, IL-24, IL-26 and low levels of IL-9 and IFN-gamma. Th22 cells are a new subset of CD4+ T cells, a combination of Th1, Th2, Th17 phenotype, and they secrete IL-13, IL-22 and TNF-alpha, but not IL-17 or IFN-gamma. The newest addition to the Th subset, Th9, was identified for its potent secretion of IL-9. Their role in MS is not yet as clear.

The role of Th1 and Th17 has been studied in MS, where they are believed to increase inflammation within the myelin damage. Th1 cells and their products are present in high levels within the demyelinating axon and CNS lesions, both in humans and in EAE. Th1 cells recognize MOG, PLP and MBP peptide epitopes presented in the context of MHC class II, HLA-DRB1*1501 and HLA-DRB1*04 alleles, leading to their activation. They proceed to crossing the BBB and inducing CNS autoimmunity. Some drug therapeutics are focused on the MHC class II-peptide-TCR complex to modulate Th1 responses to therapeutic Th2 responses. It was recently shown that dimethyl fumarate (DMF) reduced Th1, Th17 and CD8 T cells and increased Th2 cells in RRMS patients [Wu *et al.*, 2017]. Th17 cells play a crucial role in the pathogenesis of MS by inducing an inflammatory milieu. Th17 cells express high levels of CCR6, facilitating their entry through the BBB by binding to the ligand CCL20 on vascular endothelial cells. In fact, IL-17A is present at high levels in CNS lesions, cerebrospinal fluid and in the serum of patients with MS [Volpe *et al.*, 2015]. Based on these data, when AIN457 (Secukinumab) an anti-IL-17A humanized neutralizing monoclonal antibody used primarily for psoriasis and psoriatic arthritis, was injected in MS patients, reduction of lesions was noted, compared to placebo-

treated control subjects [Volpe *et al.*, 2015]. Th22 cells seem to be highly present in the CSF of patients with active RRMS. Furthermore, Th22 cells specifically recognize MBP and are resistant to IFN-beta therapy [Rolla *et al.*, 2014].

4.6 CD8 T cells

CD8 T cells or cytotoxic T cells (Tc1 cells) are T lymphocytes expressing a TCR that recognizes short antigenic 7-9-mer peptide epitopes presented on the surface of APC in complex with MHC class I. More recent classification of CD8 T cells was done based on their cytokine profile. Classical Tc1 cells secrete IFN-gamma, Tc2 secrete IL-4, Tc10 secrete IL-10, Tc17 secrete IL-17, Tc21 secrete IL-21, Tc22 secrete IL-22 and another subset is characterized by secreting TNF-alpha. Although their pathogenic role in MS is still not clear, it has been suggested that there is antigen specificity of CD8 Tc1 cells against MOG, MBP and PLP with cytolytic activity against neuronal cells in vitro [Dresser *et al.*, 1997]. Regardless of the stage and activity of disease, CD8 T cells are noted in high numbers within CNS tissues and CSF, much higher than CD4 T cells at a ratio of 10:1 CD8:CD4 T cells. CD8 T cells that are present in both acute and chronic MS lesions are found to secrete high levels of IL-17 (classed as, Tc17 CD8 T cells) [Tzartos *et al.*, 2008]. Tc17 cells also secrete TNF-alpha, but are negative for granzyme B, perforin and cytolytic activity unlike the classical CD8 Tc1 cells. In peripheral blood of patients with SPMS and RRMS elevated levels of Tc1 and Tc17 cells are noted as well as a high percentage of TNF-alpha secreting CD8 T cells [Salehi *et al.*, 2016]; Tc21 cells are increased in the remission phase of RRMS as opposed to SPMS. Although it is clear that CD8 T cells contribute to the pathogenesis of MS, it is yet unclear how such cells escape T cell tolerance and induce CNS autoimmunity.

4.7 B cells

Although MS is mainly considered a T cell mediated autoimmune disease, B cells also contribute to the pathogenesis of MS where they secrete autoantibodies and cytokines and being APC they activate T cells [Frohman *et al.*, 2006]. A known feature of the inflammatory profile of the CSF in MS patients is the presence of oligoclonal bands (OCB) in over 95% of patients. OCB is a product of clonally expanded B cells and IgG synthesis. It is clear, that abnormal activation of B cells

within the CNS of patients with MS, suggests that B cells play a role in the pathophysiology of the disease. Further studies are required on whether B cell depletion is able to restore immune function and hence, be used as a therapeutic target against MS.

4.8 Dendritic cells (DC)

DCs are the main APC which process and present antigenic peptide epitopes on their surface in complex with MHC class I or class II, resulting in CD4 or CD8 T cell stimulation respectively. Although MS is generally associated with auto-reactive T cells, emerging evidence indicates that DCs play an important role in the pathophysiology of MS, via their T cell activating and cytokine secreting properties. Once activated, DCs activate T cells specific to myelin epitopes, inducing pro-inflammatory cytokines aiding their entry through the BBB into the CNS. In the CNS, further activation of T cells and APC is enhanced, leading to demyelination. In patients with MS, DCs are highly present within inflamed lesions, CSF and in the circulation, producing high levels of TNF-alpha, IFN-gamma and IL-6 [Huang *et al.*, 1999]. In addition, co-stimulatory molecules (CD40 and CD80) that are expressed on DCs are increased in RRMS and SPMS patients, suggesting an activated pro-inflammatory state of DCs, hence their contributing role in the pathogenesis of MS.

4.9 Myeloid derived suppressor cells (MDSC)

MDSC are myeloid progenitors, the same lineage to that of macrophages, DC and neutrophils. However, they are discriminated from other myeloid types in that they have strong immunosuppressive properties rather than immune-stimulatory properties [Kong *et al.*, 2013]. Their major role is in tumor development and chronic inflammation having immune suppressive effects [90]. They interact with other immune cell types, including T cells, dendritic cells, macrophages and NK cells, regulating their functions. In animal models, MDSCs were increased following MBP immunization, acting as suppressive via inhibiting the activation of CD4+ T cells and inhibiting the development of EAE in mice [Wegner *et al.*, 2017]. It is yet unclear whether MDSCs functionality is altered in patients with MS, leading to the failure of MDSCs to suppress autoimmune T cells, as a result of disease progression.

5. Types

There are four distinct types of multiple sclerosis, primarily based on its clinical course, and categorized by increasing severity [Eckstein *et al.*, 2016; Dargahi *et al.*, 2017]. The first type is relapsing/ remitting MS (RRMS), which is the most common type, affecting 85% of all MS patients. It is characterized by clearly defined relapses followed by remission (either full recovery or with residual deficit on recovery). The periods between disease relapses are characterized by lack of disease progression. This type will finally transition to secondary progressive MS (SPMS), which consists of progression with or without occasional relapses and minor remissions. Primary progressive MS (PPMS) affects 8-10% of patients, and it is noted as continuous neurologic deterioration from onset, with occasional plateaus and minor improvements. Progressive relapsing MS (PRMS) is the least common type, affecting less than 5% of patients. It is characterized by progression from onset, with clearly defined acute relapses with or without full recovery. Contrary to RRMS, however, the periods between relapses are characterized by progression of the disease.

Other ways of categorizing the course of MS have also been proposed. In 2013 it was proposed to categorize based on whether there is evidence of activity, and when on progressive forms, whether there is ongoing progression. As a result, a RRMS patient with a relapse or new MRI lesions will be defined as RRMS with activity. A patient with PP or SP MS can be active or not active and progressive or not progressive over a defined period of time.

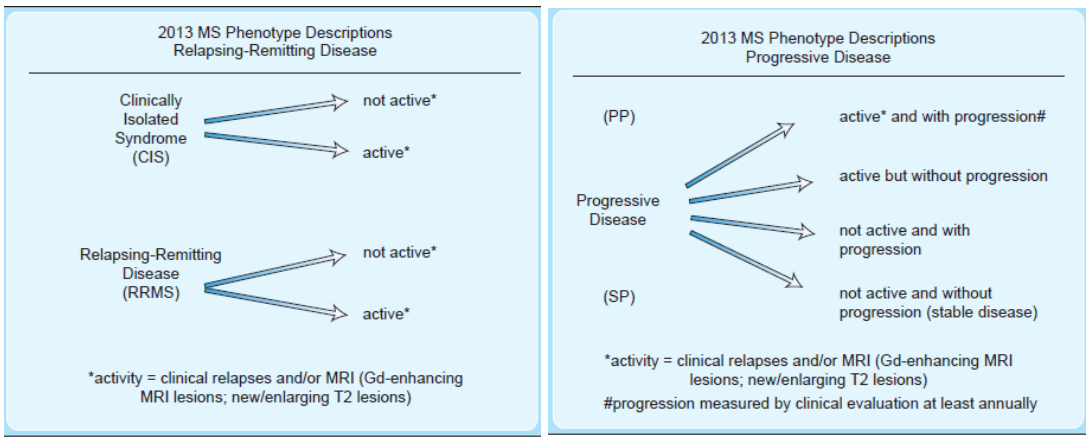

Figure 2. 2013 MS phenotype classification (adapted by Bradley's Neurology in clinical practice, 7th edition, 2016)

Clinically isolated syndrome (CIS) is a monophasic episode of neurological self-reported symptoms and objective findings, reflecting a focal or multifocal inflammatory event in the CNS, developing acutely or subacutely, with a duration of at least 24 hours, with or without recovery, in the absence of fever or infection. This seems identical to the definition of MS relapse; however, CIS occurs in a patient not previously known to have MS, when the 2017 McDonald criteria are not fulfilled. If the patient is later diagnosed with MS, CIS is considered to be the first attack [Thompson *et al.*, 2017].

5.1 Variants of MS

Rarely, patient present with more aggressive clinical or imaging profile, not meeting the criteria of the above-mentioned MS types. A rare MS variant is that of tumefactive MS, with a large (>2 cm) acute demyelinating lesion, sometimes with characteristics of a space-occupying lesion. Often, other supportive imaging criteria are not enough for confirmation that the lesion is in fact in the context of the disease, and a biopsy be the only option.

Marburg variant is another rare MS form, with a relentless, fulminant course. The presentation is multi-focal including encephalopathy, seizures, aphasia and sensorimotor deficits, due to confluent, destructive lesions. Aggressive immunosuppressive treatment is required, due to extremely poor prognosis [Capello et al., 2004].

Balo concentric sclerosis is considered an MS variant, although it could represent a separate demyelinating syndrome. Imaging studies are quite characteristic, with space occupying concentric lesions.

6. Clinical presentation

Optic neuritis (ON), inflammation at any point of the optic nerve, is one of the most common clinical presentations of MS. In fact, it is the most common first manifestation of the disease. The vast majority of the RRMS patients will experience ON at some point during the course of the disease. ON usually manifests as unilateral pain of the eye (specifically during eye movement), followed by a variable degree of visual loss and color vision. Relative afferent pupillary defect (Marcus Gunn pupil) is

also often noted at clinical examination. After an attack of acute ON, 90% of patients regain normal vision, typically over a period of 2 to 6 months. Differential diagnosis includes optic neuropathy, toxic optic neuropathy or neuromyelitis optica, especially in atypical presentation (e.g. acute loss, painless loss of visual acuity).

Ocular motor nerves are infrequently involved, with cranial nerves VI, III, involved more often than IV. More often, eye movement abnormalities reflect lesions of vestibulo-ocular connections and internuclear connections, rather than ocular motor nerves nuclei involvement. Nystagmus is a common finding in MS, the most characteristic being acquired pendular nystagmus, in which there are rapid small-amplitude pendular oscillations of the eyes in the primary position. Internuclear ophthalmoplegia (INO), defined as abnormal horizontal ocular movements with lost or impaired adduction and horizontal nystagmus of the abducting eye, is secondary to a lesion of the medial longitudinal fasciculus on the side of diminished adduction. Convergence is preserved.

Involvement of other cranial nerves is uncommon. There has been some link of trigeminal neuralgia to MS, especially in young individuals, although it remains an uncommon presenting symptom. Facial myokymia, a fine, undulating wavelike facial twitching, and hemifacial spasm can also be observed in MS. Complete hearing loss, usually unilateral, is an infrequent complaint. Vertigo is reported in 30-5% of MS patients. Isolated dysfunction of taste sensation is also rare (McGraw et al., 2012).

Sensory manifestations may be the most often reported MS symptom, also a frequent initial feature of MS. They are present in almost every patient at some time during the course of disease, reflecting spinothalamic or posterior column lesions. The sensory symptoms are commonly described as numbness, tingling, pins and needles, tightness, coldness, itching, or a feeling of swelling of limbs or trunk. Radicular sensations, unilateral or bilateral, can be present, and a bandlike abdominal sensation may be described (“MS hug”). The most frequent sensory abnormalities on clinical examination may be decrease of pain and light touch, impaired vibration and joint position sense trunk.

Impaired motor function is also a common MS manifestation, due to damage of the corticospinal tract. Most patients with weakness develop spasticity to some degree,

and it is one of the chief complaints of MS patients. This can manifest as a feeling of muscle tightness, cramping, and stiffness while walking. An increased spastic tone is identified, usually more marked in the legs than in the arms. The deep tendon reflexes of the affected limbs are exaggerated, sustained clonus may be elicited, and extensor plantar responses are observed. However, reduced reflexes can also be seen, due to hypotonia caused by cerebellar pathway lesions.

Cerebellar lesions lead to gait imbalance, difficulty performing coordinated actions with the arms, and slurred ataxic speech. Examination findings are consistent with cerebellar dysfunction, with nystagmus, ocular dysmetria and frequent refixation saccades suggesting cerebellar or cerebellovestibular connection dysfunction are common. Dysmetria, difficulty in complex and fine movements, and hypotonia, are most often observed in the upper extremities. Ataxic gait can also be identified.

Bladder and bowel dysfunction is also a common symptom in MS, which can cause great distress to the patient. There is a sense that the extent of sphincter and sexual dysfunction often parallels the degree of motor impairment in the lower extremities. The most common complaint related to urinary bladder dysfunction is that of urgency and consequent incontinence, usually the result of excessive detrusor contraction. A dyssynergic voluntary sphincter may also interrupt bladder emptying. With involvement of sacral segments of the spinal cord, however, symptoms of bladder hypoactivity may evolve (e.g., decreased urinary flow, interrupted micturition, incomplete bladder emptying). The atonic dilated bladder empties by overflow, resulting in incontinence. This may lead the patient to self-catheterization. Constipation is very common due to a number of factors; spinal cord involvement, decreased general mobility, dietary issues, and the tendency of some patients to restrict their fluid intake due to urinary urgency and incontinence, are factors that contribute to constipation. Sexual dysfunction, is frequently overlooked, but it occurs in 40%–80% of patients with MS [Schairer et al., 2014]. Complaints may be of erectile dysfunction and trouble maintaining erection. Sexual dysfunction in women manifests as inability to orgasm and decreased libido.

Fatigue is the most commonly reported symptom, and one of the most debilitating. Despite its high prevalence (80%) and significant impact, fatigue is still poorly understood and often under-emphasized because of its complexity and subjective

nature [Braley *et al.*, 2010]. It is described as physical exhaustion unrelated to the amount of activity performed.

Cognitive involvement is also a feature of MS, documented as early as 1877 by Charcot. Almost a century later, it was proposed by Kurtzke in 1981, that only 5% of patients with MS suffered from cognitive impairment. However, the Kurtzke Expanded Disability Status Scale (EDSS) focuses primarily on somatic disability measures. Recent studies report that cognitive involvement is underreported in MS, and that 34% to 65% of patients with MS have cognitive impairment [Nocentini et al., 2006]. Cognitive abnormalities can seriously affect quality of life. The most frequently reported abnormalities are with working memory, attention, and speed of information processing. Patients mention memory loss, difficulties at work or with interpersonal relations, inability to multitask, and "mental fog and fatigue."

Finally, there seems to be some kind of affective disturbance in a significant number of patients with MS. Depression is the most common manifestation, partly due secondary to the burden of coping with a chronic disease. However, it seems to be more prevalent in MS than in other chronic diseases, suggesting an organic component as well. The lifetime risk of major depression in patients with MS is up to 50%, much higher than the general population. Suicide rates are also higher in patients with MS than in the general population or when compared to patients with other chronic illnesses [Bronnum-Hansen et al., 2005]. Emotional "dyscontrol," also known as pseudobulbar affect, is quite common, with patients oscillating frequently between expressing sad and happy states, without clear precipitants.

Early or Common	Later Symptoms	Late or Rare
• Fatigue (70% of cases) and dizziness • Numbness, tingling (paresthesias) • Facial myokymia (irregular twitching) • Optic neuritis • Spasticity and muscle cramping • Depression and mood swings • Visual disturbances (blurred vision, poor contrast or color vision, and pain)	• Charcot triad of dysarthria, ataxia, and tremor • Pain (occurs in 30%-50% of patients at some time) • Bladder, bowel, and sexual dysfunction • Bilateral facial weakness or trigeminal neuralgia • Heat intolerance • Subjective cognitive difficulties • Symptoms associated with partial acute transverse myelitis (pain, paresthesias, limb weakness or paralysis)	• Bipolar disorder or frank dementia • Euphoria • Aphasia or dysphasia • Seizures

Table 1. Classic multiple sclerosis symptoms

Clinical features suggestive of multiple sclerosis
Onset between ages 15 and 50
Involvement of multiple areas of the CNS
Optic neuritis
Lhermitte sign
Internuclear ophthalmoplegia
Fatigue
Worsening with elevated body temperature

Table 2. Clinical features suggestive of MS (adapted from Bradley's Neurology in Clinical Practice, 7th edition, 2016)

7. Diagnosis of MS

7.1 Imaging features of MS

MRI is the preferred imaging method for the diagnosis and follow-up of MS patients. In T1 sequences, lesions are isointense or hypointense (T1 black holes), indicating the chronic stage of the white matter destruction. Lesions appear hyperintense in T2 weighed sequences and they might have surrounding edema. FLAIR sequence is more sensitive in detection of juxtacortical and periventricular plaques, and lesions appear hyperintense. With the administration of contrast, active lesions are enhanced with an "open ring" pattern, indicating inflammatory component. One characteristic of MS lesions is that they may demonstrate decreased diffusion in DWI. The location of the lesions can be anywhere in the CNS. A relatively specific radiological MS sign is that of Dawson fingers, with plaques arranged at right angles to the lateral ventricles along medullary veins, presenting as T2 hyperintensities.

7.2 Diagnostic criteria

The McDonald criteria, the diagnostic criteria for MS, combine clinical, imaging and laboratory evidence for the diagnosis of MS. The latest revision was of 2017, with a few changes compared to that of 2010. The recent criteria are described at Table 3. If the number of clinical events does not suffice for the diagnosis, then imaging criteria must be met. Dissemination in time, which would otherwise clinically be set in case of more than one attacks, can be demonstrated by the simultaneous presence of inactive and active lesions (based on gadolinium enhancement), or a new lesion compared to a baseline scan. Dissemination in space can be demonstrated by one or

more lesions in two or more of four areas of the CNS: periventricular, cortical/ juxtacortical, infratentorial brain regions, and anywhere in the spinal cord.

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

Table 3. The revised 2017 McDonald criteria for MS diagnosis (adapted by Thompson et al., 2017].

By the addition of OCB in the diagnosis of MS, few patients previously considered to fall in CIS category might now be considered definite MS.

The criteria for diagnosis of PPMS apply to patients with one year of disability progression, independent of clinical relapse. Two more criteria need to be met: (i) one or more T2-hyperintense lesions characteristic of MS (periventricular, cortical, juxtacortical, infratentorial); (ii) two or more lesions in the spinal cord; (iii) presence of OCB.

Special mention should be made to radiologically isolated syndrome (RIS), which is a diagnostic entity based on imaging and not clinical data. Typically, patients that fall under this category are asymptomatic patients, undergoing MRI for an unrelated reason (e.g. accident, headache syndrome) and anomalies highly suggestive of MS are detected. A study found that risk for a clinical event within 5 years is 34% (Okuda *et al.*, 2014]. Patients with a higher risk are those with enhancing lesions and spinal cord lesion. Whether these patients should start disease-modifying treatment requires further study.

7.3 CSF profile

CSF findings are supportive in diagnosing MS, important in atypical clinical syndromes, atypical or non-diagnostic MRI findings, or unusual clinical manifestations such as a course of progressive neurological impairment without

history of relapses. Grossly, CSF does not show any abnormalities; it is clear, colorless, and has a normal opening pressure. Cell counts may be slightly elevated in 15% to 20% of patients. The predominant cell type is T lymphocytes. Determining the presence of OCB is the most important diagnostic test. However, 10% to 20% of patients with confirmed CDMS do not have OCBs at any given point in time. Presence of OCBs in a patient with CIS has been consistently reported to be an independent strong predictor of the risk of conversion to clinical definite MS. For this reason, in the latest revised McDonald criteria, presence of CSF bands can substitute for the requirement of DIT, if clinical or MRI criteria of DIS are fulfilled [Thompson *et al.*, 2017]. Myelin basic protein (MBP) in the CSF is a marker of tissue damage and has been used as a measure of CNS myelin breakdown. An abnormality in CSF IgG production (as measured by the IgG index) is found in over 90% of patients with confirmed MS.

7.4 Electrophysiology studies

The three most commonly used EPs are VEPs, somatosensory evoked potentials (SSEPs), and brainstem auditory-evoked responses (BAER). VEPs are thought to be useful to determine increased risk for MS (Gronseth and Ashman, 2000). P100 wave prolongation is detected in over 90% of patients with a history of optic neuritis, even in a setting of complete restoration of vision.

8. Current treatments for MS

8.1 Treatment of relapses

MS relapses are typically defined as a new or worsening neurological deficit that lasts for 24 hours or more, in the absence of fever or infection. It is important to rule out certain conditions that could lead to so-called pseudo-exacerbations, which include fever and infections (most commonly urinary tract and upper respiratory tract infections), along with stress and heat exposure. Relapses are often associated with significant impairment and decreased quality of life. The unpredictability of MS exacerbations further compounds the impact on quality of life [Lublin *et al.*, 2003]. Relapses represent either the formation of a new demyelinating lesion or active inflammation of any previously existing lesion in any segment of the CNS [Frohman *et al.*, 2007; Repovic *et al.*, 2011]. The natural course of an MS exacerbation is

usually completed with resolution of the neurologic deficit, leading to clinical remission. However, deficit may persist and contribute to a stepwise progression of disability [Berkovich, 2012]. Patients with MS who present with a relapse are generally treated with corticosteroids intravenously, plasma exchange or adrenocorticotropic hormone injections (ACTH).

8.1.1 Corticosteroids

Intravenous methylprednisolone (IV-MP) was the second and last medication approved by FDA for the treatment of MS relapse, after ACTH. Mechanism of action of corticosteroids has been attributed to the immunologic alterations that they cause. One of them is the reduction of B-cell counts and their circulation at inflammatory sites, which could result in a decreased intrathecal IgG synthesis [Durelli *et al.*, 1986] and consequent reduction of the BBB abnormally increased permeability. Several studies were conducted in the 80's and early 90's to compare IV-MP to ACTH and placebo that proved the effectiveness of IV-MP. The dosages and route of administration of IV-MP in these studies differed: they were from as low as 40 mg/day [Milanese *et al.*, 1989] to 500 mg [Milligan *et al.*, 1987], then to 15 mg/kg/day IV [Durelli *et al.*, 1986] and finally to 1 g a day [Thomson *et al.*, 1989; Beck *et al.*, 1992]. Since the low dosages were found to be ineffective, the dosages from 500 mg to 1 g per day are now widely accepted as the preferred medical treatment. The length of treatment was also defined from these studies from the previous long 30-35 days to shorter courses of 3-7 days [Frohman et al., 2007; Repovic et al., 2011; Miller et al., 2000; Thompson et al., 1989; Beck et al., 1992]. Although the IV-MP was first proposed in a study by Barnes *et al*. in 1985, the Optic Neuritis Treatment Trial published in 1992 was the first study on a large cohort to propose the use of 1 g IV-MP for 3 days, followed by oral prednisone (1 mg/kg/day for 11 days) with significant results [Beck *et al.*, 1992]. The latest guidelines by the European Academy of Neurology (2014) suggest either treatment with 0.5 g of oral methylprednisolone for 5 days, or 1 g of IV-MP for 3-5 days in patients with underlying comorbidity (diabetes, depression) or failure of oral steroids. Latest studies suggest that oral MP is not inferior to IV-MP as to clinical improvement after 28 [Le Page *et al.*, 2015] and 30 days [Liu *et al.*, 2017] after administration, with similar safety profile.

Short-term use of steroids has been associated with only relatively minor side effects. The most frequently reported side effects are GI symptoms, weight gain, edema, mood swings, anxiety, dysphoria, anxiety, insomnia, palpitations, acne and headache [Frohman *et al.*, 2007; Repovic *et al.*, 2011; Miller *et al.*, 2000]. Among adverse effects concerning the musculoskeletal system, osteoporosis develops in at least 50% of patients required to be on long-term corticosteroid treatment [Lukert *et al.*, 1990]. In fully ambulatory MS patients, however, requiring few pulses, bone density was not affected [Schwid *et al.*, 1996]. Among psychiatric side effects, insomnia has been reported by approximately 50% of patients after pulse or oral MP. Risk for psychosis is higher within the first days of initiating treatment, and women are more prone [Fardet *et al.*, 2007]. Due to immunosuppressive effects of corticosteroids, patients are more susceptible to infections [Martinelli *et al.*, 2009; Fardet *et al.*, 2007].

8.1.2 ACTH

ACTH is a 39-amino-acid polypeptide secreted by the anterior pituitary and stimulating the adrenal cortex to secrete a number of different hormones, with a variety of physiological effects. The primary physiological and pharmacological effect of ACTH results from the secretion of adrenal cortical steroids. ACTH gel has been used for several decades for treatment of acute exacerbations of MS, with an established safety profile. Side effects that may occur with the use of ACTH are primarily due to its steroidogenic effects and are similar to corticosteroids. There may be increased susceptibility to infections or reactivation of latent infections. After prolonged treatment adrenal insufficiency may occur, along with Cushing's syndrome, hypokalemia, salt and water retention and elevated blood pressure. Psychiatric symptoms consist of euphoria, irritability, mood swings, insomnia, depression, personality changes and psychosis [Questcor Pharmaceuticals, 2011]. In patients with diabetes and myasthenia gravis, ACTH should be used with caution, since prolonged use may predispose to cataracts, ocular infection or glaucoma.

8.1.3 Second line of treatment

There are patients who do not respond to corticosteroids or ACTH during a relapse. Several alternatives have been proposed and studied, including plasmapheresis [Keegan *et al.*, 2002, Keegan *et al.*, 2005], cyclophosphamide [Barile-Fabris *et al.*,

2005; Greenberg *et al.*, 2007], intravenous IgG (IV-IG) [Noseworthy *et al.*, 2001; Tselis *et al.*, 2008; Visser *et al.*, 2004; Sorensen *et al.*, 2004; Roed *et al.*, 2005] and natalizumab [O'Connor *et al.*, 2004]. Plasmapheresis is the treatment supported by strong clinical evidence, and the one recommended by the latest American Academy of Neurology guidelines as a secondary treatment for severe flares of RRMS [Cortese *et al.*, 2011].

8.2 Long-term treatment for MS with disease-modifying agents

Drug	Brand	Dose	Number of of Injections, Route	Actions
IFN-β1a	Avonex®	7.5 mg 1st dose 15 mg 2nd dose 22.5 mg 3rd dose 30 mg all subsequent doses	1/week, i.m	Balances pro- and anti-inflammatory cytokines Decreases Th17 cells Decreases IL-17
	Rebif®	22 mg or 44 mg	3/week, s.c	
IFN-β1b	Betaseron®	62.5 mg and increase over 6 weeks to 250 mg	1/2 days, s.c	
	Extavia®	62.5 mg and increase over 6 weeks to 250 mg	1/2 days, s.c	
pegIFN-β1a	Plegridy®	63 mg 1st dose 95 mg 2nd dose 125 mg all subsequent doses	1/2 weeks, s.c	
Glatiramer acetate, EKAY	Copaxone®	20 mg or 40 mg	1/day, s.c 3/week, s.c	Blocks pMHC
Dimethyl fumarate	Tecfidera®	240 mg	2–3/day, oral	Anti-inflammatory Anti-oxidative stress
Teriflunomide	Aubagio®	7 or 14 mg	1/day, oral	Inhibits dihydroorotate dehydrogenase, T, B cells and IFN-γ secreting T cells
Fingolimod	Glenya®	0.5 mg	1/day, oral	Antagonist of SIP receptor Decrease T, B cells activates SIP signaling in CNS
Mitoxantrone	Novatrone®	12 mg/m^2	1/3 months up to 2 years	Suppresses T, B cells and macrophages. Reduces Th1 cytokines
Dalfampridine	Ampyra®	10 mg	2/day, oral	Potassium channel blocker Improves motor symptoms, i.e., walking
Humanized Monoclonal Antibody Treatments				
Natalizumab	Tysabr®	300 mg	1/28 days, i.v	Humanized anti-α4-integrin Mab. Affects cell migration, division, growth and survival
Ofatumumab	Arzerra®	3–700 mg	1/2 weeks, i.v	Humanized anti-CD20 Mab. Cytotoxic to CD20+ cells via CDC and ADCC
Ocrelizumab	Ocrevus®	300–600 mg	300 mg weeks 1 and 3, then 600 mg 1/6 months, i.v	Humanized anti-CD20 Mab
Alemtuzumab	Lemtrada®	12 mg	5 days in a row; after 1 year, 3 days	Humanized anti-CD52 Mab. Depletes T, B cells, increases Treg, Th2, decrease Th1 cells
Daclizumab	Zinbryta®	150 mg	1/month, s.c	Humanized anti-CD25 Mab.Blocks IL-2R, decreases T cells, increases NK cells

ADCC, antibody-dependent cellular cytotoxicity; CDC, complement-dependent cytotoxicity; DC, dendritic cells; EKAY, single amino acid code for L-glutamic acid, lysine, alanine, tyrosine; IFN, interferon; IL-2R, interleukin-2 receptor; i.m, intramuscular; i.v, intravenous; Mab, monoclonal antibodies; NK, natural killer cells; pegIFN, polyethylene glycol linked to IFN; pMHC, peptide-major histocompatibility complex; RRMS, relapsing remitting multiple sclerosis; s.c, subcutaneous; SIP, sphingosine-1-phosphate; Th, helper T cells; Treg, regulatory T cells ($CD4^+CD25^+FoxP3^+$).

Table 4. Disease-modifying drugs for RRMS patients (adapted from Dargahi et al., Multiple Sclerosis: Immunopathology and treatment update, Brain Sci. 2017, 7, 78).

For an extended amount of time, the only available treatment options have been limited to corticosteroids, cyclophosphamide and methotrexate. However, in mid-1990s, a big shift was carried to treatment options for the first time [Diebold *et al.*, 2016]. Disease-modifying agents target to modification of the disease course rather than improvement of the symptoms and they have been shown to reduce rate of relapses, MRI lesions and stabilize or delay MS disability. Until the approval of the first oral treatment in 2010 [Eckstein *et al.*, 2016], all MS treatments consisted of either intramuscular or subcutaneous injectable drugs. The first breakthrough disease-modifying drug for RRMS was interferon beta-1(IFNβ-1) [Kappos *et al.*, 2006, 2007]. To date, 13 FDA approved disease-modifying drugs are available for RRMS, and several more agents are in different developmental stages.

8.2.1 Interferons

Interferon (IFN) type 1 consist of a group of IFNs (IFN-α, -β, -ε, -κ, -τ, -δ, -ζ, -ω, -ν) which help regulate the immune system. IFN-β is primarily produced by fibroblasts but also by other cells such as NK cells, B cells, T cells, macrophages. IFN-β has being shown to be effective in reducing the relapse rate in patients with MS [Shirani *et al.*, 2012], through multiple actions. It balances the expression of pro- and anti-inflammatory cytokines in the brain and decreases the number of inflammatory cells crossing the blood brain barrier, leading to decreased inflammation of neurons and consequent improved neuronal survival. Moreover, IFN-β reduces Th17 population and IL-17 cytokine which, as mentioned previously, are known to be involved in the immunopathophysiology of MS [Mitsdoerffer *et al*., 2009].

IFN-β are administered subcutaneously or intramuscularly aiming to decrease the relapse rate, duration and severity, however, there is lack of solid proof for efficacy to long-term disability. Avonex was the first IFN-β injection RRMS treatment approved by FDA in 1996, after a large scale human clinical trial published in 1993, which showed that relapses rates were reduced by 34% in high dose and by 8% in lower dose and the severity of relapses was reduced. 5-year follow-up showed a 30% decrease in lesions and 50% reduction in formation of new lesions [The IFNB Multiple Sclerosis Group, 1993]. To date there are 3 approaches using IFN-β; IFN-

β1a low dosage (Avonex®), IFN-β1a (Rebif®) high dosage, and, IFN-β1b (Betaseron®, Extavia®) high dosage. Furthermore, pegIFN-β-1a (Plegridy®) has polyethylene glycol linked to IFN-β-1a allowing it to be active for longer in the body, hence fewer injections are required compared to the other treatment options. Although IFNs have no direct neuroprotective effects, their direct effect on CD4+Th1 cells and alteration of their profile results in decreased demyelination of neurons, which prevents further neuronal damage [Yong *et al.*, 2007]. Despite the proved impact of IFN-β in disease progression in RRMS there are limitations in their use, due to their side effects, which leads many patients to stop treatment. The most common is flu-like syndrome within the first 1-2 days following injection and skin reactions at the injection site. A rare but very serious skin reaction is cutaneous necrosis at site of injection for subcutaneous administration. Other symptoms include local body aches, suicidal thoughts, hallucinations, seizures and heart and liver problems [Katsara *et al.*, 2008].

8.2.2 Glatiramer Acetate

Glatiramer acetate (GA) is a synthetic 4-mer peptide (L-glutamic acid, lysine, alanine, and tyrosine) mimic of MBP, which competes with short antigenic MBP peptides in complex with MHC class II. GA was designed initially for the model of EAE, as an inducer, but instead it was proved to suppress EAE. This which quickly led to human trials with MS in order to prevent disease progression, as it bound to MHC class II and inhibited the activation of encephalitogenic T cells [Wolinski *et al.*, 2007, Ragheb *et al.*, 2001]. GA induces diversion of Th1 cells to Th2 cells leading to suppression of inflammatory responses and activation of Tregs in the periphery [Haas *et al.*, 2009]. In clinical trials, GA significantly reduced disease symptoms and development of new lesions by up to 30% in RRMS, although it showed no improvement in long-term efficacy on progression of disability [Johnson *et al.*, 1995]. Potential side effects range from minor (fever, chills) to more serious (cardiovascular, digestive, muscular, respiratory issues).

8.2.3 Fingolimod

Fingolimod was the first oral therapy (0.5 mg once daily) available for patients with RRMS. Fingolimod is a sphingosine 1-phosphate (S1P) receptor modulator, which

acts as a super agonist of S1P receptor causing receptor internalization and leading to reduced infiltration of potentially auto-reactive lymphocytes into the CNS, keeping them localized in the lymph nodes [Mandal *et al.*, 2002; Matloubian *et al.*, 2004]. As a secondary action, it targets SIP receptors on glia cells in the CNS, activating signaling pathways within the CNS [Brinkmann *et al.*, 2002; Choi *et al.*, 2011]. Phase III human clinical trials in patients with RRMS proved that fingolimod was more effective compared to first line treatment IFN-β-1a and placebo, in reducing the frequency of flare-ups, disability progression, MRI outcome measures, including brain volume loss [Calabresi *et al.*, 2014; Cohen *et al.*, 2010; Kappos *et al.*, 2010]. One of its side effects is bradycardia and 1^{st} /2^{nd} degree AV block (within 6 h after treatment initiation). For that reason, it requires cardiac monitoring protocol after the first dose, for at least 6 hours. If patient misses one dose within the first 2 weeks of treatment, first-dose protocol needs to be repeated. The same applies for discontinuation for more than 14 days after the first month of treatment. Other side effects include blurred vision, diarrhea, back pain, headache, cough and vomiting.

It should be noted as for 2015, three confirmed cases of progressive multifocal leukoencephalopathy (PML) had been reported in patients receiving fingolimod, without having received natalizumab. 17 suspected cases of PML were also reported in patients previously treated with natalizumab. PML is a rare but severe and life-threatening subacute evolving infectious disease of oligodendrocytes and astrocytes caused by a mutating, neurotrophic strain of John Cunningham (JC) virus. It is estimated that almost 60% of the population of Europe has been affected by JCV [Bozic *et al.*, 2014], thus, PML is considered an opportunistic infection exclusively associated with immunosuppression [Hunt *et al.*, 2012; Sørensen *et al.*, 2012]. This forced European Medicines Agency (EMA) to propose new guidelines for initiation of treatment with Gilenya (EMA, 2015). A baseline brain MRI is required within 3 months prior to initiation as a reference and extra caution should be given for early signs and symptoms of PML.

8.2.4 Siponimod

Siponimod is a sphingosine 1-phosphate (S1P) receptor -1 and -5 modulator, with peripheral and central effects. It acts in a similar way as Fingolimod, binding on autoreactive lymphocytes and preventing them from crossing the BBB. It is targeted

for use in SPMS, where therapeutic options are limited. First data from phase III clinical trial EXPAND revealed a decreased 3-month risk for clinical deterioration and progression by 21% compared to placebo [Kappos *et al.*, 2017]. It is not yet approved by FDA or EMA, but it is planned to be submitted as a SPMS in the first half of 2018.

8.2.5 Dimethyl Fumarate

Dimethyl fumarate (BG-12) is a methyl ester of fumaric acid that modulates immune responses. It was approved by the FDA in 2013, after being shown in phase III clinical trials to reduce relapse rate and increase the time to disability progression in patients with RRMS [Gold *et al.*, 2012]. It acts by reducing the migration of inflammatory cells through the blood brain barrier and activating nuclear factor erythroid 2-related factor (Nrf2) [Moharregh-Khiabani *et al.*, 2009]. This factor regulates anti-oxidative proteins, protecting cells against oxidative damage and inflammation, by increasing glutathione levels and suppressing pro-inflammatory cytokines from splenocytes in vitro [Albrecht *et al.*, 2012]. Side effects of BG-12 include diarrhea, abdominal pain, nausea, abnormal liver enzymes and decreased lymphocyte counts. There is only one case report of PML while on Tecfidera.

8.2.6 Teriflunomide

Teriflunomide was approved by FDA in 2012 for use in RRMS. It is an active metabolite of leflunomide (an immunosuppressive disease-modifying drug used for rheumatoid arthritis) which inhibits the proliferation of B and T cells [Palmer *et al.*, 2010]. It also inhibits IFN-gamma producing T cells while IL-4 and IL-10 producing T cells are unaffected [Korn *et al.*, 2004]. In clinical trials oral administration reduced relapse rates, MS lesions and disability progression [O'Connor *et al.*, 2011; Sanvito *et al.*, 2011; Confavreux *et al.*, 2014; Vermersch *et al.*, 2014]. Moreover, it was better tolerated compared to IFN-β-1a, with less permanent discontinuation due to adverse reactions. Side effects include, reduced white blood cell count, alopecia, hepatic effects, nausea, diarrhea, numbness in hand and feet, allergic reactions, breathing issues and increased blood pressure.

8.2.7 Mitoxantrone

Mitoxantrone is primarily used for certain types of cancer, such as non-Hodgkin's lymphoma and acute myeloid leukemia. It is a type-II topoisomerase inhibitor, which disrupts DNA synthesis and DNA repair of cancer cells, but also a potent immune suppressant, suppressing T cells, B cells and macrophages and reducing pro-inflammatory cytokines [Huang *et al.*, 2012]. In patients with SPMS, intravenous injection of 12 mg/m2 mitoxantrone every 3 months up to 2 years resulted in reduced disability progression by 84% [Hartung *et al.*, 2002]. However, due to several side effects such as nausea, vomiting, hair loss, cardiotoxicity, leukemia, infertility, infection, leukopenia and thrombocytopenia [Eckstein *et al.*, 2016], its use has significantly been reduced over time.

8.3 Humanized monoclonal antibodies

8.3.1 Natalizumab

Natalizumab is a humanized monoclonal antibody against the cellular adhesion molecule α4-integrin. Integrins are transmembrane receptors on T cells, B cells, monocytes, macrophages, NK cells DC, neutrophils and eosinophils. They enable cell-extracellular matrix adhesion activating cell signaling that regulates cell growth, division, survival, differentiation and migration. By blocking the interaction between α4-integrin and vascular endothelial adhesion molecule-1, natalizumab inhibits transendothelial migration to the CNS [Sheremata *et al.*, 2005]. It is administered intravenously once a month [144], and by reducing activated T cells within the CNS, it enhances anti-inflammatory responses and neuroprotective effects [Yong *et al.*, 2007]. In a phase III clinical trial natalizumab reduced brain lesions and the rate of disability progression up to 24 months [Klotz *et al.*, 2011; Jarius *et al.*, 2003; Miller *et al.*, 2003]. Natalizumab was firstly approved in 2004, but it was later withdrawn due to 3 cases of PML. It was re-introduced in 2006 after an extensive risk management plan. However, by 2012 a further 212 cases (or 2.1/1000) of PML were reported to be attributed to natalizumab. The risk for PML while on natalizumab can be stratified based on three main risk factors: anti-JCV antibody positive status, duration of natalizumab treatment and prior use of immunosuppressants [Fernandez, 2013].

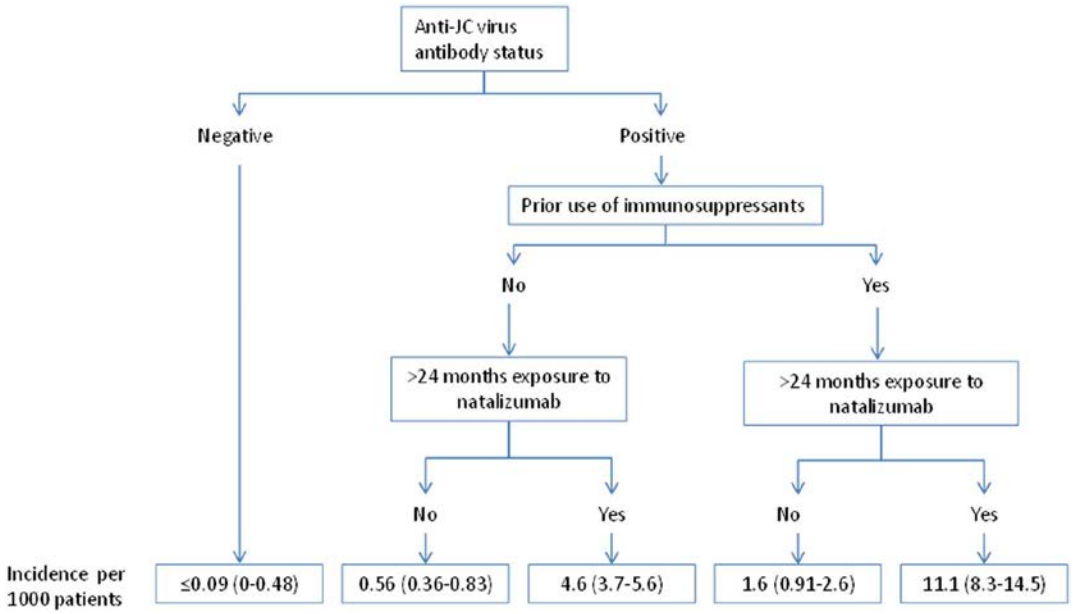

Figure 3. Stratification of PML risk in natalizumab-treated patients (adapted from Fernadez 2013)

Current guidelines suggest close monitoring both clinically and with MRI, so as to be able to detect PML as early as possible. Even in seronegative patients, seroconversion to positive status can occur (2% per year) and the test can give false negative results (2.5%) [Gorelik *et al.*, 2010]. Thus, JCV testing should be performed every 6 months. After 24 months of treatment, patient's situation and continuation of treatment should be carefully reassessed, due to known association between duration of treatment and risk of developing PML. The decision should be taken jointly by the patient and treating neurologist [Fernandez *et al.*, 2011].

Index result	PML risk estimates per 1000 patients (no prior IS use)		
	1–24 months (95% CI)	25–48 months (95% CI)	49–72 months (95% CI)
≤0.9	**0.1** (0–0.41)	**0.3** (0.04–1.13)	**0.4** (0.01–2.15)
≤1.1	**0.1** (0–0.34)	**0.7** (0.21–1.53)	**0.7** (0.08–2.34)
≤1.3	**0.1** (0.01–0.39)	**1.0** (0.48–1.98)	**1.2** (0.31–2.94)
≤1.5	**0.1** (0.03–0.42)	**1.2** (0.64–2.15)	**1.3** (0.41–2.96)
>1.5	**1.0** (0.64–1.41)	**8.1** (6.64–9.8)	**8.5** (6.22–11.38)

PML risk estimates for anti-JCV antibody index thresholds were calculated based on the current PML risk stratification algorithm (from September 2012) and predicted probabilities shown in Table 1 for the population at or below that particular index (0.9–1.5) and for the population above an index of 1.5. For index thresholds below 0.9, patient numbers were insufficient to allow for calculation of risk estimates.

Table 5. PML risk estimates by index threshold in anti-JCV antibody positive patients (adapted from Plavina et al., 2013)

Other side effects include hepatotoxicity, allergic reactions and increased risks of infection.

8.3.2 Alemtuzumab

Alemtuzumab is a humanized monoclonal antibody against CD52, a cell surface molecule expressed on B and T cells; mature NK cells, plasma cells, neutrophils and importantly, hematological stem cells do not express CD52. It was approved by FDA in 2014 for use in RRMS patients who have used two or more disease-modifying agents that failed. In phase III clinical trials in patients with RRMS, alemtuzumab showed decreased relapse rates and MRI findings (gadolinium-enhancing lesions, new or enlarging T2 lesions and brain atrophy) and were free of clinical disease longer, compared to IFNβ-1a [Cohen *et al.*, 2012; Coles *et al.*, 2012]. First admission consists of a 5-day course, followed by an anamnestic 3-day course a year later. There is not adequate data for administration more than twice; however, decision relies on clinician's opinion and patient's response to treatment. Alemtuzumab can cause serious side effects including, immune thrombocytopenia, kidney problems, serious infusion problems (trouble breathing, swelling, chest pain, irregular heart beat), certain cancers (blood cancers, thyroid cancer), cytopenia and serious infections, thus, increased vigilance is required by the clinician.

8.3.3 Ocrelizumab

In March 2017, ocrelizumab was the first drug to be approved by the FDA for use in PPMS. Phase IV clinical trials were a requirement of the FDA to be conducted in order to determine the safety of ocrelizumab in younger patients with MS, ie, risk of cancer and effects on pregnancy (study outcomes due by 2024); although clinical trials in patients with lupus and rheumatoid arthritis were halted since high rates of infections and increased risk of progressive multifocal leukoencephalopathy were observed. In addition, in patients with MS, there was an increased risk of breast cancer (6/781 females with MS on ocrelizumab compared to 0/668 females with MS in other trials) [US FDA, 2017].

8.3.4 Daclizumab

Daclizumab is a humanized monoclonal antibody against CD25, the IL-2 receptor expressed on the surface of T cells. It blocks the IL-2 receptor on T cells, preventing the activation of T cells. It is injected subcutaneously in RRMS patients, once a month. In human clinical trials, daclizumab showed 45% reduced annualized relapse rates and 54% lower in the number of new lesions [Lycke *et al.*, 2015]. However, new guidelines in 2017 require high clinical vigilance, due to severe hepatic injury.

8.3.5 Ofatumumab

Ofatumumab (OMB157) is the first fully human type 1 IgG1 kappa (IgG1κ) monoclonal antibody, licensed for the treatment of patients with chronic lymphocytic leukemia. It is currently also being developed for the treatment of MS. Ofatumumab binds on CD20, a marker present on the cell surface of B cells, inducing B-cell depletion via complement dependent cytotoxicity and antibody-dependent cell-mediated cytotoxicity causing B cell apoptosis [Blecker *et al.*, 2008]. It is currently on phase III clinical trial for patients with RRMS.

8.4 Emerging immunotherapeutic strategies against MS

Stem cells are unspecialized cells in the body that retain the ability to generate cells of undifferentiated state identical to themselves, or of differentiating into other types of body cells with specialized functions [Robey, 2000]. There are various types of stem cells such as embryonic stem cells (ESCs), hematopoietic stem cells (HSCs), neural stem cells (NSCs), mesenchymal stem cells (MSCs) and induced pluripotent stem cells (iPSCs) [13]. HSCs are isolated either from the bone marrow, umbilical cord blood or peripheral blood. Although they are more commonly used for hematological malignancies (e.g. leukemia, multiple myeloma) its application has also expanded into autoimmune diseases. The first report was in 1997 in a myelogenous patient with MS, which showed marked improvements in MS brain lesions [McAllister, 1997] quickly led to the use of HSC transplantation (HSCT) in MS patients with active RRMS, with a consequent reduced progression in about 70% of patients, decrease in number of relapses and inflammatory MRI activity [Mancardi *et al.*, 2008]. Candidates for this choice of treatment are these who have not responded to conventional therapy, whose disease is aggressive with relapsing-remitting course and who are not presenting with

high level of disability [Sormani *et al.*, 2017]. However, the clinical efficacy of HSCT long term has not been established. The mechanism by which HSCT works is that HSCT are believed to reboot the immune system, preventing further inflammation.

MSCs are isolated from an adult's bone marrow, are differentiated in vitro for 2–3 weeks and re-injected back into the patient. Most studies of MSCs in MS are in mice and EAE models, and more recently in human clinical trials. In the EAE mouse model of multiple sclerosis, MSCs systematically injected at disease onset ameliorates myelin oligodendrocyte glycoprotein (MOG)-induced EAE, and decreases the infiltration of T-cells, B-cells and macrophages into the brain and spinal cord [Zappia *et al.*, 2005]. In humans, in a phase II randomized double-blind, placebo-controlled crossover clinical trial showed lower mean cumulative number of lesions in patients receiving MSCs compared to placebo [Llufriu *et al*., 2014], with no serious adverse events. The mechanism of action of MSC includes immunomodulation, neuroprotection and neuroregeneration [Yamout *et al*., 2010]. MSCs have been reported to induce the production of IL-10 by peripheral DCs, which, in turn, trigger the generation of Tregs [Maccario *et al.*, 2005; Aggarwal *et al.*, 2005]. Tregs are believed to play an important role autoimmunity regulation by maintaining tolerance to self-antigens. As mentioned above, their function seems to be impaired in MS patients. The use of MSCs that reduce MRI parameters is a new and emerging research focus to develop new improved treatments for MS.

Other treatment options that have been studied were DNA vaccine (BHT-3009), nanoparticles (polymeric biodegradable lactic-glycolic acid, poly-ε-caprolactone), altered peptide ligands, cyclic peptide and mannan [Dargahi *et al.*, 2017].

8.5 Symptomatic management

Symptom	Management Options
Bladder dysfunction	• Oxybutynin • Solifenacin • Darifenacin • Trospium • Hyoscyamine • Fesoterodine • Propantheline • Dicyclomine • Desmopressin acetate • Prazosin, tamsulosin • Botulinum toxin • Catheterization • Mirabegron
Constipation	• Fluid intake: 8-10 cups • Dietary fiber: 15 g daily • Stool softeners, bulk formers, or laxatives
Fatigue, cognitive issues, and depression	• SSRIs • SNRIs • Amantadine • Modafinil • Methylphenidate • Dextroamphetamine
Paresthesias and neuropathic pain	• Carbamazepine, oxcarbazepine • Gabapentin • Pregabalin • Lamotrigine • SNRI (eg, duloxetine) • Tricyclic antidepressants
Poor ambulation	• Dalfampridine
Spasticity (stiffness and involuntary muscle spasms)	• Baclofen • Benzodiazepines (usually diazepam or clonazepam) • Botulinum toxin • Dantrolene • Tizanidine

Table 6. Management options for MS symptoms

8.5.1 Fampridine

Fampridine is a broad-spectrum potassium channel blocker. After approval by the FDA in 2010, it is used in MS for symptomatic improvement of walking, although the exact mechanism by which it exerts its therapeutic effect is not fully known. In animal tissue preparation it was shown to increase conduction of action potentials in demyelinating axons through inhibition of potassium channels. By blocking these

channels, it is believed that the leakage of ionic current is reduced, and the action potential formation is enhanced. Walking speed is increased by 25% in patients on prolonged-release (PR) oral treatment [Dunn et al., 2011], which was also confirmed by the phase III ENHANCE clinical trial.

9. Conclusion

Multiple sclerosis is a chronic, autoimmune, inflammatory disease of the CNS, affecting mostly young individuals at their most productive age. Each patient is unique and the course of the disease varies vastly. Some patients may fall quickly to the progressive disease, with significant disability; others may be symptoms-free for many years; one patient may respond to a certain disease-modifying treatment, another not. Since it is a chronic disease, it may put a significant economic burden for the patient, family and health system. The exact causative factor is not yet known. No treatment for complete recession of the disease is yet available. Currently available treatments target the suppression of the immune system, in an attempt to control further CNS inflammation and neuronal damage. In the past years, much research has been done to the direction of underlying the exact mechanism of pathogenesis and possible new fields for treatment. Since MS is an immensely heterogeneous disease, personalized treatment is highly desirable. Therefore, additional biomarkers are needed, in order to provide better indicators for prognosis and treatment response.

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