Identification of prognostic factors predicting the long-term clinical outcome in Multiple Sclerosis

Antonio Scalfari

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Abstract

Multiple Sclerosis (MS) evolution varies from benign to aggressive forms, and its prognosis remains largely unpredictable, especially in individual cases. Relapse frequency is commonly used as indicator of disease activity and as primary endpoint in randomized clinical trials (RCTs). However, the role of inflammatory attacks on the disease progression is still largely debated. The lack of reliable predictors of the long-term evolution prevents from applying a rational and individualized therapeutic approach. In addition, RCTs methodology is still not sufficiently rigorous for protecting against the bias due to the large variability of the clinical outcome.

The project was carried out by analysing the London Ontario (LO) database, one of the largest collections of natural history data from untreated patients, followed up for 28 years. We analysed factors affecting prognosis and predicting disease evolution up to its latest stages. We first investigated in details the relationship between relapses and long-term outcome. The analysis demonstrated poor correlation between number of attacks and the attainment of severe disability, invalidating relapse frequency as surrogate marker for late outcome. In addition, it evidenced the onset of the secondary progressive (SP) phase as the key determinant of prognosis, differentiating patients' outcome and accounting for the variability of disease course. We therefore analysed in details factors affecting the rate of conversion to SP MS, in order to calculate how the risk of becoming progressive varies with disease duration. This information can be used for designing RCTs using SP onset as primary outcome. We then extensively investigated the effect of age on the disease evolution, before and after the onset of progression. The analysis highlighted age as the strongest determinant of MS prognosis, exerting its predictive effect primarily by affecting the evolution of the relapsing remitting (RR) phase and by increasing the probability of experiencing a progressive course.

Declaration

I declare that the work described in this thesis is solely my original and that all other contributions have been referenced to my best knowledge.

Statement of copyright

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Abbreviations:

9HPT – 9-Hole Peg Test
25(OH)D– 25-hydroxyvitamin D
APC – Antigen presenting cell
ARR– Annualized relapse rate
ATP – Adenosine triphosphate
BBB – blood brain barrier
CSF – Cerebrospinal fluid
CIS – Clinically isolated syndrome
CDMS – Clinically definite MS
CNS – Central nervous system
CSF – Cerebrospinal fluid
DMT – Disease modifying treatment
DSS – Disability status scale
EAE– Experimental autoimmune encephalomyelitis
EBV – Epstein Barr Virus
EBNA – EBV nuclear antigens
EDSS – Expanded disability status scale
FLS – Flu like syndrome
FDA – Food and drug administration
FoxP3 –Forkhead box protein P3
FS – Functional system
GA – Glatiramer acetate
HLA – Human Leukocyte Antigen
HR – Hazard ratio
IFN – Interferon
Ig – Immunoglobulin
IL – Interleukin
IM – Infectious mononucleosis
IM – Intramuscular
ISR – Injection site reaction
LO – London Ontario
LTF – Long term follow up
MC – Middlesex County
MHC – Major histocompatibility complex
MBP – Myelin basic protein
MOG – Myelin oligodendrocytes glycoprotein
MRI – Magnetic resonance imaging
MRS – Magnetic resonance spectroscopy
MSFC – Multiple Sclerosis Functional Composite

NAWM– Normal appearing white matter
NAA – N-acetylaspartate
NFs – Neurofilaments
NO – Nitric oxide
NTZ – Natalizumab
NNH – Number needed to harm
OCT– Optical coherence tomography
OR – Odd ratio
PASAT– Paced Auditory Serial Addition Test
PLP– Proteolipid protein
PML – progressive multifocal leukoenchephalopathy
PP – Primary progressive
PR – Progressive relapsing
RCT – Randomized controlled trial
RIS – Radiologically isolate syndrome
RNFL – Retinal nerve fibre layer
RR – Relapsing remitting
S1P – Sphingosine-1-phosphate
SC – Subcutaneous
SLC – Silvia Lawry Centre
SO – Seen from onset
SP – Secondary progressive
T25FW – Timed 25-Foot Walk
TNF – Tumour necrosis factor
Tregs– Regulatory CD4+ T cells
TRAL –Treatment related acute leukaemia
VCAM – Vascular cell adhesion molecule
VCA – Viral capsid antigen
VDR– Vitamin D receptors

Chapter 1

General introduction

1.1 Multiple Sclerosis

Multiple Sclerosis (MS) is an immune mediated disorder of the central nervous system (CNS), characterized by acute focal inflammatory demyelination and axonal loss (Compston and Coles, 2002). It affects around 2.5 millions individuals around the world (Compston, 2005) and it is considered one of the most common cause of disability among young adults, exerting a significant burden on health care costs and quality of life of those affected.

The clinical and pathological features of MS were first described by Charcot, Carswell and others more than 100 years ago (Compston, 2005) however, its aetiology and pathogenesis remain largely unknown. The disease probably develops in genetic susceptible populations, as a results of environmental exposures (Ebers, 2008). Its clinical course is unpredictable and ranges widely from benign to severe cases (Lublin and Reingold, 1996). About 50% of patients are expected to require walking assistance within 15 years from disease onset (Compston and Coles, 2008). MS prognosis continues to puzzle clinicians and scientists around the world, engaged in the challenging task to prevent the progression of the disease.

1.1.1 Incidence and prevalence

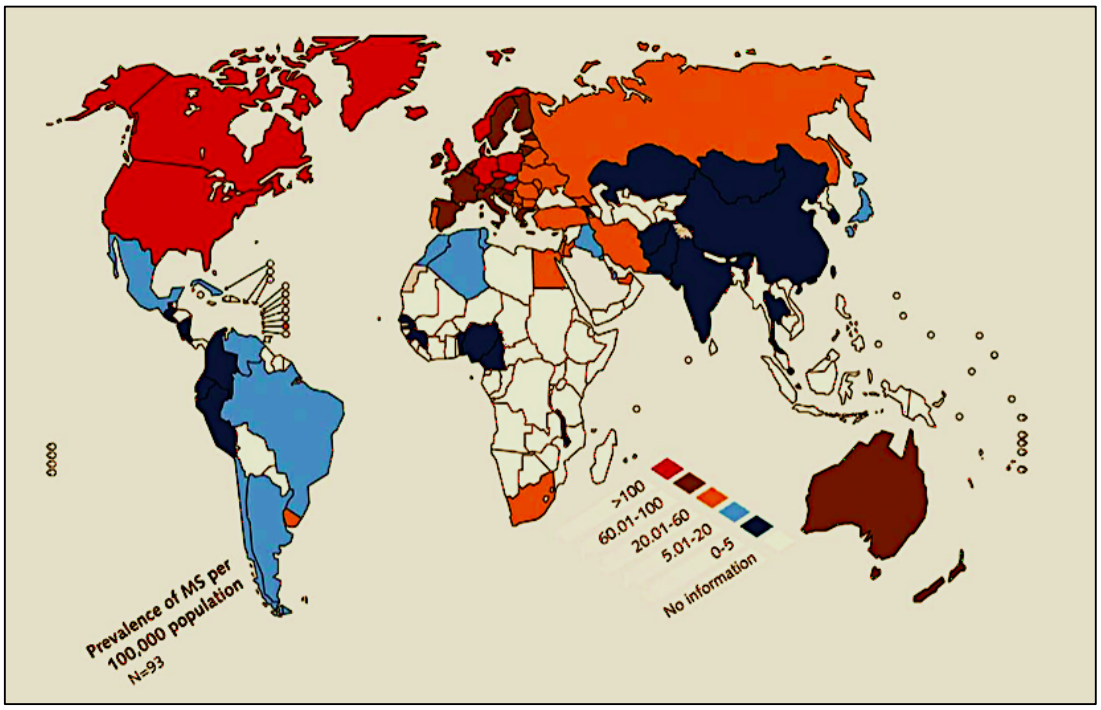
Despite the wealth of information from several prevalence studies, carried out in the past 70 years, defining the MS worldwide frequency remains difficult. The disease appears to be unevenly distributed across the world, with a higher prevalence among white people of Nordic origin, living in temperate zones and in high-income countries (Figure 1.1) (Compston, 2005).

1.1.1.1 Frequency and prevalence

The general trend seems to indicate that the disease frequency rose steadily over the years, however studies from different areas showed contradicting data. The

incidence of MS (number of new cases per year) was reported to be increased in some regions (Granieri et al., 2000; Grytten et al., 2006; Houzen et al., 2008; Larsen et al., 1984b; McLeod et al., 1994; Noonan et al., 2002) or to be unchanged/decreased in others (Svenningsson et al., 1990; Wender et al., 1987). Differences in the ascertainment methodology and the variable social, economic and genetic backgrounds of populations surveyed, make hard the comparison of data from separate geographical areas (Pugliatti et al., 2002).

Figure 1.1 The world geography of MS: prevalence for 100.000 habitants (world MS atlas 2008).



To exemplify MS distribution, ≥ 30 cases, 5-29 cases and ≤ 5 cases per 100.000 habitants are considered high, medium and low prevalence (number of patients alive at specific date), respectively (Kurtzke, 2000). Despite Europe and North America, where MS is relatively common, are disproportionally represented among epidemiological studies, the disease frequency undoubtedly varies substantially among continents. An high prevalence was consistently reported in Western Europe (Celius and Vandvik, 2001; Edland et al., 1996; Forbes et al., 1999; Gray et al., 2008; Grytten et al., 2006; Kinnunen, 1984; Kurtzke et al., 1982; Larsen et al., 1984a; Midgard et al., 1991; Poser et al., 1989; Rothwell and Charlton, 1998; Shepherd and

Downie, 1978; Sumelahti et al., 2001) and in North America (Hader, 1982; Hader et al., 1988; Hader and Yee, 2007; Helmick et al., 1989; Mayr et al., 2003; Sweeney et al., 1986; Wynn et al., 1990). Central and eastern Europe (Gross et al., 1993; Milanov et al., 1997; Pekmezovic et al., 2001; Verdes et al., 1978; Wender et al., 1985), Australia and New Zealand (Barnett et al., 2003; McLeod et al., 1994; Skegg et al., 1987) represent medium prevalence areas. Asia (Cheng et al., 2007; Houzen et al., 2008; Lau et al., 2002), Middle East and Africa (Alshubaili et al., 2005; Bhigjee et al., 2007; El-Salem et al., 2006; Radhakrishnan et al., 1985) have the lowest reported prevalence.

Scotland and its offshore Faroe Islands have the highest prevalence rates so far detected anywhere in the world, ranging from 145 to 193 cases/100.000 habitants (Forbes et al., 1999; Kurtzke and Heltberg, 2001; Rothwell and Charlton, 1998). Interestingly, it has been claimed that MS appeared in the Faroe Islands during the World War II, following the occupation by British troops, and reoccurred subsequently in 4 separate epidemics (Kurtzke and Hyllested, 1975). This led to the ambitious and questionable (Poser et al., 1988) hypothesis of a widespread asymptomatic, transmissible infection behind its aetiology (Kurtzke et al., 1993).

1.1.1.2 The "latitude effect"

The frequency of MS seems to have a distinct latitudinal variation; the risk of developing the disease is higher in areas at high geographic latitude (Simpson et al., 2011). This was observed in Australia (Hammond et al., 1988), Europe (Vukusic et al., 2007) and North America (Kurtzke et al., 1979), however other studies could not confirm the same trend (Melcon et al., 2008; Poppe et al., 2008).

In addition, in some Mediterranean European regions the prevalence of the disease is much higher than expected for their latitude, suggesting the presence of gene-environment interactions (Grimaldi et al., 2007; Nicoletti et al., 2005; Pugliatti et al., 2005; Rosati et al., 1996). The "latitude effect" hypothesis remains largely debated

(Alonso and Hernan, 2008; Koch-Henriksen and Sorensen, 2010; Rosati, 2001; Simpson et al., 2011).

1.1.1.3 The sex ratio

At the beginning of the 20th century, MS was thought to be a predominantly male disease (Brain, 1935). Since then, there has been a steady increase of its incidence among women (Bentzen et al., 2010; Debouverie et al., 2007; Hader and Yee, 2007; Hirst et al., 2009; Midgard et al., 1996; Orton et al., 2006; Sahraian et al., 2010). For instance, the sex ratio in Japan rose from 1:1 to 2.9:1 in between 1972 and 2004 (Osoegawa et al., 2009), in Canada from 1.9 to 3.2:1 in between 1940 and 1980 (Orton et al., 2006) and in northern climates it now exceeds 3.1:1 (Dyment et al., 2004).

The female preponderance, among MS patients, is now commonly agreed, with a consensus that women are about twice as likely to develop the disease than men (Compston and Coles, 2008). The reasons behind the increasing number of women with MS have been long discussed, without reaching any definitive conclusion. Smoking, known to affect the risk of MS (Hernan et al., 2001) and to have become more diffuse among women over the years, as well as the changes in lifestyle factors in women and an improved access to the health care for women, have been proposed as factors possibly contributing to the variation of the sex ratio over time (Orton et al., 2006; Sellner et al., 2011).

1.1.2 The role of vitamin D

The primary sources of vitamin D (colecalciferol) in human beings are the diet and the skin exposure to ultraviolet (UVB) radiation in sunlight. The diet provides only a modest amount of vitamin D (40-400 IU per food serving) (Brannon et al., 2008), compared to the sun exposure (20 minutes during summer produces around 10.000 IU) (Holick, 2004). UVB allows the 7-dehydrocholesterol in the skin to transform in pre-vitamin D3, which then isomerises to colecalciferol (Holick, 2003). The colecalciferol is also available from fortified foods (milk, cereals, chesses and dark fishes). It remains biologically inactive until it is converted in the liver to 25-hydroxyvitamin D, and finally to 1,25-hydroxyvitamin D, which binds and activates the vitamin D receptors (VDR).

1.1.2.1 Sun exposure

The hypothesis that vitamin D deficiency is a risk factor for MS was first proposed almost 40 years ago (Agranoff and Goldberg, 1974). Supporting evidence come from epidemiological studies, which showed an increased frequency of MS at higher latitudes (Hammond et al., 1988; Kurtzke et al., 1979; Ramagopalan et al., 2011; Simpson et al., 2011; Vukusic et al., 2007), where the population is exposed to low UVB radiations intensity and is known to have low vitamin D blood levels. In addition, other studies demonstrated that the average annual hours of sunshine during childhood inversely correlated with the prevalence of MS (Leibowitz et al., 1967; van der Mei et al., 2001; van der Mei et al., 2003). Interestingly, the association between the sun exposure during childhood and the risk of MS was also observed among monozygotic twins discordant for MS (Islam et al., 2006). Even the outdoor activity, as an occupational exposure, seems to affect the incidence of MS and its mortality rate (Freedman et al., 2000).

Migration studies further supported an association between vitamin D and the incidence of the disease. The risk of MS was shown to decrease among individuals

migrating from high to low latitude areas (Gale and Martyn, 1995; Kurtzke et al., 1985), possibly in relation to the different sun exposure intensity.

1.1.2.2 The vitamin D intake

Although the sun light exposure plays a primary role, the vitamin D diet intake becomes relevant in winter, especially at high latitudes. For instance, in Norway, a lower MS prevalence was found among coastal communities consuming greater fish than inland agricultural populations (Kampman et al., 2007; Swank et al., 1952; Westlund, 1970). Similarly, a recent longitudinal study demonstrated that a higher total vitamin D intake correlates with a significantly lower risk of MS (Munger et al., 2004). However, studying the association between the vitamin D diet intake, which is often assessed retrospectively, and the risk of MS, is difficult and potentially complicated by recall bias.

1.1.2.3 The vitamin D serum levels

Notwithstanding the methodological difficulties due to the large longitudinal assessments and the repeated blood samples collections required, the role of serum levels of 25-hydroxyvitamin D (25(OH)D), which is the main circulating form of vitamin D, in relation to MS incidence has also been studied. A 41% reduction of the risk of MS, for every 50 nmol/L increase of 25(OH)D level, was demonstrated, independently of the place of birth and of the latitude of residence during childhood (Munger et al., 2006).

In addition, recent studies found an association between an higher concentration of serum 25(OH)D and a lower risk of clinical exacerbations (Runia et al., 2012; Simpson et al., 2010), further supporting a protective effect exerted by the vitamin D. This hypothesis might be contradicted by the lower incidence of the disease among black people (Kurtzke et al., 1979), known to be vitamin D deficient (Looker et al., 2008). However, the genetic differences with white people probably compensate for the low vitamin D levels (Ascherio et al., 2010).

1.1.2.4 Immunological effects of the vitamin D

Immunological studies offer further evidence, suggesting a potential role of vitamin D in the pathogenesis of the disease. Vitamin D exerts immunomodulatory effects on a wide range of immune cells, including T-lymphocytes, B-lymphocytes and dendritic cells (Arnson et al., 2007; Deluca and Cantorna, 2001). In addition, different cells of the immune system contain VDRs (Chen et al., 2007; Veldman et al., 2000).

Indeed, the disease progression in the experimental autoimmune encephalomyelitis (EAE), the most commonly studied animal model of MS (Pachner, 2011), can be prevented or improved with the administration of vitamin D (Cantorna et al., 1996; Lemire and Archer, 1991; Nataf et al., 1996; Spach et al., 2006), and with the exposure to UVB (Hauser et al., 1984).

1.1.3 Smoking and MS

1.1.3.1 Smoking and the incidence of MS

Smoking is an important risk factor for MS. Early studies indicated a possible worsening of MS symptoms with smoking (Emre and de Decker, 1992; Perkin et al., 1975). More recently, in 3 large prospective cohorts studies, ever-smokers were shown to have a 40-80% increased risk of MS, compared to never-smokers, with a relative risk varying between 1.4 (Thorogood and Hannaford, 1998), 1.7 (Hernan et al., 2001) and 1.8 (Villard-Mackintosh and Vessey, 1993).

Given that smoking has generally become more diffuse among women over the recent years, the changes in the males/females ratio has been considered a possible confounding factor. This was recently addressed in a large study, which assessed whether the males/females incidence of MS changed concomitantly with smoking. The results demonstrated a 40% average increase in the incidence of MS among both women and men ever-smokers (Palacios et al., 2011). In addition, the risk of MS appears significantly higher, even among individuals who had smoked at some point

during their life, compared to those who never smoked. This was observed in Sweden (Odds Ratio [OR] = 1.5) (Hedstrom et al., 2009), in the U.K. (OR = 1.3) (Hernan et al., 2005) and in Norway (OR = 1.8) (Riise et al., 2003). Interestingly, the risk of developing MS in adult life was shown to remain unchanged, among offspring whose mothers smoked during pregnancy (Montgomery et al., 2008).

1.1.3.2 Smoking and the disease severity

Among MS patients, smoking was shown to affect significantly the disease severity. Being a smoker associates with a higher probability (Hazard Ratio [HR] = 3.6 (Hernan et al., 2005) and 2.5 (Healy et al., 2009)) of experiencing disease progression and of developing more severe disability (Zivadinov et al., 2009a). Neurotoxic (Smith et al., 1963) and immunomodulatory (Sopori and Kozak, 1998) effects, exerted by constituents of cigarettes smoke, are possible biological explanations of how smoking may affect the severity of MS (Palacios et al., 2011).

1.1.4 Epstein Barr Virus and MS

Epstein Barr Virus (EBV) is a potentially oncogenic herpes virus, which infects over 90% of human beings, within the first decades of life. Following the primary infection, usually occurring through contact with infected saliva, EBV remains in B-lymphocytes of the host in a "latency state" (Thorley-Lawson, 2001) and tends to be asymptomatic, especially in young children. However, in up to 40% of infected individuals, EBV leads to infectious mononucleosis (IM) during the adult life (Andersson, 1991). In addition, it can cause lymphomas in immunosuppressed subjects (Kutok and Wang, 2006).

1.1.4.1 The EBV primary infection

The EBV seronegativity is rare among MS patients (Pakpoor et al., 2012). EBV-positive, compared to EBV-negative, individuals have a much higher probability of developing MS (Ascherio et al., 2001; Ascherio and Munger, 2007; Levin et al., 2010). The risk of MS was shown to be greatly increased (OR = 13.5) by being EBV-seropositive (Ascherio and Munch, 2000) and, on the contrary, to be greatly decreased (OR = 0.06 (Ascherio and Munger, 2007) and 0.18 (Pakpoor et al., 2012)) by being EBV-seronegative. Interestingly, in a large cohort of EBV-negative young adults, the incidence of MS was observed to increase sharply following the EBV infection; all MS patients had seroconverted before the onset of symptoms, strongly indicating the EBV infection precede the immune deregulation, which leads to the development of the disease (Levin et al., 2010).

1.1.4.2 EBV antibodies

Among EBV infected healthy individuals, the incidence of MS appears to rise proportionally with the serum titres of EBV related antibodies (Ascherio et al., 2001; DeLorenze et al., 2006; Levin et al., 2005). From a large meta-analysis, the calculated ORs for MS risk, in relation to seropositivity for antibodies anti EBV nuclear antigens (EBNA) complex, anti-EBNA-1 and anti viral capsid antigen (VCA), were 5.4, 12.1 and

5.5, respectively (Santiago et al., 2010). Interestingly, the association between anti-EBNA antibodies titres and the risk of MS was shown to be independent of the HLA-DRB1*1501 allele status (De Jager et al., 2008; Simon et al., 2010; Sundstrom et al., 2008), but to vary according to the smoking status, being about two folds greater among ever-smokers compared to never-smokers (Simon et al., 2010). In addition, young adulthood appears to be the potential window for the risk of MS. Anti EBNA and EBV antigen antibodies were found to occur in similar titres in cases and control, but to increase dramatically after the age of 25 among those who later developed the disease (Levin et al., 2005)

Antibodies anti EBV might also associate to the disease severity and progression. Two independent studies reported a positive correlation between increased titres of anti-EBNA and anti-VCA antibodies and the brain volume at magnetic resonance imaging (MRI) and the disability severity (Farrell et al., 2009; Zivadinov et al., 2009b). These results suggest that immunological parameters of EBV infection could be potentially used as biomarker in MS, however further investigations are needed to better explore this hypothesis and to elucidate whether EBV contributes to the disease progression (Ascherio and Munger, 2010).

1.1.4.3 Mononucleosis

The hypothesis linking EBV infection (IM) and the risk of MS was first driven by their similarities in the geographical and socioeconomic distribution (Ascherio and Munger, 2007; Disanto et al., 2012; Warner and Carp, 1981). Both diseases affect mostly young adults, are more prevalent among women, follow a similar latitude gradient and are more frequent in high-income populations. Despite several evidences from epidemiological, laboratory and pathology studies, supporting a role of EBV infection in the pathogenesis of MS, definitive conclusions on whether EBV is simply a concomitant epiphenomenon of an immune derangement or a causative risk factor for MS, have never been reached.

Independent studies cohere in demonstrating that MS risk is 2-3 folds higher among people with a history of IM (Ahlgren et al., 2009; Nielsen et al., 2007; Ramagopalan et al., 2009c; Zaadstra et al., 2008). In a recent large meta-analysis, combining case control and cohort studies, the calculated relative risk (RR) of MS for a past history of IM was 2.17 (Handel et al., 2010c). The risk seems to increase proportionally with the age at infection (Hunter and Hafler, 2000), and to be much larger among patients HLA-DRB1*1501 allele positive (Nielsen et al., 2009), which is the strongest genetic predictor of MS.

1.1.4.4 EBV infection and MS pathogenesis

The mechanisms linking the EBV infection and the pathogenesis of MS have been long debated and remain controversial (Ascherio and Munger, 2007; Ascherio and Munger, 2010; Lassmann et al., 2011; Lucas et al., 2011). It has been postulated that the EBV infection, asymptomatic in early life, might indirectly trigger the autoimmune reaction, which leads to the development of MS in adult life, among susceptible individuals (Ascherio and Munger, 2007; Hunter and Hafler, 2000). However, this attractive hypothesis cannot sufficiently explain why MS remains relatively rare, despite the widespread diffusion of the EBV infection in the adult population. This suggests that genetic determinants possibly influence the susceptibility to both EBV infection and MS (Ascherio and Munger, 2007; Handel et al., 2010b).

Pathological studies offer contradicting evidences and cannot help to further elucidate these hypotheses. In nearly 100%, of 22 brains analysed, a high proportion of EBV-infected B-lymphocytes was found in meningeal follicles, infiltrating the MS lesions (Serafini et al., 2007). These findings might indicate that antigen-presenting EBV-infected B-cells infiltrate the central nervous system (CNS) and trigger a cytotoxic response, resulting in the chronic inflammatory process and in the tissue damage (Pender, 2011). However, results from Serafini et al. could not be replicated in other studies, using similar technologies, but showing no evidence of EBV infection in the CNS (Peferoen et al., 2010; Sargsyan et al., 2010; Willis et al., 2009).

In an attempt to identify the reason underlying these discrepant results, a joint meeting of all research groups involved in the EBV infection and MS brains analyses was recently held (Vienna, July 2010) (Lassmann et al., 2011). It was concluded that the differences among studies are probably due to technical issues and different interpretations of the immunocytochemistry (Lassmann et al., 2011). The EBV infection in the CNS, when present at all, is probably restricted to a very low number of B cells and current evidence are not sufficient for judging if EBV is concomitant or causal to MS (Lassmann et al., 2011).

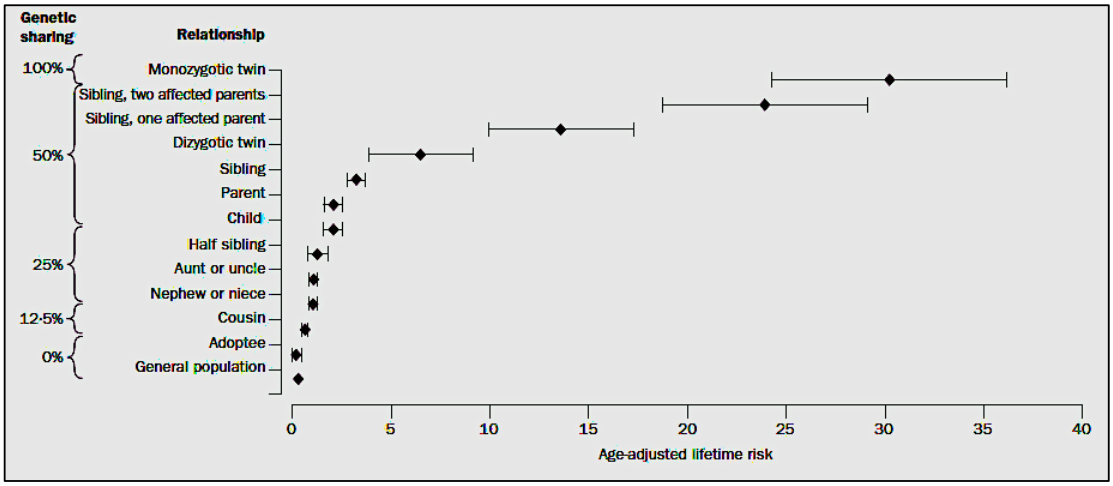
The lack of convincing evidence of the presence of EBV, within the CNS of MS patients, possibly indicates that EBV plays an indirect role in the disease pathogenesis, through a T-cells activation (Ascherio and Bar-Or, 2010). Other studies highlighted the intriguing hypothesis of a common pathogenic mechanism between EBV and vitamin D, both exerting an adverse effect on the risk of MS, through changes in the level of interleukin 10 (IL-10) (Hayes and Donald Acheson, 2008) or through an activation of auto-reactive T cells, which is facilitated by a low vitamin D status (Holmoy, 2008).

1.1.5 Genes and MS

1.1.5.1 The familial risk of MS

A genetic influence on MS susceptibility was first suggested many decades ago by observations of the disease aggregation in families (Mackay, 1950; Schapira et al., 1963), later confirmed in other studies (Ebers et al., 2004; Robertson et al., 1996; Willer et al., 2003). First-degree relatives have 10-25 times greater risk of MS than the general population; this risk correlates directly with the degree of kinship (Figure 1.2) (Compston and Coles, 2002). Further supporting evidence come from twin studies from different populations, consistently showing that a monozygotic twin of an affected individual has much higher risk of developing the disease than a dizygotic twin (Hansen et al., 2005; Willer et al., 2003).

Figure 1.2 The recurrence risk for MS in families: age adjusted recurrence risk for different relatives with MS. Pooled data from population-based survey. Estimated 95% CIs are shown (Compston and Coles, 2002).



1.1.5.2 HLA and MS susceptibility

For the first time in the early 70s it was highlighted that the Human Leukocyte Antigen (HLA) genes, residing within the major histocompatibility complex (MHC) region, play a primary role in determining the genetic susceptibility to MS (Jersild et al., 1973). HLA-class II types exert the strongest effect, accounting for 20-60% of the

genetic risk (Haines et al., 1998), however the association is not straightforward and changes in relation to the geographic areas. Some haplotypes (HLA-DRB1*03, HLA-DRB1*10, HLA-DRB1*11, HLA-DRB1*14) are known to correlate with the risk of MS in northern Europe (Barcellos et al., 2003), with a predominant role played by the HLA-DRB1*15 in virtually all populations (Caillier et al., 2008; Lincoln et al., 2005; Ramagopalan et al., 2009a). However, different haplotypes affect the susceptibility in other populations, such as Sardinians (HLA-DRB1*03, HLA-DRB1*04, HLA-DRB1*13) (Marrosu et al., 2001), Canadians (HLA-DRB1*11, HLA-DRB1*14) (Ramagopalan et al., 2007), Japanese (HLA-DRB1*09) (Matsuoka et al., 2008) and Spanish (HLA-DRB1*03, HLA-DRB1*08, HLA-DRB1*14) (Pina et al., 1999).

MHC class II molecules are cell surface glycoproteins, presenting the antigen CD4+ to T-helper cells and therefore implicated in the maintenance of the immune system self tolerance and in its adaptive response to pathogens (Watts, 2004). HLA-DRB1 alleles are thought to affect the risk of developing the disease risk, by influencing T-cell repertoires, which are involved into the disease pathogenesis (McFarland and Martin, 2007) However, this hypothesis requires further clarifications (Ramagopalan et al., 2009b). Other studies have identified, within HLA-class I alleles, some other few loci affecting MS susceptibility, independently of class II loci (Bergamaschi et al., 2010; Cree et al., 2010; Link et al., 2010).

Complex gene-to-gene interactions (epistasis) affect the MS susceptibility in different ways. For instance, while carrying the HLA-DRB1*15 allele roughly triplicates the risk of MS, when this is associated with the HLA-DRB1*14 allele, the risk dramatically drops to 1 (Dyment et al., 2005; Ramagopalan et al., 2007). Similarly, although the HLA-DRB1*08 allele on its own exerts a very modest effect, its association with a single copy of the HLA-DRB1*15 allele doubles the probability of developing MS (Dyment et al., 2005).

1.1.5.3 Genome wide association

The MHC is not the only genetic region associated with the risk of MS. Recent genome wide association (GWA) studies highlighted the role of multiple non-MHC loci, affecting the risk of developing the disease (Baranzini et al., 2009; Sawcer et al., 2011). The majority of these genes are involved in the immune system, in agreement with the immune-mediated pathogenesis of the disease: interleukin (IL)-2 and IL-7 receptors, CD6, CD58, IRF8 genes. However, all of them were found to exert a rather modest effect and their exact role in the disease aetiology and pathogenesis remains to be elucidated.

1.1.5.4 Genes and the disease severity

The role of genes affecting the disease severity is extremely controversial. The HLA-DRB1*01 allele was found to exert a protective effect against a severe disease course, as it was overrepresented among patients with benign, compared to those with severe outcome (DeLuca et al., 2007). Similar results were reported by an Australian study (Stankovich et al., 2009). However, in a Spanish MS cohort, the HLA-DRB1*01 allele associated with a more rapid disability development (Romero-Pinel et al., 2011). In addition, in a large French study, the HLA-DRB1*15 allele positively correlated with a faster disease progression (Cournu-Rebeix et al., 2008). Differences in the study design and methodology might partially account for the discrepancies among studies, and further analyses are needed for validating the hypothesis that the disease severity is regulated, to some extent, by a genetic control (Ramagopalan et al., 2008).

1.1.6 Environmental factors

Results from migration studies (Gale and Martyn, 1995), the geographical gradient of the distribution of the disease (Simpson et al., 2011) and the high rates of discordancy in identical twins (Islam et al., 2006; Willer et al., 2003), suggest that environmental factors, such as the sunlight exposure or viral infections, play a relevant role in the development of MS. Whether such factors act as triggers or are directly involved into the pathogenesis of the disease remain unknown and has therapeutic implications.

1.1.6.1 The time of exposure

The timing of exposure is of crucial importance with regards to the potential prevention of the disease. Environmental factors might act in utero and affect the susceptibility to MS later in life. For instance, some studies suggested a "month of birth effect"; the risk of developing MS was shown to be higher among those born in May and lower among those born in November (Willer et al., 2005). This association could be mediated by the sunlight exposure and its effect on vitamin D levels, known to be low among pregnant women and among new born during winter (Newhook et al., 2009). This hypothesis is reinforced by other studies, supporting a maternal parent-of-origin effect on the MS susceptibility (Chao et al., 2009). By analysing the parent-child concordance in MS, it has been demonstrated an excess of mother-daughter pairs and a paucity of father-son pairs (Sadovnick et al., 1991a). In addition, family studies showed a much higher concordance for MS between maternally related half siblings than between paternally related ones (Ebers et al., 2004; Herrera et al., 2008).

Migration studies helped to better address the role of the timing of exposure on the risk of developing the disease. It has been suggested that, by migrating from a high to a low incidence area, individuals acquire a lower risk of MS only if migration occurs in early adolescence, within the age of 15 (Dean and Kurtzke, 1970; Kurtzke et

al., 1970), or at latest within the third decade of life (Hammond et al., 2000). Interestingly, among migrants to Canada from Europe, features of sex ratio from the original country are retained if migration occurs after the age of 21 only (Orton et al., 2010). In addition, the level of summer sun exposure during early stages of life correlates inversely with the probability of developing MS (Kampman et al., 2007; van der Mei et al., 2003), even among monozygotic twins discordant for MS (Islam et al., 2007).

Taken together, these data indicate that there is a "cut off period of susceptibility", most likely early in life. Environmental factors, probably during childhood and adolescence, interact with genetic factors and determine the overall susceptibility to MS (Goodin, 2009). EBV infection is unambiguously present among MS patients (Ascherio and Munger, 2007) and vitamin D deficiency early in life might alter the immune response and eventually predispose the individual to develop MS (Hayes and Donald Acheson, 2008). Other factors are likely to play a relevant role during different stages of life, as confirmed by the wide range of age at which the disease becomes clinically evident (Handel et al., 2010b).

1.1.6.2 Epigenetic

The epigenetic effect implies heritable changes in gene expression caused by mechanisms not involving the underlying DNA sequence (Urdinguio et al., 2009). As they can be modulated by the environment, these mechanisms provide the bridge between external factors and internal genetic system (Handel et al., 2010a). Environmentally induced changes to the epigenome can persist through generations. The exact role of epigenetics in MS aetiology remains unknown. However, several observations lend support to the hypothesis that epigenetic modifications, by mediating the effect of the environmental factors, affect the disease susceptibility and pathogenesis. Identical monozygotic twins discordant for MS (Islam et al., 2006; Willer et al., 2003), the month of birth effect (Willer et al., 2005), the maternal parent-of-origin effect (Chao et al., 2009) and the associations between vitamin D deficiency (Munger et al., 2006), EBV infection (Levin et al., 2010) and smoking

(Palacios et al., 2011), and the incidence of MS, imply changes to the epigenome induced by the environment.

Epigenetic effect has also been observed in in the HLA region of MS patients. Among aunt/uncle/niece/nephew MS pairs, the frequency of the HLA-DRB1*1501 allele was found to be different between the first and the second generation of females. Affected aunts had a significantly lower HLA-DRB1*1501 allele frequency compared to their affected nieces (Chao et al., 2009). Therefore, as the HLA-DRB1*1501 allele frequency remains unchanged among affected males, it has been suggested that epigenetics modifications might play a role in the MS preponderance among women (Hoppenbrouwers and Hintzen, 2011).

The complexity of MS aetiology derives from the interaction between the external environmental risk factors and the internal genetic landscape. The epigenetic mechanisms are therefore likely to play a primary role in the MS susceptibility, which still needs to be fully elucidated (Burrell et al., 2011)

1.2 Clinical presentation and disease course

MS follows different patterns of evolution and the rate of disability accumulation largely varies, among patients. The disease course and the clinical phenotypes are characterized by relapses and progression, which present alone or in combination.

For the majority of patients, during the initial stages of the disease, relapses are the exclusive clinical feature, defining the relapsing remitting (RR) phase. This is followed, in most of the cases, within a variable lapse of time, by a steady and progressive accumulation of irreversible disability, which characterizes the secondary progressive (SP) phase. A minority of patients experience a primary progressive (PP) course, featured by the accumulation of unremitting disability since the disease onset. Relapses can present during both the PP and SP phase, in a variable percentage of patients.

1.2.1 Relapses

Relapses are episodic, acute neurological symptoms, lasting for more than 24 hours, in patients otherwise free from concomitant illness (typically an infection producing fever) (Lublin and Reingold, 1996). They represent the clinical manifestation of on-going inflammatory activity and can virtually manifest with any kind of neurological symptom, according to where the inflammation localizes, within the CNS (Compston and Coles, 2008). It is commonly agreed that events occurring within 1-month period are considered part of the same acute episode (McDonald et al., 2001; Poser et al., 1983). The onset of the functional impairment is commonly acute or sub-acute and can be followed by a partial or complete remission over weeks (Bethoux et al., 2001; Lublin and Reingold, 1996) and, in some cases, even after 12 months (Kremenchutzky et al., 2006a). A permanent severe motor disability (requiring unilateral walking assistance or worse) only rarely results from a lack of recovery (Bejaoui and Rolak, 2010; Tutuncu et al., 2012).

1.2.2 Progression

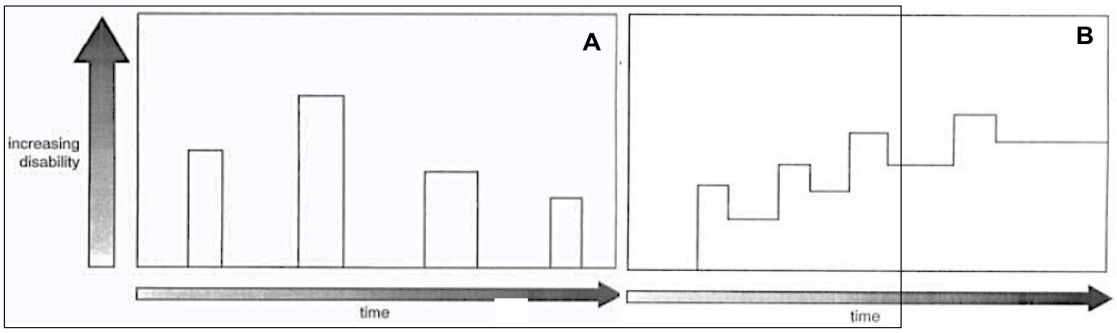
Progression is characterized by a steady accumulation of irreversible disability, for at least 1 year, although occasional plateaus and minor temporary improvements can be observed (Lublin and Reingold, 1996). This definition remains controversial and it is often mistaken with the worsening of disability from baseline (Liu and Blumhardt, 2000). In the majority of patients, progression initially presents clinically with a long tract involvement of lower limbs, causing deterioration of ambulation (Kremenchutzky et al., 2006b). The date at which progression starts is invariably assigned in retrospect, once the required 12 months duration of continuous worsening is confirmed. Nevertheless, in some cases relapses with lack of complete recovery can cloud the onset of progression, making the task of pinpointing when the progressive phase begins particularly challenging (Kremenchutzky et al., 2006b).

1.2.3 The disease course

The lack of clear biological markers, allowing to distinguish the various forms of MS, contributed, over the years, to the difficulties of finding unanimous definitions of course patterns. The long debate came to an end in 1996 when, following the Advisory Committee on Clinical Trials of New Agents in MS survey, definitions and terminologies used to describe the disease courses were standardized (Lublin and Reingold, 1996). It was finally agreed to classify four different clinical subtypes of MS.

1.2.3.1 Relapsing-remitting MS

Figure 1.3 RR MS is characterized by clearly acute attacks with full recovery (A) or with residual deficit (B). Periods between relapses are characterized by lack of disease progression (Lublin and Reingold, 1996).



Approximately 80% to 85% of MS patients experience a RR phase (Confavreux and Vukusic, 2006a; Kantarci et al., 1998; Leray et al., 2010; Phadke, 1987; Poser et al., 1983; Runmarker and Andersen, 1993; Scalfari et al., 2010; Tremlett et al., 2009b). This is characterized by clearly defined relapses, followed by full or partial recovery, with a stable disease course between attacks (Figure 1.3) (Lublin and Reingold, 1996).

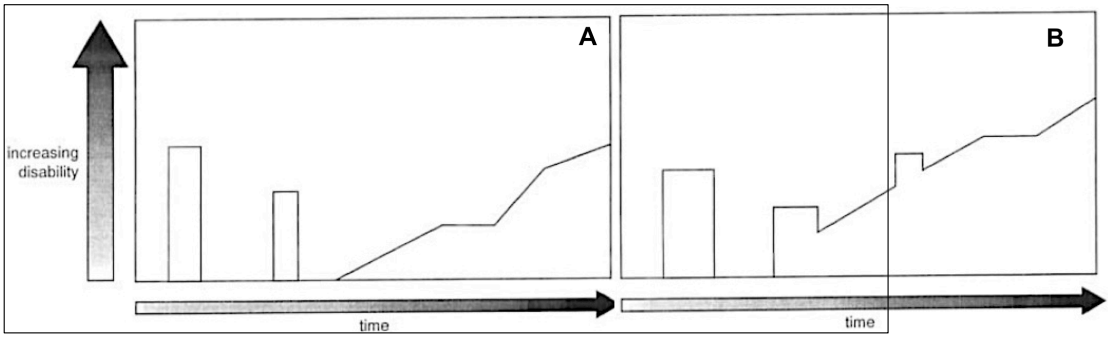
Motor, sensory and visual disturbances represent the most commonly observed symptoms at the onset of the RR phase. However, these figures vary among natural history cohorts, reflecting different methodologies of studies (Confavreux and Vukusic, 2006a; Kantarci et al., 1998; Leray et al., 2010; McDonald et al., 2001; Phadke, 1987; Poser et al., 1983; Runmarker and Andersen, 1993; Tremlett et al., 2009b). The type of symptoms featuring the relapses over the course of the RR phase has never been systematically ascertained.

The frequency of the attacks averages 1 relapse/year in the early stage of the disease (Confavreux et al., 1980; Goodkin et al., 1989; Patzold and Pocklington, 1982), and decreases over time, due to the “regression to mean” phenomenon (Patzold and Pocklington, 1982; Tremlett et al., 2008b). For instance, in the British Columbia database population, annualized relapse rate reduced by 17% every 5 years of disease duration (Tremlett et al., 2008b). In addition, 77% of patients stayed

free of relapses for at least 5 years, highlighting the unpredictable pattern of the evolution of inflammatory attacks (Tremlett et al., 2008b).

1.2.3.2 Secondary progressive MS

Figure 1.4 SP MS begins with an initial RR course, followed by progression at variable rate (A). Occasional relapses and minor remission can occur (B) (Lublin and Reingold, 1996).



The RR course can be followed by the SP phase, which is characterized by progression, with or without superimposed, occasional relapses, minor remission and plateau (Lublin and Reingold, 1996). It has been estimated that more than 80% of patients, with an initial RR course, convert to SP MS after 25 years from the disease onset (Weinshenker et al., 1989b). However, a minority can remain progression free even after 50 years of disease course (Skoog et al., 2012).

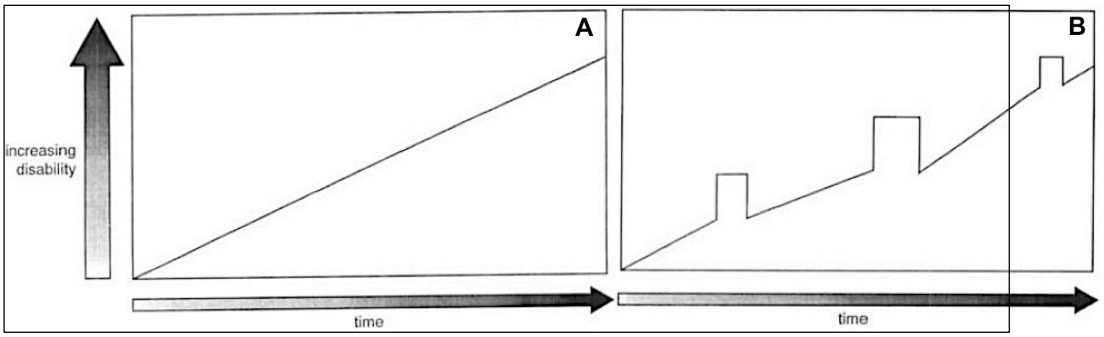
It is commonly accepted that, once the baseline between relapses begins to progressively worsen, the patient has switched to the secondary progression (Lublin and Reingold, 1996). Nevertheless, the boundaries between the RR and the SP phases remain somehow arbitrary. Establishing when the SP phase begins can be a challenging task, particularly when patients start to progress, while still experiencing relapses, as the progression of disability is not discretely attributable either to lack of recovery from the inflammatory attack or to the underlying progression (Kremenchutzky et al., 2006b).

The rate of worsening varies among SP MS patients, although the evolution of the progressive phase, eventually, invariably leads to the attainment of severe disability

(Kremenchutzky et al., 2006b; Tutuncu et al., 2012). Relapses can still occur during the SP phase in about 40% of patients, without significantly impacting on its evolution (Confavreux et al., 2000).

1.2.3.3 Primary progressive MS (PP-MS)

Figure 1.5 PP MS is characterized by progression of disability from onset (A) with occasional plateaus and temporary minor improvements allowed. Clear acute relapses can overlap the PP course (B) (formerly progressing relapsing [PR] MS) (Lublin and Reingold, 1996).



A minority of patients, ranging from 15% to 20% (Confavreux and Vukusic, 2006a; Kantarci et al., 1998; Leray et al., 2010; Phadke, 1987; Poser et al., 1983; Runmarker and Andersen, 1993; Tremlett et al., 2009b), experiences a PP course. This is characterized by progression from onset, with occasional plateau and temporary minor improvements allowed (Lublin and Reingold, 1996). The essential requirement in PP MS cases is a gradual, nearly continuously worsening and accumulation of disability, with minor fluctuations, but no distinct relapses.

A progressive disease course from onset, overlapped by clear acute relapses, with full or partial recovery, and with continuous progression in between relapses, characterizes the progressive relapsing (PR) forms of MS (Lublin and Reingold, 1996). It has been estimated that about 30-40% of PP patients can experience superimposed relapses (Confavreux et al., 2000; Kremenchutzky et al., 1999b), mostly occurring within 10 years from disease onset, although in a minority even after 20 years (Kremenchutzky et al., 1999b). In view of the similar clinical and demographic features and of the same long-term outcome between PR and PP

patients, it has been suggested that the two subgroups should be considered a single clinical entity (Confavreux and Vukusic, 2006a; Kremenchutzky et al., 1999b).

1.2.3.4 Clinically isolated syndrome

Clinically isolated syndrome (CIS) is a term that describes a first clinical episode, lasting at least 24 hours, with features suggestive of MS (Miller et al., 2008). The episode generally presents acutely or sub-acutely, and reaches its peak within 2-3 weeks. To be termed CIS, symptoms have to occur in absence of fever or infection and should not have features of encephalopathy (Miller et al., 2008). CIS is generally a mono-focal syndrome that can affect the optic nerve (more commonly), the spinal cord, the brainstem or the cerebellum however, and, in a minority of cases, presents with multi-focal involvement (Miller et al., 2008).

The risk of conversion to clinically definite (CD) MS, among patients with CIS, varies according to the type of clinical presentation (Miller et al., 2012). MS was reported to develop in 10%-85% of patients presenting with optic neuritis (2008), in 41%-61% of patients presenting with transverse myelitis (Young et al., 2009), in 53%-60% of patients presenting with brainstem symptoms (Tintore et al., 2010). These different figures might be accounted for by the different geographic location of studies and the different length of follow up (Miller et al., 2012).

1.3 The immunology of Multiple Sclerosis

Initial evidence of the autoimmune and inflammatory nature of MS date back many decades ago when, for the first time, high levels of immunoglobulins (Ig) were detected in the cerebrospinal fluid (CSF) of patients (Kabat et al., 1948). The development of the disease is likely to be preceded by an activation, in the peripheral immune system, of reactive CD4+ Th1 cells, which subsequently migrate across the blood brain barrier (BBB) into the CNS, targeting myelin components. Sclerotic plaques, involving the white matter of the CNS, are the pathological hallmark of MS (Lassmann et al., 2001) and represent the end stage of a process primarily driven by inflammation and demyelination, and accompanied by remyelination, astrocytosis and axonal degeneration (Lassmann et al., 2001). White matter lesions are characterized by a perivascular T lymphocytes infiltration, which tends to spill into the surrounding parenchyma (Lassmann et al., 2001). However, the presence of IgG oligoclonal bands into the CSF of up to 95% of patients (Freedman et al., 2005) indicates also an abnormal B cells activation, highlighting the great complexity of the MS immunopathogenesis.

1.3.1 T-Lymphocytes

1.3.1.1 The antigen specificity

T-lymphocytes migrate into the CNS, trigger an immune response, targeting specifically myelin and non-myelin antigens components, and expand clonally into the perivascular space, causing cytotoxic damage (Kivisakk et al., 2009). However, the initiation of the antigen-specific response remains largely unclear and environmental factors are considered the most likely triggers (Marrie, 2004; Sospedra and Martin, 2005).

Myelin basic protein (MBP) and the proteolipid protein (PLP) are the two most abundant myelin proteins in the CNS (30-40% and 50%, respectively). They are involved into the maintenance of the myelin structural compactness and they are found in significant quantities in both the central and peripheral nervous system (Seamons et al., 2003). Their key role as inductors of the inflammation into the CNS, along with other antigens candidates, such the myelin oligodendrocytes glycoprotein (MOG), has been extensively demonstrated in the MS animal model (EAE) (Kennedy et al., 1990; Mendel et al., 1995; Wekerle et al., 1994). Nevertheless, studies failed to show significant differences of myelin reactivity between MS patients and healthy donors (Burns et al., 1983). It has been hypothesized that, in MS patients, myelin specific T cells are of "higher avidity" and skewed towards the pro-inflammatory phenotype, compared to healthy controls (Bielekova et al., 2004), explaining their pathogenic role.

1.3.1.2 CD4+ T cells

The hypothesis that CD4+ T-cells are major players into the MS pathogenesis is confirmed by observations in the experimental model of the disease (EAE) (Goverman et al., 1993; Zamvil and Steinman, 1990). EAE can be caused by the injection of defined protein components of the myelin sheath into susceptible animals, but cannot be transferred by antibodies, highlighting the primary role played by T cell-mediated autoimmune mechanisms (Pettinelli and McFarlin, 1981). EAE can also be transferred to naïve animals, by injecting myelin-specific CD4+ T-cells reactivated in vitro (Zamvil and Steinman, 1990). In addition, genetic studies demonstrate that disease susceptibility strongly associates with MHC class II products, normally required for presenting auto-antigens to T lymphocytes and therefore implicated into the immune system self tolerance (Watts, 2004).

CD4+ Th1 circulating cells are found abundant in the patients' blood and receptors specific for MBP are present in MS human brains (Lassmann et al., 2001; Oksenberg et al., 1993). Once CD4+ T cells accumulate into the CNS, the macrophage-mediated immune response is amplified by pro-inflammatory cytokines such as interferon

(IFN)-γ and tumour necrosis factor (TNF)-α (Olsson et al., 1990; Seder and Ahmed, 2003). High levels of Th1 cytokines are found in the MS patients' blood and the disease activity seems to correlate with IFN-γ and interleukin (IL)-12 levels (Balashov et al., 1998).

More recently, CD4+ Th17 cells have also been highlighted as important drivers of the CNS inflammation (Korn et al., 2009; Tzartos et al., 2008). Through secretion of IL-17 and IL-22, disrupting the BBB, these cells penetrate into the brain and cause the tissue damage (Kebir et al., 2007; Tzartos et al., 2008). Indeed, Th17 cells are found abundant in MS brain active lesions (Lock et al., 2002) and are in a much higher blood concentration among MS patients, compared to healthy controls (Matusevicius et al., 1999). In addition, the neutralization of IL-17 by blocking antibodies was shown to have a beneficial effect in EAE (Rohn et al., 2006).

Both Th1 and Th17 cells are therefore major players into the autoimmune cascade behind the disease pathogenesis. Interestingly, in EAE, Th1:Th17 ratio seems to correlate with distribution (Stromnes et al., 2008) and type (Kroenke et al., 2008) of CNS lesions. However, the extent of their contribution to inflammation in MS brains remains to be fully elucidated.

1.3.1.3 CD8+ T cells

For many years CD4+ T cells were considered the only player in the pathological events responsible for initiating and maintaining the immune response in MS. This hypothesis was challenged when depletion of CD4+ T cells, in MS patients, was found not to associate with any improvement of the disease activity parameters (van Oosten et al., 1997). In contrast, global depletion of CD8+, as well as CD4+ T cells, with an anti-CD52 monoclonal antibody (Campath), led to a marked suppression of the inflammatory component of the disease. Since then, the potential role of CD8+ T cells in the disease pathogenesis has received increasing attention (Lassmann and Ransohoff, 2004; Steinman, 2001).

It has been hypothesised that, after initiation of the autoimmune cascade, driven by CD4+ T cells, CD8+ T cells predominantly cause the tissue damage in the CNS (Friese and Fugger, 2005). Indeed, in EAE, activated CD8+ T cells are able to migrate into the CNS, to induce local tissue damage, without the help of CD4+ T cells (Cabarrocas et al., 2003), and to replicate some of the MS features normally not seen in CD4+ T cells models (Sun et al., 2001). However, it remains unclear when and where the activation of CD8+ T cells occurs. Expanded CD8+ T cells have been isolated in brain lesions as well as in the blood and in the CSF of MS patients (Annibali et al., 2011; Junker et al., 2007; Skulina et al., 2004). It has been shown that, once migrated into the CNS, activated CD8+ T cells can recognize their antigens on MHC class I expressing cells, expand clonally and trigger the tissue damage (Ramakrishna et al., 2004). Interestingly, the extent of the injury to axons and oligodendrocytes was shown to be proportionate to the number of infiltrating cells (Bitsch et al., 2000; Saxena et al., 2008). This can be caused by several mechanisms, including the release of lytic enzymes (Harty et al., 2000) and of pro-inflammatory cytokines such as IFN-γ, TNF-α (Harty and Badovinac, 2008) and IL-17 (Tzartos et al., 2008).

1.3.1.4 Regulatory T cells

The presence of MBP-reactive T cells in both MS patients and healthy controls (Burns et al., 1983) strongly indicates that the failure of local regulatory mechanisms accounts for the development of inflammatory lesions. Both CD4+ and CD8 + T cells bear suppressor or regulatory properties. Therefore, a lack of balance between protective and deleterious effects is most likely implicated in the disease pathogenesis.

The main types of regulatory CD4+ T cells (Tregs), expressing the transcription factor forkhead box protein P3 (FoxP3) and the IL-2 receptor CD25, have been mostly studied in mice (Hori et al., 2002; McGeachy et al., 2005). The adoptive transfer of CD4+ CD25+ T was shown to prevent the development of spontaneous EAE (Hori et al., 2002). In addition, the inactivation or the depletion of Tregs cells in mice results in an increased susceptibility to EAE (McGeachy et al., 2005), and their accumulation

in the CNS correlates with a recovery from EAE (Korn et al., 2007; McGeachy et al., 2005). Suppressive CD8+ T cells, in EAE, were also found to protect against the disease exacerbations (Jiang et al., 1992) and to prevent the disease recurrence, by inhibiting MBP-activated CD4+ T cells in the periphery (Jiang et al., 2003). Studies in vivo demonstrated that MS patients' Tregs have an impaired capacity to suppress activated T cells as compared to healthy controls (Viglietta et al., 2004).

1.3.2 B-Lymphocytes

The presence of IgG oligoclonal bands in vast majority of the patients' CSF strongly suggests that B cells and antibodies are important players in the disease pathogenesis (Freedman et al., 2005). Although their exact contribution remains unclear, their role is supported by several pathology studies.

Antibodies deposits have been observed in MS brains active lesions (Lassmann et al., 2001), and B cells, producing anti-myelin antibodies, have been detected in the patients' CSF (Mancardi et al., 2000). Indeed, the number of B cells in the CSF seems to correlate with the disease severity (Berger et al., 2003) and with the degree of brain inflammation, detected by MRI (Kuenz et al., 2008). Plasma cells are observed in large numbers in the perivascular spaces, within the demyelinated plaques (Henderson et al., 2009). In addition, meningeal B cells "lymphoid-like follicles" have been recently identified in a subset of MS patients (Magliozzi et al., 2007; Serafini et al., 2004), and their presence associates with a more rapid disease course (Howell et al., 2011). Finally, therapeutic B cells depletion was shown to improve the disease activity (Hauser et al., 2008), further highlighting their important contribution to the demyelination.

B cells participate to the MS pathology primarily by producing autoantibodies. These accumulate in the brain demyelinated areas and participate to the BBB dysfunction (Barnett et al., 2009; Lassmann et al., 2001). However, despite a variety of antibodies against CNS antigens have been detected in MS patients (Archelos et al., 2000),

these are also found in healthy controls (Karni et al., 1999), leaving their pathological role controversial. In addition to the antibodies production, B cells might contribute to the inflammation by acting as antigen presenting cells (APCs) (Sospedra and Martin, 2005), by recruiting auto-reactive T cells to the CNS (Lou et al., 2000) and by producing pro-inflammatory cytokines (Alter et al., 2003).

1.3.3 Cytokines

Cytokines have a primary role in driving all phases of the immune responses. To maintain homeostasis, a balance between pro- and anti-inflammatory cytokines is required. IFN-γ and TNF-α are the two most important pro-inflammatory cytokines and they are produced by both inflammatory cells and by tissue components. They are known to participate to the pathogenesis of MS, by activating certain components of the immune system and by propagating the immune reaction and inflammation.

1.3.3.1 TNF-α

TNF-α is produced by a variety of cell types, including T cells, astrocytes and microglia (Hanisch, 2002; Locksley et al., 2001). Elevated serum (Hohnoki et al., 1998) and CSF (Maimone et al., 1991) levels have been reported in MS patients and its concentration was shown to correlate with the disease severity (Sharief and Hentges, 1991). In addition, TNF-α expression has been detected in active MS lesions, within the CNS (Hofman et al., 1989; Selmaj et al., 1991a). Studies in vitro demonstrated that its pathological effect is primarily exerted by damaging the myelin and by promoting apoptosis (Selmaj et al., 1991b; Selmaj et al., 1990). TNF-α was also shown to cause neurotoxicity, leading to the block of the axons conduction (Redford et al., 1997).

1.3.3.2 IFN-γ

The results of a therapeutic trial with IFN-γ in MS patients demonstrated a worsening of the disease course (Panitch et al., 1987). Therefore, IFN-γ has a pathogenic role in the disease, which is probably exerted by increasing MHC class II expression and by preventing the remyelination (Lin et al., 2006). However, data on the IFN-γ blood concentration in MS patients are contradictory. Some studies showed higher levels compared to normal controls (Hohnoki et al., 1998) others found no differences (Nguyen et al., 1999).

IFN-γ expression in the CNS of EAE was found to increase during disease exacerbations and to decrease during remissions (Begolka and Miller, 1998). Nevertheless, IFN-γ knockout mouse were shown to develop a more aggressive form of EAE (Ferber et al., 1996), suggesting a potential regulatory role of IFN-γ on other anti T cell proliferative cytokines (Badovinac et al., 2000) and on T cells apoptosis (Chu et al., 2000).

1.4 The pathology of Multiple Sclerosis

Since its first description, MS has been recognized as an inflammatory disease of the CNS, with focal destruction of myelin sheaths (Charcot, 1880). The demyelinating process, primarily driven by Th1 cells, may occur at any place, within the CNS, and it is accompanied by astrocytosis, leading to the formation of sclerotic plaques (Lucchinetti et al., 2000). Although myelin represents the primary target of the autoimmune reaction, axons, nerve cells and astrocytes can also be affected to a lesser degree (Kornek and Lassmann, 1999; Lassmann et al., 2001).

The demyelination causes impairment of the physiological saltatory nerve conduction, eventually leading to the appearance of acute symptoms. Neurodegenerative processes, causing axonal loss, drive the accumulation of the chronic, irreversible disability (Bitsch et al., 2000; Trapp et al., 1998).

1.4.1 The white matter pathology

The demyelinated, sclerotic, plaques are the pathological hallmark of MS. Lesions present as areas of focal damage, involving the white matter, widely varying in size and location. Although normally distributed throughout the CNS, inflammatory lesions show a predilection for the optic nerves, the periventricular regions, the corpus callosum, the cerebellum and the cervical cord (Lassmann et al., 2001).

1.4.1.1 Stage dependent features

The pathological features of demyelinating plaques are stage dependent and, to some extent, reflect the evolution of the disease course (Frischer et al., 2009; Lassmann et al., 2007). The inflammation dominates during the RR phase and leads to the formation of new white matter lesions. When the disease enters in the progressive phase, new inflammatory lesions become rare and the diffuse atrophy,

accompanied by changes of the normal appearing white matter (NAWM), are the prominent features (Lassmann et al., 2007).

A**ctive lesions** characterize the pathology of acute MS and RR MS (Lassmann et al., 2007). Their boundaries are irregular and ill defined, and the inflammation is accompanied by a large disruption of the BBB, allowing the peripheral inflammatory cells to enter and to target the CNS (Hochmeister et al., 2006; Kirk et al., 2003). High numbers of lymphocytes and pro-inflammatory cytokines are observed, indicating an on going demyelination (Lassmann et al., 2007). The tissue damage results from the cytotoxic activity and from the deposition of antibodies. Macrophages and microglial cells are responsible for the removal of the myelin debris (Frohman et al., 2006b). Axonal damage can be found within the early active lesions (Trapp et al., 1998).

Chronic active lesions are mainly observed during the progressive phase of the disease (Lassmann et al., 2007). They display a sharp edge, with perivascular cuffs of infiltrating cells, macrophages, astrocytosis and some degenerating axons (Frischer et al., 2009). In contrast with acute lesions, demyelination is primarily associated with Ig deposition, and an increased number of oligodendrocytes, indicating remyelination, can be observed. The centre is relatively hypo-cellular and contains naked axons, surrounded by a matrix of scaring astrocytes (Frohman et al., 2006b).

Chronic inactive lesions are found in the latest stage of the disease. The edge appears much sharper and the astroglial scar tissue is abundant. Only few microglia activated cells contain myelin degradation products, suggesting a slow rate of on going demyelination (Lassmann et al., 2007). The number of demyelinated axons and oligodendrocytes is greatly reduced, and blood vessels walls appears thickened, surrounded by occasional lymphocytes (Frohman et al., 2006b).

"Shadow lesions" are areas of remyelination, commonly occurring during the early stage of RR MS and becoming less frequent as the disease progresses (Franklin, 2002). These lesions are typified by thinner myelin sheaths, and are associated with extensive macrophages infiltration (Prineas et al., 1993).

1.4.1.2 Active lesions, mechanisms dependent features

The patterns of demyelination are different among MS subgroups, implying multiple mechanisms, accounting for the heterogeneity of the lesions. These include macrophages activation (Bruck et al., 1995; Ulvestad et al., 1994), involvement of cytotoxic cytokines (Hofman et al., 1989) and deposition of demyelinating antibodies or complement components (Mancardi et al., 2000; Serafini et al., 2004; Storch et al., 1998). In addition, the pathogenesis of the demyelination is also conditioned by other factors responsible for the amplification of the chronic inflammatory reaction (Lassmann et al., 2001).

Indeed, a profound heterogeneity characterizes the immunopathological appearances of MS active lesions (Lucchinetti et al., 2000). An extensive work on a large series of biopsy and autopsy material, carried out by Lucchinetti et al., allowed to characterize four different types of lesion. Classification was based on the distribution and the extension of the myelin loss, the pattern of the oligodendrocytes destruction and the extent of the Ig and of the complement deposits distribution (Lucchinetti et al., 2000).

Type I lesions: the demyelination is mainly characterized by a T-cells and macrophages infiltration, without evidence of significant antibodies or complement deposition. Lesions show the typical perivenous distribution of inflammatory cells and the extent of tissue damage correlates with the amount of cytokines.

Type II lesions: the active perivenular demyelination is characterized by T-cells and macrophages, with an extensive antibodies deposition and accentuated Ig reactivity. The large amount of complement C9 neoantigen, at the sites of the myelin destruction, highlights the relevant role played by antibodies in the development of this type of lesions.

Type III lesions: similarly to type I and II lesions, the inflammatory infiltrate is mainly composed by T lymphocytes and macrophages, but Ig deposition is absent. Typically,

the demyelination is not centred on inflamed blood vessels. The myelin rim remains preserved around the vessels within the plaques, and the contours of the lesions are well defined.

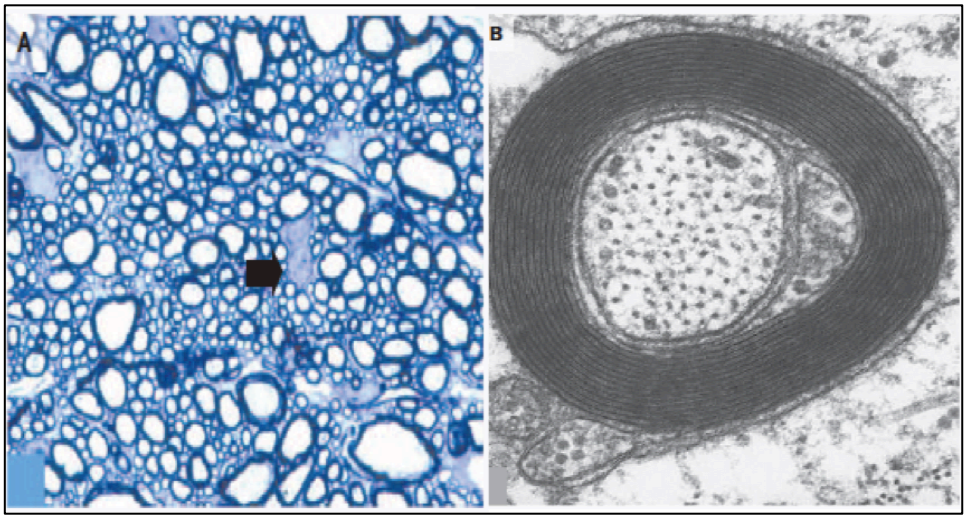
Type IV lesions: are exclusively present in PP MS patients. They present, similarly to type I and II lesions, with a T cells and macrophages inflammation, mainly distributed around blood vessels. They lack Ig deposition and they are typically characterized by a large extent of oligodendrocytes damage.

Interestingly, a unique study reported pathological features of 12 patients who died during the RR phase, shortly after the onset of a relapse. These allowed to gather important information on mechanisms leading to the development of inflammatory lesions during the very active stages of the disease, and responsible of the acute symptoms (Barnett and Prineas, 2004). Some of the lesions associated with a fatal outcome and, unlike what observed in EAE, were characterized by an extensive oligodendrocytes apoptosis and microglia activation, but few or no infiltrating lymphocytes (Barnett and Prineas, 2004).

1.4.1.3 Axonal injury

Within the CNS, oligodendrocytes-myelin-axon units represent the key functional structures. Myelin, produced by the oligodendrocytes, spirally wraps the axons (Figure 1.6), creating periodic gaps (nodes of Ranvier). Its main physiological function is to accelerate the conduction velocity of the axonal action potential, propagating by a saltatory mode (from one node to another) (Hartline and Colman, 2007). In addition, oligodendrocytes and the myelin exert a protective and a trophic effect on the axons (Brady et al., 1999).

Figure 1.6 A) The myelin sheaths (dark blue rings), surrounding axons in the normal white matter. The arrow indicates the oligodendrocytes, producing the myelin. **B**) The myelin sheath in a cross section, wrapping the axon (Frohman et al., 2006b).



Although the demyelination has been identified as the hallmark feature of MS since the earliest report (Charcot, 1880), it is now well established that the acute axonal pathology occurs in active demyelinating lesions and the axonal density is reduced in chronic plaques (Trapp et al., 1998). The axonal loss is considered the essential cause of the development of unremitting clinical disability and of the disease progression (Trapp and Nave, 2008). Imaging studies with MRI (Bakshi et al., 2008; Filippi et al., 2003) and with magnetic resonance spectroscopy (MRS) (Narayana, 2005; Tartaglia and Arnold, 2006), and pathological studies (Bjartmar et al., 2000; Stadelmann and Bruck, 2008; Trapp et al., 1998) demonstrated that the axonal damage occurs already during the early phase of the disease. The axonal degeneration takes relatively long time, compared to myelin disruption, reflecting the variable axonal density observed in inactive lesions, ranging from nearly normal to a loss of 80%, averaging 60-70% (Bjartmar and Trapp, 2001; Mews et al., 1998).

1.4.1.3.1 Mechanisms of axonal loss

Mechanisms causing the neurodegeneration and the axonal loss remain unclear (Dutta and Trapp, 2011). It has been hypothesized that the axonal injury might be partially explained by the increased expression of sodium channels in the membranes, attempting to re-establish the normal conduction (Waxman et al.,

2004). The sodium in excess slows the nerve conduction and triggers an intracellular cascade of calcium-mediated injury, eventually leading to the neurodegeneration (Waxman et al., 2004). An excessive production of glutamate has also been shown to damage the oligodendrocytes and the axons by promoting a toxic calcium intracellular accumulation (Micu et al., 2006). Indeed, sodium-channel blocker (phenytoin) was shown to preserve axons in EAE (Lo et al., 2003), lending support to this hypothesis. These analyses put the basis for the "mitochondrial theory", which is based on the assumption that a rise in the intracellular calcium level causes a mismatch between the energy demand and the reduced supply of adenosine triphosphate (ATP), ultimately leading to a mitochondrial dysfunction and to the degeneration of chronically demyelinated axons (Dutta et al., 2006).

Infiltrating inflammatory cells may also have a direct toxic effect on axons by releasing proteolytic enzymes, cytokines and oxidative products (Trapp and Nave, 2008). An immunological attack on the axon is suggested by the correlation between the inflammation and the axonal transection (Trapp and Nave, 2008; Weiner, 2009), and between the number of infiltrating CD8+ T cells and the extent of the axonal loss (Dutta and Trapp, 2011; Frischer et al., 2009). This might be mediated by TNF-α and by Interferon-γ, both stimulating the release of nitric oxide (NO) from the astrocytes and from the macrophages (Redford et al., 1997; Smith et al., 2001). However, another study failed to find a correlation between the axonal damage and the TNF-α/NO expression, indicating a possible dissociation between mechanisms causing the demyelination and those responsible for the axonal injury (Bitsch et al., 2000).

1.4.1.3.2 Axonal loss in the normal appearing white matter (NAWM)

Whether inflammatory mechanisms are essential for the axonal damage remains a matter of large debate. Some studies reported a strong correlation between the number of inflammatory cells and the axonal damage, even in the NAWM, during both the early and the late stage of the disease (Frischer et al., 2009; Howell et al., 2011). However, extensive axonal loss can also occurs within the NAWM, in the absence of inflammation (Bjartmar et al., 2001; DeLuca et al., 2006; Evangelou et al.,

2000; Frischer et al., 2009; Trapp et al., 1998), suggesting that axons can degenerate independently of the active demyelination (Bitsch et al., 2000).

Trapp and colleagues demonstrated axonal transection in the demyelinated white matter and in the NAWM of patients with variable disease duration, ranging from 2 weeks to 27 years (Trapp et al., 1998). Similarly, Bitsch et al., by analysing a large group of brain biopsies from MS patients, reported axonal damage in areas of the NAWM and within remyelinated lesions, during all the disease stages (Bitsch et al., 2000). Furthermore, Evangelou et al. demonstrated a substantial (50%) loss of both axonal density and volume in the NAWM of the corpus callosum, among 8 patients with 21 years mean disease duration (Evangelou et al., 2000). Axons degeneration in the NAWM, distal to a fatal brainstem lesion, was also described in a patient with very aggressive and short disease course (Bjartmar et al., 2001). Finally, the axonal injury in the NAWM appears to be more diffused among progressive patients, compared to patients with acute or relapsing disease (Kutzelnigg et al., 2005).

Spinal cord pathological analyses offered further evidences. Lovas et al. studied samples from the spinal cord of SP MS patients', where one side contained a completely demyelinated plaque and the contralateral corresponding area had NAWM. This approach allowed to compare the axon density, which was found to be similarly reduced in both the demyelinated and the normal white matter (Lovas et al., 2000). A much larger study (n = 55) showed a lack of correlation between the plaques (active and inactive) load both, in the cerebral hemispheres and in the spinal cord, and the extent of the axonal loss at any level of the cortico-spinal tract (DeLuca et al., 2006). In addition, this study confirmed a similar extent of the axonal damage between chronic plaques and the NAWM (DeLuca et al., 2006).

1.4.2 Grey matter pathology

The cortical demyelination in MS has been described for decades (Brownell and Hughes, 1962), however its importance was initially underestimated. This is because grey matter lesions are not easily detectable in autopsy studies, due to their hypo-cellularity (Dutta and Trapp, 2007), or by routine MRI procedures, due to the poor contrast resolution and the relatively lack of BBB disruption (Kidd et al., 1999).

Although they can be detected throughout the CNS, grey matter lesions seem to have a predilection for the cingulate gyrus and for the temporal and frontal lobes (Bo et al., 2003; Gilmore et al., 2009). The extent of the grey matter pathology does not appear to correlate with the extent of the white matter demyelination (Bo et al., 2007; Kutzelnigg et al., 2005), suggesting that the two processes might occur independently.

Following a study on autopsy tissues, from 50 MS patients, three types of cortical lesions have been described, based on their localization in the cortex (Peterson et al., 2001):

- **Type I lesions** accounted for 34% of the cortical lesions. They typically encompass both the white matter and the cortex and they can be rather extensive.
- **Type II lesions** accounted for 16% of the cortical lesions. They are much smaller and reside entirely within the grey matter.
- **Type III lesions** are the most abundant, accounting for 50% of the cortical lesions. They extend from the pial surface into the cortex and they are large, sometimes involving all cortical layers.

The grey matter pathology occurs in the absence of BBB disruption (Bo et al., 2003; Kidd et al., 1999; van Horssen et al., 2007) and, compared to the white matter pathology, it associates with much lower inflammatory infiltrating cells and very few perivascular cuffs (Peterson et al., 2001). In addition, in the grey matter damaged areas there is extensive axonal loss (Peterson et al., 2001), which is though to be

driven by activated microglia (Bo et al., 2003; Peterson et al., 2001) and it was found in increased density at the leading edge of active type I cortical lesions (Peterson et al., 2001).

The cortical demyelination was reported to be strikingly different in relation to the type of the disease course (Kutzelnigg et al., 2005). It was rare or absent in patients with acute or RR MS, but was described as a prominent feature in patients with SP and PP MS, where it was associated with high degree of meningeal inflammation (Howell et al., 2011; Kutzelnigg et al., 2005). These results suggested that the cortical pathology might be the main driver of the disability accumulation, among progressive patients (Howell et al., 2011; Kutzelnigg et al., 2005).

However, a recent large biopsy study demonstrated variable degree of cortical demyelination, with diffuse inflammatory infiltrates, in nearly 40% of patients during the early stage of the disease (Lucchinetti et al., 2011), implying that the cortex might be an early target of the MS pathogenic mechanisms. Indeed, cortical lesions and atrophy are detected by the MRI in early disease (Amato et al., 2004a; Calabrese and Gallo, 2009), and might represent the pathological substrate of the early cognitive impairment (Calabrese et al., 2009a).

1.4.2.1 Meningeal inflammation

Since B-cell follicle-like structures have been described in the meninges of a significant number of SPMS patients (Howell et al., 2011; Magliozzi et al., 2007; Magliozzi et al., 2010; Serafini et al., 2004), the meningeal inflammation has gathered increasing attention as important player in the induction and propagation of the cortical pathology (Lassmann, 2012).

Follicles were mainly found in the frontal, temporal, parietal lobes and in the cingulate gyrus. They contained prominent inflammatory cells, comprising CD3+ T cells, CD20+ B cells, plasma cells and large perivascular B-cells aggregates (Magliozzi et al., 2007). Importantly, follicles positive patients, compared to follicles negative,

were younger at the clinical onset, were younger at the time they became wheelchair bound and were younger at death (Magliozzi et al., 2007), indicating that the presence of follicles associates with a worse clinical outcome. In addition, follicles were also accompanied by a significantly increased number of grey matter lesions and by a larger extension of grey matter demyelination (Magliozzi et al., 2007; Magliozzi et al., 2010). Furthermore, activated microglia and the axonal loss were significantly increased in cortical lesions of follicles positive patients (Magliozzi et al., 2010). These results were lately expanded and confirmed by assessing more cases from the same series (Howell et al., 2011). The analysis was also repeated in a subgroup of PP cases only, where follicles were not found, but the diffuse meningeal inflammation correlated with the degree of the cortical demyelination and of the axonal loss (Choi et al., 2012). Similarly, a separate analysis from Lassmann's group confirmed that the diffuse inflammation in the meninges strongly associates with the extent of the grey matter pathology, in progressive MS (Frischer et al., 2009).

Overall, these data indicate that the meningeal inflammation is the key driver of the cortical demyelination. By the time the disease enters in the progressive phase, the inflammation becomes increasingly compartmentalised in the perivascular and subarachnoid spaces, behind a relatively intact BBB (Frischer et al., 2009; Meinl et al., 2008). In addition, the inflamed meninges, by diffusing proinflammatory and cytotoxic factors, activate the microglia, which participate to sustain the cortical damage (Frischer et al., 2009; Magliozzi et al., 2007).

However, this conclusion was lately challenged by results from a Dutch study (Kooi et al., 2009). Among 28 patients with progressive disease, the percentage areas of subpial demyelination did not correlate with the extension of the meningeal inflammation. In addition, authors failed to demonstrate a significant increase of leptomeningeal B-cells follicles, leaving their pathogenic role still unclear (Kooi et al., 2009).

1.4.3 Primary versus secondary progression

The axonal loss is considered the pathological substrate of the progressive accumulation of disability (Trapp and Nave, 2008). It was described at the sites of white matter demyelination (Frischer et al., 2009; Trapp et al., 1998) but it is also known to occur within the NAWM (Bitsch et al., 2000; Bjartmar et al., 2001; DeLuca et al., 2006; Evangelou et al., 2000). The controversial relationship between inflammation and neurodegeneration has been largely debated (Lassmann, 2012; Trapp and Nave, 2008). Within this context, whether PP MS shares the same pathological features of SP MS becomes particularly relevant.

Pathology assessments confirmed that brain inflammatory lesions characterize both subtypes, indicating that also PP MS is an inflammatory disease (Revesz et al., 1994). The pattern of tissue injury observed in the progressive subtypes is generally similar and it is characterized by demyelination, loss of oligodendrocytes and axons, remyelination and gliosis (Frischer et al., 2009; Prineas et al., 2001). A recent study directly compared pathological features of PP and SP MS, by assessing the extension of the axonal loss in the cervical cord's cortico-spinal tracts of 47 patients, who had reached similar levels of disability before death (Tallantyre et al., 2009). Patients with SP MS were found to have a larger extent of the white and grey matter demyelination, however the extent of the axonal damage within the demyelinated areas was larger in PP MS patients. Interestingly, the degree of the axonal loss within the NAWM was similar between the two subtypes (Tallantyre et al., 2009).

In contrast, by comparing 35 SP and 13 PP brains, Lassmann's group found no differences in the extent of axonal damage within demyelinated areas and within the NAWM (Frischer et al., 2009). More importantly, this study established a correlation between the number of inflammatory cells and the acute axonal damage, in both progressive subtypes, suggesting that the inflammation, compartmentalized behind a repaired BBB, is the driver of the disability accumulation during the late disease stage (Frischer et al., 2009). Finally, a large biopsy study, analysing samples from 45

patients at different stages of the disease course, found more pronounced axonal damage within the demyelinated areas among SP MS patients and very limited among PP MS patients (Bitsch et al., 2000). In addition, authors observed a partial dissociation between the extent of inflammatory infiltrates and the axonal loss, suggesting that the two processes might be driven by different mechanisms (Bitsch et al., 2000).

1.5 The MRI

The use of conventional magnetic resonance imaging (MRI) has revolutionized the management of MS. It is sensible to MS-related abnormalities, it is non-invasive and it is reproducible. Therefore, this technique now plays a pivotal role in diagnosing the disease, and in monitoring the disease progression and the efficacy of treatments.

1.5.1 MRI and the MS pathology

Although the MRI allows to estimate the extent of the focal and diffuse CNS involvement in MS patients, it does not specifically mirror the pathological substrates of the disease (Filippi et al., 2012). The T2-weighted scans can easily detect the white matter demyelinated areas, which appear as foci of hyper-intensity, however they lack specificity for the changes in the axonal density (van Waesberghe et al., 1999). In addition, the focal white matter lesions have limited pathological specificity and can be seen in other diseases (Barkhof and van Walderveen, 1999). Any alteration of the tissue composition, due to inflammation or to axonal injury, causes an increased T2 signal (Barkhof and van Walderveen, 1999). Therefore, when assessing the radiological features of MS, complementary information on the lesions shape, location, and size has to be considered (Fazekas et al., 1999). MS lesions are commonly round or ovoid shaped and their size ranges from few mm to more than 1 cm. They typically locate in the brainstem, the cerebellum, around the corpus callosum and in the periventricular white matter. The confluence of peri-venular lesions results in irregular extensive areas of signal abnormality (Fazekas et al., 1999).

In contrast to the T2-weighted scan, the T1-weighted scans allow to identify the axonal damage and the areas of severe tissue destruction with higher reliability (Bruck et al., 1997; van Waesberghe et al., 1999; van Walderveen et al., 1998). These appear as hypo-intense foci, also known as persisting “black holes” (Fazekas et al.,

1999). Biopsy (Bruck et al., 1997) and autopsy (van Walderveen et al., 1998) studies showed that the degree of hypo density correlates with the degree of loss of axons. However, the T1 hypo-intensities have variable duration: a third lasts for more than 4 years, while half disappear within 1 year (Bagnato et al., 2003). In addition, it has been demonstrated that at least 25% of gadolinium (GAD)-enhancing lesions will eventually resolve to become a T1 lesion (Bagnato et al., 2003). Different factors contribute to the increase of extracellular free water and, consequently, to the increase of the T1 hypo-intensity: active demyelination, cellular infiltration, oedema and astrogliosis (Bruck et al., 1997). Therefore only "black holes" that stay persistent over time reflect an irreversible tissue destruction (Bitsch et al., 2001). The magnetization transfer ratio (MTR) is also highly sensitive to changes of the axonal density and is a reliable marker of the tissue damage (Barkhof and van Walderveen, 1999; Schmierer et al., 2007; van Waesberghe et al., 1999)

On the T1-weighted images the acute and chronic lesions can be differentiated, after the administration of GAD. Following the BBB breakdown, resulting from the inflammatory process, the GAD penetrates the CNS and is enhanced by the active lesions (Barkhof and van Walderveen, 1999; Filippi et al., 2012). Many methodological factors can potentially affect the gadolinium enhancement: the dose of GAD, the timing of the scan and the slices thickness (Filippi, 2000; Thompson et al., 1992). The pattern of the enhancement depends on the size and on the intensity of the inflammation. It can be nodular or ring-like, it can persist for 2 to 6 weeks (Filippi, 2000; Thompson et al., 1992) and it can change in the same lesion within short time (Filippi et al., 2012). Active lesions predominate in the RR phase and are the pathological substrate of the clinical attacks (Frank et al., 1994; Kappos et al., 1999; Weiner et al., 2000), while chronic slowly expanding lesions are typical features of the progressive phase, and do not show any contrast enhancement (Filippi, 2000). During the late stages of the disease, when the BBB damage is missing or less prominent and the GAD cannot penetrate the CNS, the MRI becomes less able to detect any active inflammation (Filippi et al., 2012). In addition, conventional MRI has limited power to detect the demyelination occurring in the grey matter, as the focal cortical demyelinating lesions are difficult to be visualized on scans because

of their poor contrast with the surrounding normal grey matter (Geurts et al., 2012; Seewann et al., 2011). However, the use of double inversion recovery (DIR) sequences allows to better characterize the extension of the cortical damage (Calabrese et al., 2007b; Geurts et al., 2011).

1.5.2 MRI and the diagnosis of MS

The MS inflammatory lesions develop at different times and involve different anatomic locations within the CNS. Therefore, the dissemination in time and space of the demyelinating processes, and the exclusions of other conditions potentially mimicking MS, are the three essential requirements for the diagnosis of the disease.

Before the advent of the MRI, Schumacher was the first to formulate internationally accepted criteria for diagnosing MS (Schumacher et al., 1965). Clinically definite MS was defined by objective signs of CNS dysfunction, evidence of two or more neurological sites involvement and at least 2 distinct (separated by at least 6 months) inflammatory episodes or slow progression over 6 months (Schumacher et al., 1965). Following the increasing availability of the neuroimaging in the late 1970s, the MRI was recognized as important tool for assessing the disease burden (Young et al., 1981) and, for the first time, it was introduced into the diagnostic process by Poser (Poser et al., 1983). The MRI was considered a “paraclinical” test, which could be used in support of the diagnoses of clinically definite MS, laboratory supported definite MS and clinically probable MS (Poser et al., 1983)

1.5.2.1 The McDonald criteria

The rapid recognition of the MRI as important diagnostic tool led to the development of the first radiological diagnostic criteria. The fulfilment of these criteria required the presence of at least 3 lesions located in the periventricular and infratentorial regions, within the CNS white matter (Fazekas et al., 1988; Paty et al., 1988). In 1997, Barkhof et al., by conducting a 3 years prospective study on CIS

patients, identified the MRI features which associated with an increased risk of conversion to clinically definite MS: at least one GAD enhancing lesion, at least one infratentorial lesion, at least one subcortical lesion and three or more periventricular lesions (Barkhof et al., 1997). These criteria were lately modified by Tintore' et al. It was concluded that, if GAD enhancing lesions are not present on the scan, the occurrence of at least 9 T2 lesions would have the same predictive value. In addition, it was proposed that the fulfilment of at least 3 of the 4 Barkhof's parameters allowed an accurate prediction of the conversion to clinically defined MS (Tintore et al., 2000). These guidelines put the basis for the development of the McDonald criteria (McDonald et al., 2001), which dramatically redefined the diagnostic process. The widespread use of DMTs, exerting most of their efficacy in the early stage of the disease, brought attention to the need of an early diagnosis, among those patients without a second clinical attack (CIS). According to the new diagnostic guidelines, the Barkhof and Tintore' MRI criteria (Barkhof et al., 1997; Tintore et al., 2000) could be used for demonstrating that the demyelinating process involved at least two distinct anatomic locations and occurred in two separate times, at least 3 months apart (McDonald et al., 2001) (Table 1.1).

The incorporation, in the diagnostic algorithm, of the MRI criteria was welcomed but also received some criticisms. The radiological criteria had poor specificity for making a correct differentiation from other diseases (Poser and Brinar, 2004). Furthermore, they were derived from patients with CIS, and therefore were predictive of the risk of having MS but not truly diagnostic. The lack of specificity about the qualitative description of the lesions and the lack of systematic consideration of MRI findings at the spinal level represented additional drawbacks (Poser and Brinar, 2004). Indeed, when retrospectively applied to other CIS cohorts, the 2001 McDonald criteria showed a high specificity for conversion to clinically definite MS but a modest sensitivity for detecting and treating most of those at risk of MS (Dalton et al., 2002; Tintore et al., 2003).

Table 1.1 MRI criteria for dissemination in time and in space incorporated in the McDonald diagnostic criteria 2001 (McDonald et al., 2001).

MRI criteria for dissemination in space	MRI criteria for dissemination in time
Three of four of the following	1) If a first scan occurs 3 months or more after the clinical onset, the presence of a GAD-enhancing lesion is sufficient to demonstrate dissemination in time, provided that it is not at the site involved at the onset event. If there is no GAD-enhancing lesion at this time, a follow up scan is required after 3 months. A new T2- or GAD enhancing lesion at this time then fulfills the criterion for dissemination in time.
1) One GAD-enhancing lesions or nine T2-hyperintense lesions if there is no GAD-enhancing lesion	
2) At least one infratentorial lesion	2) If the first scan is performed less than 3 months after the onset of the clinical event, a second scan done 3 months or more after the clinical event showing a new gadolinium-enhancing lesion provides sufficient evidence for dissemination in time. However, if no enhancing lesion is seen at this second scan, a further scan not less than 3 months after the first scan that shows a new T2 lesion or an enhancing lesion will suffice.
3) At least one juxtacortical lesion	
4) At least three periventricular lesions	

Based on this concerns, the McDonald criteria were revised in 2005. The spinal cord lesions were included among the parameters that could be used for confirming the dissemination in space (Polman et al., 2005b). In addition, less restrictive criteria were applied for demonstrating the dissemination in time, which could be confirmed by the appearance, after at least 30 days, of new T2 lesions (Polman et al., 2005b). The 2005 McDonald criteria had an improved sensitivity, while maintaining a good specificity (Swanton et al., 2007).

1.5.2.2 The current guidelines

The key to all diagnostic criteria remains the absence of an alternative diagnosis in the context of an appropriate clinical presentation. The MAGNIMS (European Network for Magnetic Resonance Research in MS) criteria were recently developed with the aim of simplifying the diagnostic process, without reducing its specificity (Montalban et al., 2010). According to the new guidelines, for demonstrating the dissemination in space only one parameter is sufficient: the occurrence of at least one T2 lesion in more than two of the four typical locations (periventricular, subcortical, infratentorial and spinal cord). For demonstrating the dissemination in time two criteria can be applied: a) the simultaneous presence of gadolinium enhancing and non enhancing lesions at any time from the clinical onset; b) one new T2 lesion and/or new gadolinium enhancing lesion on follow up MRI at any time from the clinical onset (Montalban et al., 2010).

After the publication of the 2005 McDonald criteria, further studies emphasized the importance of a new T2 lesion, irrespective of whether this was detected within or after 30 days from the onset attack, to predict the conversion to clinically definite MS (Swanton et al., 2007; Tur et al., 2008). For this reason, the MAGNIMS diagnostic criteria were developed for allowing to gather evidence of the dissemination in time and space on a MRI scan performed at any time after the clinical onset (Montalban et al., 2010). The new criteria could be applied even when a new GAD enhancing lesion was considered a proof of the dissemination in time, with important practical implications for the diagnosis of MS in CIS patients (Montalban et al., 2010). The simultaneous presence of GAD enhancing and non enhancing lesions, on the first MRI scan, can substitute for a follow up scan to confirm the dissemination in time and to make the diagnosis. Based on these new MRI parameters (Table 1.2), in 2010 the McDonald criteria were modified again and now represent the most updated guidelines for diagnosing the disease (Polman et al., 2011).

Table 1.2 MRI criteria for dissemination in time and in space incorporated in the McDonald diagnostic criteria 2010 (Polman et al., 2011).

MRI diagnostic criteria	
Dissemination in space of lesions	**Dissemination in time of lesions**
More than one T2 lesion in at least 2 of 4 typical locations for MS:	
Pervientricular Subcortical Infratentorial Spinal cord	Simultaneous presence of asymptomatic GAD enhancing and non enhancing lesions at any time A new T2 lesion and/or GAD enhancing lesion(s) on follow up MRI, with reference to a baseline scan, irrespective of the timing of the baseline MRI

1.5.3 Monitoring the treatment response

There are no internationally accepted guidelines on the use of MRI for monitoring the disease activity. It is common practice in some medical systems to arrange a brain MRI scan in patients who are to start a new therapy or to change therapy (Rocca et al., 2013). However, the MRI criteria for assessing the response to the

treatment and the definition of treatment failure vary among practitioners (Giacomini et al., 2009). Some consider the worsening of the MRI activity not relevant to the decision to change therapy, when it occurs in the context of a clinically stable disease. Others argue that even a single new MRI lesion indicates an active disease and therefore a poor response to the therapy.

Indeed, the response to IFN beta, based on MRI inflammatory parameters, is highly heterogeneous (Kappos et al., 2006c; Rio et al., 2008; Rudick et al., 2004; Sormani et al., 2005), reflecting the variable definitions of "responders". Among patients enrolled in the original IFN beta-1a trial (Jacobs et al., 1996a), the presence of more than 1 new gadolinium enhancing lesion or more than 3 new T2 lesions was associated with a higher risk of experiencing disability progression over a period of 2 years (Rudick et al., 2004). These data were lately extended and confirmed after 15 years follow up (Bermel et al., 2013). Similar result were obtained by analysing data from the PRISMS trial (1998a). The median number of T2 lesions accumulated over 8 years from the commencement of the therapy was found to be an indicator of the EDSS worsening (Kappos et al., 2006c). Another study demonstrated that the risk of poor response to the treatment (increased disability after 2 years of treatment) was higher among those with 3 or more new lesions within 12 months from the commencement of IFN beta (Rio et al., 2008). Chiu et al. longitudinally assessed, with monthly scans for 3 years, 15 patients treated with IFN beta-1b, highlighting the difficulties of measuring the disease activity with conventional MRI. The study confirmed the expected large heterogeneity of the response to the therapy at individual level. Only half of the patients experienced a reduction of the total number of GAD enhancing lesions (Chiu et al., 2009). In addition, the MRI responsiveness to the treatment changed over time. In some cases, the suppression of the active lesions was not sustained for long time, while in other patients with initial MRI activity it occurred after months from the commencement of the therapy (Chiu et al., 2009).

Among 222 RR patients treated with IFN beta-1b, Rio et al. assessed the MRI variables, which could predict the risk of experiencing a clinical worsening. Within 1

year from the commencement of the therapy, the presence of two or more active lesions (new or enlarging T2 lesions, or contrast enhancing lesions) associated with a higher probability of experiencing a new relapse and/or an accumulation of disability (Rio et al., 2009a). Based on these data, it was developed a decision making algorithm for the individual therapeutic management (Rio et al., 2009b). Patients should undergo neuroimaging assessments at the beginning of the treatment and at 6 and 12 months follow up. According to the algorithm, a change in the treatment strategy is recommended in patients developing three or more active lesions and/or experiencing a clinical deterioration (new relapses and/or disability progression). A worsening of the MRI parameters, in patients free of clinical activity, warrants a closely monitoring over time (Rio et al., 2009b).

1.5.4 The association between clinical and MRI activity

Among MS patients, the severity of the clinical picture does not always correlate with the extent of the CNS radiological involvement (Barkhof, 2002; Miller et al., 1996). New T2 lesions are known to occur 5-10 times more frequently than the clinically evident inflammatory attacks (Willoughby et al., 1989), indicating that the MRI inflammatory activity does not necessarily mirror the clinical activity (Barkhof, 2002; McDonald et al., 1994; Miller et al., 1996). This discrepancy became apparent in the early MRI studies (Thompson et al., 1990) and was then confirmed during the first trial of the IFN beta-1b, which profoundly reduced the MRI outcome measures but only modestly the relapse rate (Paty and Li, 1993). In addition, with the recent widespread use of the MRI in clinical practice, MRI abnormalities, highly suggestive of demyelinating disease, are often incidentally identified among subjects who never experienced clinical symptoms (radiologically isolated syndrome [RIS]) (Okuda et al., 2009). For these reasons, the prognostic relevance of an increased MRI inflammatory activity and the use of MRI for evaluating the disease course and the treatments efficacy is still debated among some researchers (Daumer et al., 2009).

Despite this, in the context of clinical trials, MRI remains widely used as surrogate marker for monitoring the disease activity (Cohen et al., 2012b) and for assessing the progression of disability (Sormani et al., 2011). By detecting the asymptomatic pathological changes of the disease, the MRI gives the opportunity to monitor the subtle degenerative process, much before it becomes clinically evident. The brain atrophy is considered a measure of tissue damage and it was consistently shown to occur at significantly faster rate among MS patients, compared to the healthy population (Luks et al., 2000; Rudick et al., 1999; Simon et al., 1999). Interestingly, the grey matter atrophy develops during the very early stage of the disease, independent of the enhancing lesions load (Calabrese et al., 2007a; Chard et al., 2002). Similarly, a significant cord atrophy was found in a large percentage of patients presenting with CIS (Brex et al., 2001) and significant grey matter lesions were found even in subjects with RIS (Giorgio et al., 2011).

The proton magnetic resonance spectroscopy allows the quantification of the extent of the axonal loss, by measuring the changes in the signal intensity of N-acetylaspartate (NAA), which decreases proportionally to the axonal damage (De Stefano et al., 1995). This technique allowed to confirm that the axonal pathology is an early event in the course of the disease (Trapp et al., 1998) and does not correlate with the baseline inflammatory lesions volume (De Stefano et al., 2003; Filippi et al., 2003). In addition, a decrease of brain NAA level was recently shown also in a group of subject with RIS, suggesting that axonal damage can be evident even when the disease has not clinically manifested yet (Stromillo et al., 2013). Taken together, this evidence strongly indicates that the degenerative process starts much before than its clinical counterpart and highlights the important role of the MRI for monitoring the disease activity since its early stage.

1.5.5 MRI and long-term prognosis

1.5.5.1 The T2 lesions

Although the baseline MRI T2 lesions load is a good predictor of the risk of conversion to CD MS (Barkhof et al., 1997), the MRI inflammatory measures only modestly correlate with the development of severe disability in the long term.

The predictive values of the number and the volume of T2 lesions were assessed in several studies. Among 30 patients originally enrolled in the IFN beta-1a trial (Jacobs et al., 1996a), an increased T2 lesion volume, over 2 years from onset, was associated with a more severe disability measured by the MSFC at 13 years follow up (Rudick et al., 2006a). In a cohort of 140 CIS patients, who never received DMT (Ormerod et al., 1987), clinical and MRI follow-ups were performed at different time points. The EDSS score at 14 years was available for 71 patients and it modestly correlated with the baseline lesions volume ($r = 0.48$) and number ($r = 0.47$) (Brex et al., 2002). From the same cohort (Ormerod et al., 1987) 107 patients were reassessed after 20 years, which represented the longest observation period ever reported in a CIS population with MRI examinations (Fisniku et al., 2008a). At the end of the follow up, 82% patients with abnormal baseline brain MRI scan had developed CD MS. Within this group, the spectrum of disability and the clinical course at year 20 largely varied: 42% had converted to SP MS and 39% had accumulated minimal disability (EDSS ≤ 3). The study demonstrated only a modest correlation between the number of T2 lesions at baseline and the EDSS score. There was a trend indicating that patients with a higher number of T2 lesions were somewhat more likely to be disabled after 20 years (18% with 1-3 lesions versus 45% with ≥ 10 lesions were scored at EDSS 6 or more), suggesting that lesions number has some predictive effect for late disability. However, 35% of those with ≥ 10 lesions accumulated not more than moderate disability and 18% had not developed CD MS, indicating that the predictive effect of MRI T2 lesions number at CIS presentation remains limited in the long term. The rate of lesions volume growth

was found to be a more reliable indicator of disease activity; those developing SP MS had a greater increase of lesions size, compared to patients who remained in the RR phase, and this difference was already evident 5 years after the CIS presentation. Data therefore suggested that slowing the lesions volume increase might delay or prevent the conversion to SP MS (Fisniku et al., 2008a). The 15 years follow up study of the patients enrolled in the Optic Neuritis Treatment Trial (ONTT) (Beck and Cleary, 1993) reported similar results. The EDSS was available for 113 subjects and the degree of disability was found to be unrelated to the number of baseline T2 lesions ($r = 0.07$).

The location of the MRI lesions was shown to influence the short-term disease evolution. Two separate studies found a correlation between the presence of T2 lesions in the infratentorial region on the baseline scan an the EDSS score at 5 years (Filippi et al., 1994) and at 10 years (Sailer et al., 1999) follow ups. Similarly, in a cohort of 42 patients followed up for 8 years, the risk of reaching moderate disability was significantly higher among those with at least two T2 infratentorial lesions (Minneboo et al., 2004).

1.5.5.2 The T1 lesions

The poor correlation between the long term disability accumulation and the T2 lesions is probably explained by their lack of pathological specificity (Barkhof, 2002; Filippi et al., 2012). In contrast, the T1 hypo-intense lesions have much higher sensitivity and specificity for the axonal damage (Bruck et al., 1997; van Waesberghe et al., 1999; van Walderveen et al., 1998) and were found to correlate more strongly with the development of disability. In a small group of 24 patients, the total number of T1 lesions was found to be associated significantly with the EDSS score ($r = 0.65$). The level of disability was higher among those with a larger “black holes” volume (Parry et al., 2002). Similarly, in a cohort of 46 patients, a higher T1 lesion load at baseline and a faster accumulation of hypo-intense lesions predicted a higher EDSS score at 40 months follow up (Truyen et al., 1996). However, the same authors failed to demonstrate a significant association between the T1 lesion volume and the EDSS

score in a separate study involving a larger number (n = 138) of patients (van Walderveen et al., 2001).

1.5.5.3 The grey matter pathology

The grey matter pathology is considered an important determinant of the irreversible disability (Geurts et al., 2012; Pirko et al., 2007). Several studies have demonstrated that the grey matter damage (Calabrese et al., 2007b; Calabrese et al., 2009b; Calabrese et al., 2013; Roosendaal et al., 2009) and the grey matter atrophy (Bakshi et al., 2001; De Stefano et al., 2003; Fisniku et al., 2008b; Roosendaal et al., 2011; Tedeschi et al., 2005) have a good correlation with the clinical outcome. Among 203 patients observed for 5 years, an increased number and volume of cortical lesions at baseline associated with a significantly higher EDSS score (Calabrese et al., 2012). Authors noted that, among those with worse outcome, the cortical involvement increased significantly more quickly over time, suggesting that the evolution of the cortical lesions might be used for monitoring the disease evolution (Calabrese et al., 2012). Indeed, a separate study from the same group demonstrated that, patients with a benign disease course (EDSS ≤ 3 after 15 years from onset) had a significantly lower number and volume of cortical lesions and a slower accumulation of the cortical damage over time, compared to patients with similar degree of disability but much shorter disease duration (Calabrese et al., 2009b). More recently, in a 5 years longitudinal study of 334 RR MS patients, those who entered the SP phase during the observation period were shown to have a significantly higher number of cortical lesions and a significantly lower cortical volume (Calabrese et al., 2013). The authors hypothesized that during the RR phase the grey matter pathology remains clinically silent and, after a threshold is reached, it determines the changing course of the disease (Calabrese et al., 2013). Similar conclusions were reached by analysing a group of CIS patients observed for 20 years after the first attack. A significantly greater grey matter atrophy predicted the conversion to SP MS and the development of severe disability (Fisniku et al., 2008b). These results strongly suggested that grey matter atrophy is a good indicator of the

clinical status and correlates with the clinical disability in the long term (Fisniku et al., 2008b).

1.6 Measures of disability

1.6.1 The expanded disability status scale

The disability status scale (DSS) was introduced more than 50 years ago (Kurtzke, 1955) and its expanded version (EDSS) was created 30 years ago (Kurtzke, 1983). It remains the most widely used tool for assessing disability, among MS patients, in both clinical practice and clinical trials. The score is calculated by taking into account dysfunctions in a wide range of neurological functional systems (FS): pyramidal, cerebellar, brainstem, visual, cerebral, bladder/bowel (Table 1.3) (Kurtzke, 1983).

Table 1.3 Functional systems score in the EDSS (Kurtzke, 1983).

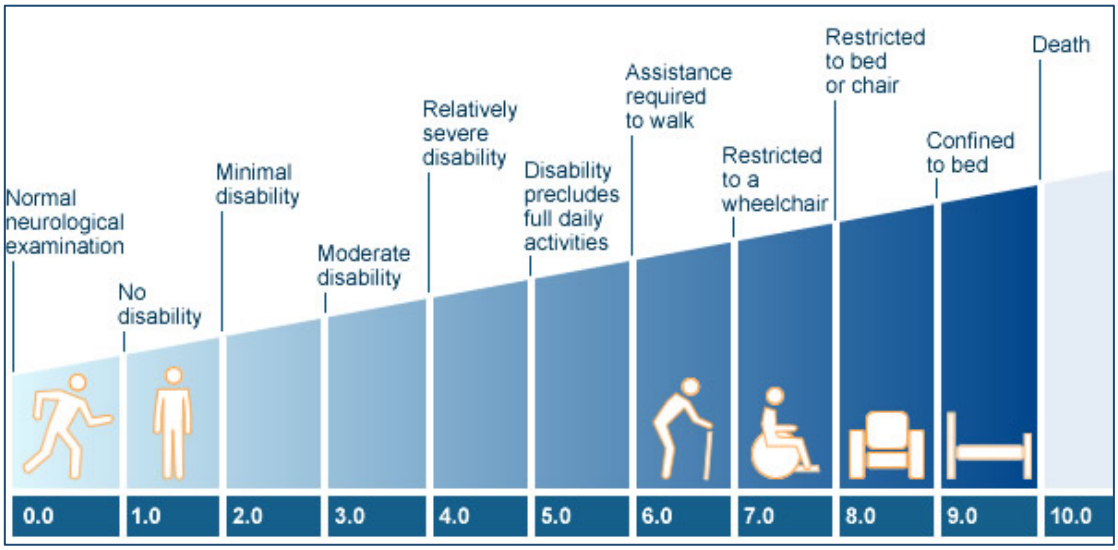
Score	Pyramidal Functions	Cerebellar Functions	Brainstem Functions
0	Normal	Normal	Normal
1	Abnormal signs without disabililty	Abnormal signs without disability	Signs only
2	Minimal disability	Mild ataxia	Moderate nystagmus
3	Mild/moderate paraparesis; severe monoparesis	Moderate truncal or limb ataxia	Severe nystagmus; marked ocular weakness
4	Marked paraparesis; moderate quadriparesis	Severe ataxia	marked dysarthria
5	Quadriplegia	Unable to perform coordinated movements	Inability to swallow or speak
	Sensory functions	**Bowel/bladder Functions**	**Visual Function**
0	Normal	Normal	Normal
1	Vibration decrease in one or two limbs	Mild urinary hesitancy, urgency or retention	Scotoma with visual acuity better than 20/30
2	Mild decrease in touch in one or two limbs	Moderate bowel or bladder urgency or retention	Scotoma with visual acuity 20/30 to 20/59
3	Moderate decrease in touch or lost of vibration sense	Frequent urinary incontinence	Large scotoma with visual acuity 20/60 to 20/99
4	Marked decrease in touch	Almost constant catheterization	Marked decrease of fields and visual acuity 20/100-200
5	Loss of sensation in one or two limbs	Loss of bladder function	Maximal visual acuity less than 20/200
6	Sensation lost below the head	Loss of bowel and bladder functions	Grade 5 + acuity in better eye of 20/60 or less

The function score in each system is calculated according to the clinical findings from the neurological examination. Pyramidal, cerebellar and brainstem functions are rated from 0 to 5, based on the degree of weakness, ataxia, ocular movements abnormalities and speech/swallowing difficulties, respectively. Sensory, visual and bowel/bladder functions are rated from 0 to 6, based on the degree of sensory and visual impairment, and sphincter dysfunction (Table 1.3). The combination of scores in each functional system allows to calculate the final disability score, which ranges from 0 (normal) to 10 (dead) (Figure 1.7), with 0.5 steps increments. Death due to

MS (EDSS = 10) is defined as *"…due to brainstem involvement or to respiratory failure, or consequent to the chronic bedridden state with terminal pneumonia, sepsis, uremia, cardio-respiratory failure. Antemortem, the patient will ordinarily be scored EDSS 9, sometimes 8…"* (Kurtzke, 1983).

The EDSS is familiar to virtually all MS clinicians, it allows making comparisons between patients and within a single patient over time, and it is relatively easy to administer. However, it is disadvantaged by several shortcomings and has received large critiques (Cohen et al., 2012b; Whitaker et al., 1995; Willoughby and Paty, 1988). It is based on the standard neurological examination, which is inevitably subjective. It relies heavily on the ambulation and it has poor assessment of the upper limbs function. In addition, the scoring of the FS dysfunctions is rather ambiguous (Table 1.3), making difficult to determine the overall score.

Figure 1.7 The EDSS score. Adapted from (Kurtzke, 1983)



Steps	Definition	Ambulation
1	No disability, minimal signs in one FS (grade 1)	Fully ambulatory
1.5	No disability, minimal signs in more than one FS (grade 1)	Fully ambulatory
2	Minimal disability in one FS (grade 2)	Fully ambulatory
2.5	Minimal disability in two FS (grade 2)	Fully ambulatory
3	Moderate disability in one FS (grade 3) or mild disability in 3/4 FS (grade 2)	Fully ambulatory
3.5	Moderate disability in one FS (grade 3) and 1/2 FS grade 2, or2 FS grade 3, or 5 FS grade 2	Fully ambulatory
4	Relatively severe disability: 1 FS grade 4	Able to walk without aid or rest some 500 mts
4.5	Relatively severe disability: 1 FS grade 4	Able to walk without aid or rest some 300 mts
5	Severe disability: 1 FS grade 5	Able to walk without aid or rest some 200 mts
5.5	Severe disability: 1 FS grade 5	Able to walk without aid or rest some 100 mts
6	More than 2 FS grade 3+	Unilateral assistance (cane) to walk about 100 mts
6.5	More than 2 FS grade 3+	Bilateral assistance (cane) to walk about 100 mts
7	More than 1 FS grade 4+	Unable to walk beyond 5 meters, essentially wheelchair restricted
7.5	More than 1 FS grade 4+	Unable to walk more than few steps, wheelchair restricted
8	Several FS grade 4+	Restricted to bed or chair, with effective use of arms
8.5	Several FS grade 4+	Restricted to bed or chair, with some effective use of arms
9	Most FS grade 4+	Helpless bed bound, can communicate and eat
9.5	Most FS grade 4+	Totally helpless bed bound, unable to communicate or eat
10	Death due to MS	

1.6.1.1 Limitations of the EDSS

Several studies highlighted the poor reliability of the scale by demonstrating that both the FS and EDSS are often affected by high inter-examiner and intra-examiner variability, (Amato et al., 1987; Amato et al., 1988; Goodkin et al., 1992; Noseworthy et al., 1990). In a small study involving 4 MS physicians, examining 24 patients, the degree of inter-examiners agreement was reported to be unacceptably low (30-50%), demonstrating that a single point change on the EDSS was not a sufficient evidence of the disease progression (Amato et al., 1988). These findings were confirmed by the Canadian Cooperative Study group, showing a considerable inter-examiners variability of the disability scores, even when 2 experienced clinicians examined the patient consecutively (Noseworthy et al., 1989). The authors concluded that the poor agreement among clinicians may often account for changes of a single EDSS step (Noseworthy et al., 1990). This was shown to be mainly evident in the lower scores of the scale (EDSS 1-3.5), where the inter-examiners variation occurred in up to 40% of assessments (Goodkin et al., 1992). The low EDSS scores are more difficult to be calculated because definitions of the FS dysfunction are particularly ambiguous. In contrast, the scoring from 4 to 7.5 is based on the more objective ambulatory endurance (distance the patient can walk with or without assistance).

More than 20 years ago, it was recommended that a change of at least 1 EDSS point, preferably validated by a second confirmatory examination, is required to detect a significant clinical change in the context of a trial (Noseworthy et al., 1989). The unremitting disability, defined by 0.5 or 1 EDSS point increase, confirmed at 3 or 6 months, has become the key therapeutic target in modern MS trials. The validity of these definitions of disability accumulation was assessed in a large study involving 1344 patients from several randomized controlled trials (RCTs) placebo arms, pooled together. Not even the most stringent 1 EDDS point increase, confirmed at 6 months, was found to be a valid surrogate marker for late disability. The worsening on the EDSS scale was shown to occur as frequently as the improvement (Ebers et al., 2008). These data suggested that the variation of the disability, and therefore the

treatment failure, observed in placebo arms is likely to be strongly affected by the random variation of the EDSS score (Ebers et al., 2008). Authors recommended that the confirmation at 1 year, of at least 1-2 EDSS point change, is needed for detecting a meaningful disability (Ebers et al., 2008). During the last International Conference on Disability Outcomes in MS (May 2011), it was finally agreed that 3 months confirmation of EDSS increase is not a reliable indicator that disability worsening has occurred and it was recommended to use a confirmatory assessment at 6 months (Cohen et al., 2012b).

An additional drawback of the EDSS is the lack of linearity, as the clinical importance of a 1-point change varies according to the starting score. Indeed, the rate of progression experienced by an individual patient through the scale varies over time. For instance, among the London Ontario database population, the distribution of the DSS scores was bimodal, with peaks at levels 3-4 and 6-7 (Weinshenker et al., 1991b). These factors greatly complicate the interpretation of score changes over time, especially within the context of clinical trials.

1.6.1.2 Possible improvements of the EDSS

The EDSS remains widely used in RCTs, mainly because of its acceptance by regulatory agencies (Cohen et al., 2012b). Almost 20 years ago the EDSS was already considered inadequate for reliably assessing the disease progression. Following an international workshop on outcome measures in clinical trials, large improvements of the scale were suggested (Whitaker et al., 1995), however, since then, only small changes have occurred. A modern version is now available, on CD-ROM or online from *Neurostatus*. The reliability of the scale was improved by using standardized neurological examinations and scoring rules, and by also questioning the patients when calculating the scores (Cohen et al., 2012b). During the last International Conference on Disability Outcomes in MS (May 2011), it was advised that modifications should be implemented to improve the linearity of the measurement, although it remains unclear how this refinement can be accomplished (Cohen et al., 2012b).

1.6.2 Multiple Sclerosis functional composite

The Multiple Sclerosis Functional Composite (MSFC) was developed as an alternative disability assessment scale, able to overcome the EDSS shortcomings (Cutter et al., 1999; Rudick et al., 1997). It is simple to administer, as it is not based on neurological examination, but implies the use of 3 separate quantitative tests (Cutter et al., 1999). 1) The 9-Hole Peg Test (9HPT) assesses arms and hands functions, by measuring the time needed for inserting and removing 9 pegs from a board; the mean time for both hands represents the final score (Goodkin et al., 1988). 2) The Timed 25-Foot Walk (T25FW) evaluates changes in ambulatory functions; at least a 20% time increase indicates a clinically relevant ambulatory impairment (Kaufman et al., 2000). 3) The Paced Auditory Serial Addition Test (PASAT) measures the cognitive function by asking the patient, while listening 61 spoken numbers at 2-3 seconds interval, to add each number to the previous one; the number of correct addictions defines the final score (Gronwall, 1977). The final MSFC composite score, combining the score from the three tests, is computed as Z-score (the number of standard deviation units a patient's score is below or above the average score). Therefore lower scores, compared to baseline or to previous assessment, is interpreted as a neurological deterioration (Cutter et al., 1999). The calculation of the Z-score is based on the comparison with a reference population (e.g. a standard MS population or healthy controls), which influences the weighting of the individual components.

The overall MSFC score, as well as the performance in the individual components, were found to decline with disease duration and to correlate well with the EDSS and its changes (Cutter et al., 1999). Not unexpectedly, the correlation of the MSFC with the EDSS derives mainly from the T25FW test (Hoogervorst et al., 2002; Kalkers et al., 2000), with the additional advantage of taking into account the arms and the cognitive functions (Polman and Rudick, 2010). A modest correlation between the MSFC and MRI measures was also reported (Kalkers et al., 2001). In addition, the MSFC was shown to have excellent intra-examiner and inter- examiners reliability, especially when examiners were previously trained (Cohen et al., 2000). This was

also confirmed when it was used as primary clinical endpoint in a RCT (Cohen et al., 2001).

However, the MSFC is not free of limitations. It does not include assessment of the visual function and changes, in both the composite Z-score and in the individual domains Z-scores, are difficult to interpret (Polman and Rudick, 2010). In addition, both the 9-HPT and the PASAT are affected by a practice effect; therefore improved scores can be wrongly interpreted as a clinical improvement (Solari et al., 2005). Finally, the choice of the reference population influences the weighting to different tests, limiting the comparison among different studies (Uitdehaag et al., 2002). Overall the MSFC cannot be easily clinically interpreted and for this reason has not been accepted by regulatory agencies as an alternative to the EDSS in RCTs.

1.6.3 Surrogate markers

Surrogate markers are defined by the FDA as "…laboratory measurement or physical signs that can be used in therapeutic trials as a substitute for a clinically meaningful endpoint that is expected to predict the effect of the therapy…" (Katz, 2004; Temple, 1999). As MS generally evolves over 30-40 years, with an unpredictable clinical course, validated surrogate markers, predicting the clinical changes in the long term and able to detect a treatment effect during the short duration of a clinical trial, are desperately needed.

1.6.3.1 MRI

The use of MRI, as marker of disease activity, is mainly based on its ability of detecting the subclinical inflammatory activity (McDonald et al., 1994). Imaging outcomes are currently well accepted as secondary endpoints in RCTs. Some authors claimed to have validated the individual-level surrogacy of MRI lesions for the EDSS worsening at trial level, among patients under immunomodulatory treatments (Sormani et al., 2011). However, the modest correlation between the clinical and

the radiological activity (Barkhof, 2002; Miller et al., 1996) limits the use of MRI as para-clinical outcome measure of the inflammatory activity. One strategically placed lesion (e.g. in the lower medulla) can make a patient quadriplegic, whereas several lesions distributed periventricularly can remain completely asymptomatic (Barkhof, 2002). In addition, MRI inflammatory lesions number was shown to predict conversion from CIS to clinically defined MS (Fisniku et al., 2008a) however, correlated only modestly with the disability development in the long term (Fisniku et al., 2008a; Khoury et al., 1994). Although the MRI inflammatory activity appears a strong surrogate endpoint for clinical relapses, it remains largely unclear which MRI features can be predictive of the disease progression and of disability changes (Cohen et al., 2012b). In contrast, both the grey matter damage (Calabrese et al., 2007b; Calabrese et al., 2009b; Calabrese et al., 2013; Roosendaal et al., 2009) and the grey matter atrophy (Bakshi et al., 2001; De Stefano et al., 2003; Fisniku et al., 2008b; Roosendaal et al., 2011; Tedeschi et al., 2005) correlate reasonably well with the clinical outcome, and might be used as surrogate markers for late disability.

1.6.3.2 Optical coherence tomography

Optical coherence tomography (OCT) is a non-invasive procedure, able to generate high resolution retinal images (Huang et al., 1991). Because of its ability of quantifying the retinal nerve fibre layer (RNFL), it has recently gained interest, among MS experts, as marker of neurodegeneration and for monitoring the axonal loss (Petzold et al., 2010).

Following an optic neuritis (ON), the RFNL thickness is significantly lost (Costello et al., 2006). However, the RNFL loss can also be a consequence of other causes (Choi et al., 2008), representing a limitation of the technique. An association between the RNFL thinning and the MS pathology is supported by several studies, demonstrating a significant RNFL loss in MS patients with no evidence of ON, compared to healthy controls (Albrecht et al., 2007; Bock et al., 2010; Fisher et al., 2006; Siger et al., 2008). In addition, the RNFL thickness was reported to decrease with the increasing EDSS score (Sepulcre et al., 2007; Siger et al., 2008) and with the increasing cognitive

disability (Gordon-Lipkin et al., 2007; Toledo et al., 2008), and to associate directly with the brain atrophy (Grazioli et al., 2008; Siger et al., 2008).

Furthermore, the RNFL loss in MS patients worsens with the disease duration (Pueyo et al., 2008; Siger et al., 2008), although some studies could not confirm this observation (Henderson et al., 2008; Klistorner et al., 2008). These discrepancies might be accounted for by ascertainment differences. Indeed, a large longitudinal study described an average 2-µm RNFL thinning for each year of follow up, totalling 3.2% loss after 2-3 years (Talman et al., 2010). These data suggested that a minimum 2 years follow up would be required for detecting significant changes, making the OCT still not a reliable outcome measure during the phase 2 trials, normally lasting 4-6 months (Petzold et al., 2010). Overall, the OCT holds great potential for monitoring the treatment effect in trials testing the efficacy of neuroprotective agents and its role in the MS research will receive increasing attention in future (Petzold et al., 2010).

1.6.3.3 CSF markers

Degenerating axons release in the extracellular space their protein contents, which accumulate in the cerebrospinal fluid (CSF), where they can be detected. Neurofilaments (NFs) are the major structural protein component of neurons, and are particularly abundant in long projection axons (Gresle et al., 2011). Such components, especially the subunits light (NFs-L) and heavy (NFs-H), can be potentially used as biomarkers of the axonal degeneration, for monitoring the disease progression and for assessing the efficacy of drugs with neuroprotective effect.

The utility of NFs-L as marker of the disease activity was first suggested 15 years ago, following a study demonstrating increase CSF levels among RR MS patients, and a moderate correlation with the disability (Lycke et al., 1998). Since then, findings were confirmed, both in RR and progressive MS (Haghighi et al., 2004; Kuhle et al., 2011; Malmestrom et al., 2003; Semra et al., 2002), by a large number of studies.

Some observed higher levels of NFs-L during acute relapses, decreasing within 3 months from the acute attack (Kuhle et al., 2011; Lycke et al., 1998; Malmestrom et al., 2003). In addition, elevated CSF NFs-L levels were found to correlate with a significantly higher risk of experiencing a severe disease course and of converting to SP MS, suggesting a potential role as prognostic markers (Norgren et al., 2004; Salzer et al., 2010). Recently, NFs-L levels were used for assessing the effect of Natalizumab on the tissue damage. The analysis showed a three folds reduction of CFS NFs-L levels after treatment, highlighting a potential neuroprotective effect of Natalizumab (Gunnarsson et al., 2011). NFs-H levels are also considered a useful measure of the neurodegenerative activity, because high levels were found to associate with a progressive disease course (Petzold et al., 2005). Overall, the measurement of CSF NFs holds substantial promises for monitoring the axonal loss in future MS clinical trials.

1.7 Disease modifying treatments

With the approval of the first disease modifying treatment (DMT) in 1993 the new treatment era in MS began, marking a milestone in the history of the disease. The therapeutic management, which until then was only based on treating acute exacerbations, dramatically changed. Currently there are 5 approved therapies for MS: interferons beta, galtiramer acetate, mitoxantrone, natalizumab and fingolimod. These agents have been shown to suppress the relapse frequency and to reduce the inflammatory load detected by the MRI. However, their impact on the severe disability accumulation in the long term remains largely unknown.

1.7.1 Interferon beta

Interferon (IFN) beta, along with other subtypes, belongs to the type I IFN family with immune-modulatory and anti-infectious effects (Gonzalez-Navajas et al., 2012). Its efficacy is exerted by contrasting many of the pathological processes underlying the MS pathogenesis, reflecting different possible mechanisms of actions, still to be clarified. IFN beta is believed to favour the Th2 migration, to block the Th1 migration and to shift the balance pro/anti-inflammatory cytokines towards the latter, preventing the release of IL-12, IL-17, IL-23 as well as of IFN gamma and TNF (Kieseier, 2011). In addition, IFN beta increases the serum concentration of soluble vascular cell adhesion molecule-1 (sVCAM). This increase probably inhibits the leukocytes migration into the CNS by blocking their adhesion to the cerebral endothelial surface (Prat et al., 2005).

1.7.1.1 Interferon beta in RR MS

IFN beta-1b was approved by the food and drug administration (FDA) in 1993, following the positive results from a large randomized, placebo controlled, double blind trial, testing its efficacy in RRMS patients (1993; Paty and Li, 1993). Among patients treated with subcutaneous (SC) injections of IFN beta-1b every other day,

compared to placebo, there was statistically significant 34% reduction of the annualized relapse rate (ARR) and a significantly lower disease activity detected by the MRI. In 1996, weekly injections of intramuscular (IM) IFN beta-1a received FDA approval for treating RRMS patients. Decision was taken in view of a randomized, placebo controlled, trial results, showing a significant ARR reduction (32%) among treated patients, compared to placebo (Jacobs et al., 1996a). Finally, the PRISM (prevention of relapses and disability by IFN beta-1a subcutaneously in MS study group), a randomized, placebo controlled trial, testing the efficacy of SC injections of IFN beta-1a (22 μg and 44 μg) three times a week, demonstrated a 27% and a 33% reduction of the ARR, among patients treated with 22 and 44 μg, respectively, compared to placebo (1998a). In 2002 SC both doses of IFN beta-1a received approval from the FDA for the treatment of RRMS.

Since then, IFN beta preparations have been successfully used for treating RRMS worldwide. Although results of the pivotal trials indicated that the magnitude of the therapeutic effect is similar, among the three formulations, methodological issues, complicating the interpretation of results, do not allow to draw definitive conclusions on which compound exerts the largest effect (McGraw and Lublin, 2012). However, it clearly emerged that the efficacy of the IFN is dose dependent. A comparative study demonstrated that IFN beta-1a SC 44 μg three times a week associates with a 90% increased probability (OR = 1.9; p = 0.0005) of remaining relapse free, compared to IFN beta-1a IM 30 μg once a week (Panitch et al., 2002). Similarly, another study demonstrated superiority (larger proportion of patients free from relapses) of the IFN beta-1b 250 μg every other day on the IFN beta-1a IM 30 μg once a week (Durelli et al., 2002).

1.7.1.2 Interferon beta in CIS

Following the IFNs pivotal trials, further study were undertaken in order to test their effect early in the disease course, among patients with CIS. The CHAMPS (controlled high risk subjects Avonex MS prevention study) evaluated the efficacy of weekly IM injections of IFN beta-1a in patients with a first, single demyelinating attack and MRI

features suggesting MS. A significantly lower percentage (35%) of patients in the treated group, compared to the placebo group (50%), developed clinically definite MS (CDMS), defined by the occurrence of a new acute attack (Jacobs et al., 2000). Similar results were obtained by the ETOMS (early treatment of MS study group) study, demonstrating a 9% difference in the proportion of patients experiencing a second attack, between the group treated with SC IFN beta-1a injections (three times a week) and the placebo group (34% versus 45%, respectively) (Comi et al., 2001a). Finally, SC IFN beta-1a every other day was also proven to be effective in delaying the conversion to CDMS (28% versus 45% experiencing a second attack in the treated and placebo groups, respectively) in the BENEFIT (Betaferon in Newly Emerging MS For Initial Treatment) study (Kappos et al., 2006b).

Overall, results from CIS trials demonstrate a beneficial impact of IFNs beta early in the disease course. However, large controversy still exists on whether patients should start treatment at the time of diagnosis (Frohman et al., 2006a; Pittock et al., 2006; Pittock, 2007). Given that a considerable proportion might remain progression free for decades without treatment (Skoog et al., 2012), and given the difficulty of adhering to an injectable drug for a long time (Giovannoni et al., 2012), the potential clinical benefit should be carefully evaluated. One of the main limitations of CHAMPS, ETOMS and BENEFIT trials is the limited observation period (2 years). No clear evidence support the assumption that delaying the second attack by 6 months prevents the long-term disability accumulation (Pittock et al., 2006). More than half of patients in the placebo groups of both CHAMPS and ETOMS studies did not have a second attack during the observation period. In addition, even in the BENEFIT extension study (5 years), the effect of early versus delayed treatment on the probability of converting to CDMS (46% versus 57%) and on the disability progression (25% versus 29%) was clinically rather small; it was calculated that 9 and 25 patients needed to be treated early, to avoid one additional patient to have a second attack and to experience disease progression (increase of 1 EDSS score), respectively (Kappos et al., 2009). Similarly, in the CHAMPS extension study the effect of early initiation versus delayed treatment on the probability of converting to CDMS remained small at 10 years (58% versus 69%) (Kinkel et al., 2012). The limited

impact of delayed conversion to CDMS on the long-term disability accumulation leaves the question whether patients should start treatment early in the disease course still open (Pittock, 2007; Pittock, 2009).

1.7.1.3 Interferon beta in progressive MS

In 1998, for the first time, The European Study Group of IFN beta-1b in SP MS assessed the effect of SC IFN beta-1b every other day in SPMS. Patients recruited in the study had had 6 months of neurological deterioration, independent of relapses, and a previous history of RR MS. Treatment efficacy was tested by using the time to 3 months confirmed disability, defined as a sustained increase of 1 EDSS point (or 0.5 point if baseline EDSS was 6 to 6.5), as outcome measure. At the end of the 3 years observation period, the study demonstrated a significantly smaller number of treated patients (38.9%) experiencing sustained disability, as compared to placebo (49.8%) (1998b). These results led to the approval of SC IFN beta-1b every other day for the treatment of SP MS.

However, a subsequent trial in North America failed to replicate these results, and demonstrated no significant effect exerted by SC IFN beta-1b every other day in SP patients. In this study, it was used a slightly different definition of disease progression (time to 6 months sustained disability, defined as an increase of 1 EDSS point, or 0.5 point if baseline EDSS was 6 to 6.5) (Panitch et al., 2004). It was concluded that the differences in outcome measures and the recruitment of patients with earlier SPMS, and therefore more active disease, in the European study (1998b), accounted for the different results observed in the two trials (Kappos et al., 2004).

In addition, another study (SPECTRIMS: The Secondary Progressive Efficacy Trial of Recombinant Interferonbeta-1a in MS) compared two different doses (22 μg and 44 μg) of SC IFN beta-1a three times a week versus placebo, among patients with SP MS. No significant differences were observed between groups, in the proportion of patients experiencing a 3 months confirmed disability progression, defined as an

increase of 1 EDSS point (or 0.5 point if baseline EDSS was 6 to 6.5) (2001a). Similar results were obtained in The Nordic SPMS group study, which showed no significant impact of IFN beta-1a SC 22 μg once a week on disease progression (6 months time of sustained disability) (Andersen et al., 2004).

Finally, The International MS Secondary Progressive Avonex Controlled Trial (IMPACT) assessed the effect of IM IFN beta-1a in SPMS patients by using the MSFC as primary outcome measure. Although in the treatment group there was a large (40%) reduction in the MSFC worsening, the study failed to demonstrate significant differences in the proportion of patients experiencing EDSS progression confirmed at 3 months (Cohen et al., 2002). For this reason IM IFN beta-1a never received FDA approval for treating SPMS.

Based on the results of the European study (1998b), IFN beta-1b SC every other day remains the only IFN approved for SPMS. Interestingly, a recent Cochrane review pooled together data from the 5 trials, totalling 3082 SPMS patients treated with IFNs or placebo; IFN beta-1b (1998b; Panitch et al., 2004) and IFN beta-1a (2001a; Andersen et al., 2004; Cohen et al., 2002). The risk of 6 months sustained disability accumulation was found to be not significantly different between the treatment and the placebo group (65% and 67% of patients experienced disease progression, respectively). Same results were obtained when considering number of patients with progression confirmed at 3 months. Overall, results showed that IFNs are not effective in reducing the risk of disease progression in SPMS (La Mantia et al., 2012).

1.7.1.4 Interferon beta long-term follow up studies

The impact of IFN on the disability development in the long term remains largely unclear. The validity of the findings from clinical trials, typically lasting 2 years, has been questioned (Gout, 2008; Koch et al., 2008). The debate has mainly focused on trials methodological issues, including immortal time bias (Renoux and Suissa, 2008; Suissa, 2008), selection bias (Dimick and Livingston, 2010; Trojano et al., 2007), small sample sizes (Coppola et al., 2006; Pozzilli et al., 2005), insufficient follow up

(Coppola et al., 2006; Milanese et al., 2003) and outcome measures (Daumer et al., 2009; Ebers et al., 2008; Ebers et al., 2011; Ebers et al., 2012). Long term follow up (LTF) studies have recently gained increasing attention as potential tool for addressing the controversial point of the effect of DMTs on the accumulation of severe disability (Freedman, 2011).

The longest study so far published, reported results from the original cohort of patients treated with SC IFN beta-1b every other day (1993), which was followed up for 16 years (Ebers et al., 2009) and subsequently for 21 years (Ebers et al., 2010; Goodin et al., 2012). Although patients treated with IFN continued to show a sustained reduction of the AAR and a slower disease progression, no statically significant differences were observed between the treated and the untreated group in the proportions reaching SP and EDSS 6, after 16 years (Ebers et al., 2009). However, the 21 years follow up study, with nearly complete ascertainment (366 patients identified from the original 372), demonstrated a significant reduction of all cause mortality, among those treated with IFN, compared to placebo (Goodin et al., 2012). Nevertheless, the missing data between when patients were last observed in the trial and their time to death represents a main limitation, especially for characterizing the clinical course over time before demise.

From the original cohort treated with IM IFN beta-1a weekly (Jacobs et al., 1996a), 70% of patients were followed up for 15 years (Bermel et al., 2010). At the end of the observation period, those who had chosen to continue staying on IM IFN had lower EDSS score and experienced slower disease progression, as compared to those who had switched to other drugs (Bermel et al., 2010). However, these results might be biased by the better adherence to therapy and by the better disease outcome among those who had remained on treatment.

The original 2 years study, evaluating the efficacy of SC IFN beta-1a three times a week (1998a), was extended initially to 4 years (2001b) and eventually to 6 years (Kappos et al., 2006c). The study confirmed a dose dependent effect, showing a slower disease progression, among patients treated with 44 μg versus those treated

with 22 μg. In addition, the early treatment associated with a delayed onset of the progressive phase and a delayed attainment of EDSS 6 (Kappos et al., 2006c). However, the frequency and the timing of assessments were not consistent over time and many patients had a single examination during the follow up, increasing the chance of false positive when assessing the disability progression (Freedman, 2011).

A large study carried out by the British Columbia group evaluated the long term clinical benefit of IFN therapy by comparing the disease evolution of treated patients and two untreated natural history cohorts. The exposure to IFN beta was found not to influence the risk of requiring walking assistance (EDSS 6), and authors concluded that the administration of IFN beta was not associated with a reduction of the disability progression (Shirani et al., 2012). However, methodology of this study has been largely criticized, leaving the debate still open (Derfuss and Kappos, 2012).

1.7.1.5 Interferon beta safety

The occurrence of adverse events represents an important factor, which affects the adherence to the therapy, and it is the reason most commonly reported by patients for discontinuing IFN. The rate of discontinuation largely varies among studies, averaging from 22% in SC IFN beta-1a trials to 30% and 34% in IM IFN beta-1a and SC IFN beta-1b trials, respectively (Giovannoni et al., 2012).

Flu-like syndrome (FLS) and injection site reaction (ISR) are the side effects more frequently observed. In a large review, including 151 studies, it has been calculated that FLS was reported in 57%, 40% and 32% of patients receiving IM IFN beta-1a, SC IFN beta-1a and SC IFN beta-1b, respectively (Giovannoni et al., 2012). ISRs were more frequently observed among patients treated with SC IFN beta-1a (65%) than patients treated with IM IFN beta-1a (22%) and with SC IFN beta-1b (33%) (Giovannoni et al., 2012). The incidence of both FLS and ISR does not seem to be affected by the treatment duration and does not diminish with the extended administration of the drugs. Headaches, myalgia and fatigue are often reported,

more frequently among patients receiving IM/SC IFN beta-1a, than patients on SC IFN beta-1b (Giovannoni et al., 2012).

1.7.2 Glatiramer acetate

Despite glatiramer acetate (GA) was first described more than 40 years ago (Abramsky et al., 1977), its mechanisms of action remain poorly understood. It has been hypothesized that GA exerts its therapeutic effect by promoting the T cells shifting from pro-inflammatory (Th1) to anti-inflammatory state (Th2) (Blanchette and Neuhaus, 2008). In addition, GA may restore the function of Treg and therefore increase the suppression of auto-reactive lymphocytes (Hong et al., 2005). Beside its anti-inflammatory properties, GA may also have neuro-protective effect. Indeed, in animal model it was shown to stimulate the release of neurotrophic factors (Aharoni et al., 2005).

1.7.2.1 Glatiramer acetate in RR MS

GA was approved in 1996 for the treatment of RR MS following results of a 2 years, double blind, placebo controlled trial. In the group treated with GA 20 mg SC injections daily, compared to placebo, a significantly lower (29%) ARR was observed, however, no effect on the disability progression was detected (Johnson et al., 1995). Results were confirmed in the extension study, reporting 32% reduction of the ARR after 2 more years of observation (Johnson et al., 1998). To address the GA effect on MRI parameters, a larger study assessing 239 RR MS patients over 9 months with monthly MRIs was undertaken. Patients treated with GA had a significant reduction (29%) of MRI gadolinium enhancing lesions (Comi et al., 2001b).

GA was also shown to delay the time to the second attack and therefore the conversion to CDMS, among patients with CIS (Comi et al., 2009). Although it was originally planned to last for 3 years, given the clear efficacy demonstrated in the group treated with GA (45% reduction of the risk of converting to CDMS,) the study

was interrupted after 2.3 years, and patients in the placebo group were offered the possibility of starting the active treatment.

1.7.2.2 Glatiramer acetate in progressive MS

The efficacy of GA was tested among patients showing evidence of a chronic progressive course over at least 18 months (Bornstein et al., 1991). No statistically significant differences were observed between the treated and the placebo group in the proportion of patients experiencing 3 months sustained increased disability (1 EDSS point, if baseline was 5 or greater, or 1.5 EDSS point, if baseline was less than 5) (Bornstein et al., 1991). In addition, GA was shown to exert no effect on the disability progression among PPMS patients (Wolinsky et al., 2007).

1.7.2.3 Glatiramer acetate long-term follow-up studies

The cohort recruited in the GA pivotal study (Johnson et al., 1995) was given the opportunity to remain in an open label long term-follow up study and was observed for up to 15 years (Ford et al., 2010). The GA treated group appeared to maintain a lower ARR and to experience a slower disease progression. However, 131 patients, from the 251 included in the original study, had discontinued the treatment, most of them because of "perception of disease worsening", strongly biasing results.

1.7.2.4 IFN versus GA

Trials comparing the efficacy of high dose IFN versus GA, showed no large differences between the two products. The time to the first attack was similar between the two groups treated with IFN beta-1a SC 44 μg three times a week and with GA SC 20 mg daily (Mikol et al., 2008). Similar efficacy of the two compounds was also demonstrated with regards to MRI active lesions (Cadavid et al., 2009), relapse free risk and changes in the EDSS score (O'Connor et al., 2009). Interestingly, the combination of IFN beta-1a IM 30 weekly and GA daily was shown not to be superior to GA alone, but to provide a larger reduction of the risk of experiencing

relapses than the IFN alone, possibly indicating a higher efficacy of GA (McGraw and Lublin, 2012).

1.7.2.5 Glatiramer acetate safety

GA is probably better tolerated than IFNs, although the adherence remains similar, as the average rate of discontinuation was reported to be 36% (Giovannoni et al., 2012). Patients on GA only rarely report FLS but in large percentage (61%) develop ISR. In addition, a significantly higher proportion of patients, compared to placebo, experience a short lasting, harmless, but rather frightening, sensation of flushing and chest tightness after each injection (Johnson et al., 1995).

1.7.3 Natalizumab

Natalizumab (NTZ) is a humanized monoclonal antibody antagonist to the α4-subunit of α4β1-integrin, expressed on the surface of all leukocytes. By binding to α4-integrin, it inhibits the adhesion of leukocytes to vascular cells receptors, preventing their migration across the BBB to the inflammatory sites, within the CNS. In addition, it was shown to have a down regulating effect on activated lymphocytes (Khademi et al., 2009; Rice et al., 2005).

1.7.3.1 Natalizumab efficacy

The efficacy and the safety of NTZ in MS was tested in two 2-year studies, the AFFIRM (Natalizumab Safety and Efficacy in RR MS) (Polman et al., 2006) and the SENTINEL (Safety and Efficacy of Natalizumab in Combination with Interferon beta-1a in Patients with RR MS) (Rudick et al., 2006b). Results from the two trials led to the approval of NTZ for treating active forms of RRMS (Kappos et al., 2011). In the AFFIRM study patients received intravenous (IV) infusions of NTZ 300 mg every 4 weeks, up to 116 weeks, and were compared to placebo. In the SENTINEL study patients had received, for at least 12 months before randomization, weekly

injections of IM IFN beta-1a and were randomized to receive NTZ (300 mg IV every 4 weeks, up to 116 weeks) in addition to IFN or IFN alone. Both studies used the relapse rate at 1 years and the 3 months sustained disability progression (increase of 1 EDSS point, if baseline score ≥ 1, or of 1.5 EDSS point, if baseline score = 0) over two years, as primary clinical outcome measures. MRI measures (number of T2 lesions and number of gadolinium-enhancing lesions) were used as secondary endpoints.

The relapse suppression exerted by NTZ was much greater than what observed in patients treated with IFNs. In the AFFIRM study NTZ mono-therapy significantly decreased the ARR at 1 year by 68%, compared with placebo (ARR 0.26 versus 0.81), maintaining similar effect at 2 years (ARR 0.23 versus 0.73). In addition, the proportion of patients free from attacks was significantly larger in the treatment group (77% versus 56%). The therapy with NTZ was also associated with a 42% reduction of the risk of disability progression sustained for 3 months; 17% versus 29% of the treatment and the placebo group, respectively, experienced disease progression. A post-hoc analysis showed a significant reduction (64%) of the risk of 6 months sustained progression, among treated patients with highly active RRMS (≥ 2 attacks 1 year before study entry and ≥ 1 gadolinium-enhancing lesion at MRI) (Hutchinson et al., 2009). NTZ also exerted a large (83%) suppression of MRI lesions (new T2 lesions at 2 years: treatment group = 1.9 versus placebo group = 11.0) (Polman et al., 2006).

The SENTINEL study showed similar results. Patients treated with NTZ + IM IFN, compared with those treated with IM IFN alone, had 54% and 55% reduction of the ARR at 1 and 2 years, respectively. NTZ + IM IFN also reduced the risk of 3 months sustained disability progression by 24% however, no differences between the two groups were observed in the risk of 6 months sustained disability. In addition, patient receiving NTZ had significantly lower number of T2 MRI lesions, which were reduced by 83% over 2 years (Rudick et al., 2006b). Similarly to what observed in the AFFIRM study, a post-hoc analysis in treated patients with highly active disease,

showed a 58% reduced risk of 6 months sustained disability progression (Hutchinson et al., 2009).

1.7.3.2 Natalizumab safety

Overall, NTZ was well tolerated in both studies. Infusion reactions (within 2 hours from the infusion) were reported in 24% of treated patients. The most common complaints were headache and hypersensitivity reactions (allergic or anaphylactic) (Polman et al., 2006; Rudick et al., 2006b). Adverse events were generally mild and included headache, fatigue, and urinary tract infections.

In February 2005, the clinical trials were temporarily suspended, following reports of 3 cases of progressive multifocal leukoenchephalopathy (PML) in 2 MS patients from the SENTINEL study (Kleinschmidt-DeMasters and Tyler, 2005; Langer-Gould et al., 2005) and in 1 patient affected by Crohn's disease (Van Assche et al., 2005), treated with NTZ. PML is a serious, and potentially fatal, demyelinating disease of the CNS, normally occurring among immune-compromised patients, which is caused by reactivation of the latent JC virus (Major et al., 1992). Following the temporary withdrawal, an extensive safety report showed no additional PML cases, among 3116 patients treated with NTZ (Yousry et al., 2006), leading to the reintroduction of the drug in the market in 2006. Since then, as of June 2011, 133 new cases of PML (24 [18%] deaths) have been reported, among MS patients, who had received NTZ mono-therapy for more than 1 year, with a 2 mean years exposure to the drug. Accordingly, the estimated risk of PML is 1.51/1000 patients. This appears to be higher among those who had previous immunosuppression. PML incidence was reported to be low in the first 12 months of treatment and to reach its peak at 36 months (Kappos et al., 2011).

NTZ is currently recommended for patients with RRMS, responding poorly to, or not tolerating, an alternative MS treatment. However, its use is also allowed as first line treatment in US (Kappos et al., 2011). In the UK, according to the NICE guidelines, NTZ can be administered as first line therapy to patients with rapidly evolving severe

RR MS, defined as two or more disabling relapses in 1 year, and one or more MRI GAD-enhancing lesions or a significant increase in the T2 lesions load, compared with a previous MRI.

1.7.4 Fingolimod

Fingolimod, also known as FTY720, is the first oral drug approved by the FDA in 2010 as first line treatment for RRMS, and by the European Medical Agency (EMA) in 2011 as second line therapy for patients with high disease activity, not responding to IFN treatments. It is structurally similar to sphingosine-1-phosphate (S1P) receptors, which are present on the surface of lymphocytes and, because of its lipophilic structure, it can easily enter the CNS. By binding to S1P receptors, Fingolimod prevents the migration of T and B cells from the lymph nodes to the circulation (Conway and Cohen, 2010). This ultimately results in a dramatic reduction (more than 90%) of circulating lymphocytes, including IL-17 producing T cells, known to be key players in the inflammatory mechanisms underlying the disease pathogenesis (Mehling et al., 2010).

1.7.4.1 Fingolimod efficacy

The initial trial assessing the efficacy of Fingolimod was a phase 2, 6 months study, in 281 patients, which were randomized to 1.25 mg, 5 mg, or placebo (Kappos et al., 2006a). The primary outcome measure was the cumulative number of gadolinium enhancing lesions and the new T2 lesions, which were both shown to be significantly reduced in the treatment group versus placebo (Kappos et al., 2006a).

The FREEDOMS (FTY720 Research Evaluating Effects of Daily Oral Therapy in MS) was the first phase 3, double blind, randomized, placebo-controlled trial assessing the effect of Fingolimod in RRMS: 1272 patients were randomized to receive Fingolimod 1.25 mg or 0.5 mg daily for 2 years (Kappos et al., 2010). The ARR was significantly reduced by 60% in the 1.25 mg group and by 54% in the 0.5 mg group, compared to

placebo (ARR = 0.16, 0.18 and 0.40 respectively). A beneficial effect was also observed in the time to 3 months sustained disability accumulation (1 EDSS point or 0.5, if the EDSS baseline was 5.5). The percentage of patients experiencing disease progression was significantly lower in the high (16.6%) and low (17.7%) dose groups, compared to placebo (24.1%). Finally, the number of new T2 MRI lesions was significantly reduced among treated patients, and almost 90% were free of gadolinium enhancing lesions at the end of the 24 months follow up.

The TRANSFORMS (Trial Assessing Injectable Interferon Versus FTY720 Oral in RR MS) study compared the two Fingolimod doses (1.25 mg and 0.5 mg) to IM IFN beta-1a, over 12 months (Cohen et al., 2010). The AAR was significantly lower in the two Fingolimod doses groups (1.25 mg = 0.20, 0.5 mg = 0.26), compared to the IFN group (0.33); this resulted in a 52% and 38% AAR reduction, respectively. However, the time to sustained disability was not significantly different in the 3 groups. In the 1-year extension study, totalling 24 months of follow up, the larger effect on the AAR exerted by Fingolimod was confirmed (Khatri et al., 2011).

1.7.4.2 Fingolimod safety

The safety of Fingolimod in the long term remains unclear due to limited data available. During the TRANSFORM trial two deaths were reported among patients treated with high dose; one for herpes zoster infection and one for herpes simplex encephalitis. The role of Fingolimod in the two deaths remains unclear however, given its mechanism of action, it is plausible to suspect a link (Pelletier and Hafler, 2012).

The most commonly reported adverse effects were cardiovascular events (bradycardia and 1st degree atrio-ventricular block), which occurred in 1-3 % of patients. Most of these events are normally observed during the first dose and resolved within the first 24 hours. In addition, less than 1% developed macular oedema.

1.7.5 Mitoxantrone

Mitoxantrone is an antineoplastic agent currently approved for the treatment of acute myeloid leukaemia and of hormone-refractory prostate cancer (Shenkenberg and Von Hoff, 1986). The rationale for its use in MS is based on its immunosuppressive effect, leading to the inhibition of T and B cell and macrophage proliferation (Chan et al., 2005; Lublin et al., 1987).

1.7.5.1 Mitoxantrone efficacy

The drug was approved for treating aggressive RRMS and SPMS by the FDA in 2000, following results from a phase III double blind, placebo-controlled multicentre study (MIMS) (Hartung et al., 2002). A total of 124 patients, with a 1 EDSS point worsening over the previous 18 months, received Mitoxantrone at low (5 mg/m2) and high (12 mg/m2) doses, every 3 months for 2 years, and were compared to patients treated with placebo. In the high dose group there was a significant reduction of the disease progression, measured using a composite score, which included the EDSS assessment, the ambulatory index, the number of treated relapses and the time to first relapse. Furthermore, among patients treated with 12 mg/m2, compared to placebo, a lower percentage (8% versus 25%; $p = 0.013$) had a one point EDSS worsening and their annualized relapse rate was 66% lower (0.35 versus 1.02; $p = 0.001$). Nevertheless, because of the lack of blinding amongst the physicians carrying out the clinical assessments, information on the primary and secondary outcomes was rated as only Class III evidence of efficacy by the Therapeutic Technology Assessment (TTA) Subcommittee of the American Academy of Neurology (AAN) (Goodin et al., 2003; Marriott et al., 2010). In addition, the strength of evidence supporting its beneficial effect in SPMS was considered very weak, questioning the validity of the rationale use of Mitoxantrone in progressive patients (Goodin et al., 2003).

Following its approval by the FDA, Mitoxantrone was tested in specific sub-cohorts of patients with aggressive disease course. A large open study, involving 100 patients with aggressive RRMS (3 mean relapses and one point worsening at EDSS in the previous 12 months) from the Rennes EDMUS database (Confavreux et al., 1992b), tested the effect of monthly doses of Mitoxantrone (20 mg + 1 g of methylprednisolone) for six months (Le Page et al., 2008). At 12 months it was observed a marked reduction of the relapse rate (91%) and of the MRI activity (78%). In addition, 78% of patients had been free of attacks and 64% of cases had had an improvement of 1 point or more on the EDSS. More importantly, the effect on the relapse rate and on the disability accumulation was prolonged up to 5 years from the start of the study (Le Page et al., 2008).

1.7.5.2 Mitoxantrone safety

Unfortunately, the use of Mitoxantrone is limited by its cardiac toxicity, causing cardiomyopathy (De Castro et al., 1995; Strotmann et al., 2002). Because of this, it is recommended a cumulative dose of not more than 140 mg/m2. This implies that the therapy, at the standard dose of 12 mg/m2 every 3 months, should last no longer than 2-3 years (Marriott et al., 2010). In addition, the potential risk of late malignancy should be considered. An increasing number of studies have documented cases of treatment related acute leukaemia (TRAL) and it was reported that at least 29% of MS patients who developed TRAL died (Marriott et al., 2010). The overall incidence of TRAL is now estimated as 0.8% of treated cases, and the number needed to harm (NNH) as 123 patients (Marriott et al., 2010). Given the potential serious adverse effects, the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology has recommended that the use Mitoxantrone should not be preferred to other immunomodulatory drugs, and should be limited to those patients with a rapidly advancing disease course, who have failed other therapies (Marriott et al., 2010).

1.7.6 Teriflunomide

Leflunomide, the prodrug of teriflunomide, is approved for the treatment of rheumatoid arthritis (Schattenkirchner, 2000). It is an oral drug that is rapidly converted into its active metabolite (teriflunomide) in the intestinal mucosa (Fox et al., 1999). The actions of teriflunomide are wide: it inhibits the migratory capacity of T cells, it prevents the switch of B cells to IgG1 (Korn et al., 2004) and it targets neutrophils and macrophages by modulating their cytokines secretion (Claussen and Korn, 2012).

The first phase II trial testing the efficacy and safety of oral teriflunomide involved 179 relapsing onset MS patients (157 RR and 22 SP). Two doses (7 mg and 14 mg per day) were tested against placebo, and were shown to reduce by more than 61% the MRI activity (O'Connor et al., 2006). In addition, Teriflunomide in combination with IFN-beta was associated with a significant reduction of the GAD enhancing lesions, compared to the IFN alone (Freedman et al., 2012). The Teriflunomide MS Oral (TEMSO) trial was a large phase III study, involving 1088 relapsing MS patients, treated with the two doses. Compared to placebo, the ARR was significantly reduced in both treated arms (31% relative risk reduction), and the risk of sustained progression was significantly reduced by 30% in the 14 mg group only (O'Connor et al., 2011). The trial also confirmed the positive effect on the formation of new GAD enhancing lesions (O'Connor et al., 2011), detected in the previous study (O'Connor et al., 2006).

A separate phase III trial (TOWER: Teriflunomide Oral in People With RR MS) involved 1169, randomized for receiving the two doses of teriflunomide or placebo. The group receiving 14 mg teriflunomide had a 36.3% ($p < 0.001$) reduction in ARR and 31.5% ($p = 0.044$) reduction in the risk of 12 weeks sustained accumulation of disability compared to placebo (results presented at ECTRIMS 2012).

Data on safety are rather extensive, as leflunomide has been licensed for rheumatoid arthritis. Common adverse event are predominantly gastrointestinal,

skin rashes and hypertension (Schattenkirchner, 2000). Serious adverse event included elevated liver enzymes and neutropenia (Mladenovic et al., 1995). Teriflunomide (14 mg) was approved for the treatment of MS by the FDA in September 2012.

1.7.7 BG-12

BG-12 is a oral dimethyl fumaric acid (DMF) ester (FAE) compound already licensed as second line therapy for psoriasis (Mrowietz et al., 1999). It has been recently (March 2013) approved by the FDA for its use in MS. Its exact mechanism of action is poorly understood. Experimental studies have suggested anti-inflammatory effects on the immune system: increase production of anti-inflmmatory cytokines IL-10 and IL-1 (Asadullah et al., 1997) and reduction of TNF production (Lee et al., 2008).

Its efficacy in MS was assessed in a 24 week phase II trial, randomizing 257 RR MS patients into 4 groups: BG-12 120 mg daily, BG-12 120 mg three times daily (total dose 360 mg), BG-12 240 mg three times daily (total dose 720 mg) or placebo. The treatment with the higher dose (720 mg) associated with a 70% reduction of GAD enhancing lesions, but no differences were found in the other two treatment groups (Kappos et al., 2008; Kappos et al., 2012). Subsequently, two phase-III trials were carried out. The DEFINE (Determination of the Efficacy and Safety of Oral Fumarate in RR MS) involved 1237 RR MS patients, randomized for receiving 240 mg twice a day, 240 mg three times a day or placebo (Gold et al., 2012). Both doses were superior to placebo in reducing the ARR (53% and 48% for the lower and higher dose, respectively) and in reducing the number of GAD enhancing lesions. In addition, BG-12 reduced the disability progression at 12 weeks, by 38% (low dose) and by 34% (high dose). In the second phase-III trial, CONFIRM (Comparator and an Oral Fumarate in RR MS), 1430 RR MS patients were randomized for receiving BG-12 (480 mg daily or 720 mg daily), GA or placebo (Fox et al., 2012). In both BG-12 doses arms there was a significant reduction of the ARR (51% and 41%, respectively) and of

the number of new T2 lesions (73% and 71%, respectively), compared to GA (Fox et al., 2012).

Adverse events more frequently occurring in BG-12 treated patients were gastrointestinal symptoms and flushing (typically within 30 minutes from the administration).

1.7.8 Treatments in advance clinical development

1.7.8.1 Alemtuzumab

Alemtuzumab is a monoclonal antibody targeting the CD52 antigen on the surface of more than 95% of T and B cells, monocytes and macrophages, and causing leukopenia (Flynn and Byrd, 2000). In US and Europe is approved as first line treatment for B cell lymphoma.

Its therapeutic effect in MS has been tested in several trials. In an open label study, 25 SPMS patients received a single dose of Alemtuzumab and were followed up for 18 months. Although there was a significant decrease of relapses rate and inflammatory activity detected by the MRI, most of patients experienced disease progression and an increase in brain atrophy (Paolillo et al., 1999). However, when tested in active RRMS (high relapse rate), along with a strong relapse suppression (90%), patients also experienced disability improvement, suggesting different mechanisms driving disease evolution before and after the onset of the progressive phase (Coles et al., 1999). Recently, a phase II trial (CAMMS223) compared the efficacy of Alemtuzumab (annual infusions 12 mg or 24 mg) versus SC IFN beta-1a in patients with early RRMS (disease duration < 3 years), followed up for 36 months. It was observed a significant reduction of ARR (74%) and of sustained disability accumulation (71%), compared to IFN. In addition, the EDSS score improved by 0.39 points in Alemtuzumab treated patients, whereas worsened by 0.38 points in patients treated with IFN (Coles et al., 2008). These finding were partially confirmed

by the 2 subsequent phase III trials. Comparison of Alemtuzumab with SC IFN beta-1a (CARE-MS1) did not shown any significant difference in the progression of disability, between the two compounds (8% of Alemtuzumab group and 11% of the IFN-beta group experienced sustained increase in the EDSS score). However, the study confirmed the Alemtuzumab superiority in reducing the relapse frequency (Cohen et al., 2012a). Results from the second phase III trial (CARE-MS2) were more encouraging: Alemtuzumab, compared to SC IFN beta-1a, reduced the relapse rate by 49% ($p<0.001$) and the risk of a 6-month sustained accumulation of disability (increase of 1 EDSS point from the baseline, or at least 1.5 point if the baseline was 0) by 42% ($p = 0.008$). In addition, 29% of Alemtuzumab treated patients experienced an improvement of the EDSS score (Coles et al., 2012a)

The principal adverse effect was autoimmunity caused by lymphopenia, presenting months or even years after treatment. About 20-30% developed hyperthyroidism and about 3% developed idiopathic thrombocytopenia purpura (ITP).

1.7.8.2 Autologous haematopoietic stem cell transplantation

Immune ablation followed by autologous hematopoietic stem cell transplantation (HSCT) is under evaluation as an experimental therapeutic approach in MS and other autoimmune diseases. The rationale of HSCT in MS is to eliminate the existing immune system that harbours disease-associated cells and regenerate a new and healthy immune system (Muraro et al., 2003). Heterogeneous patient populations and transplantation regimens, small number of patients, and lack of a control arm makes difficult to draw definitive conclusions about its efficacy in MS (Mancardi and Saccardi, 2008).

HSCT clearly exerts a suppression of relapses and of Gadolinium-enhancing MRI lesions (Mancardi et al., 2001; Saccardi et al., 2005; Saiz et al., 2001), much stronger than other treatments (Mancardi and Saccardi, 2008). The largest data set currently available is a retrospective analysis from the European Group for Blood and Marrow Transplantation (EBMT), last updated in 2006 (Saccardi et al., 2006). The analysis

reported a progression-free survival in 60-70% of patients after 3 years and 50-60% after 6-8 years. In addition, Burt et al. recently treated 21 RRMS patients with mild to moderate disability (EDSS 2.0-5.5) with a reduced-intensity conditioning HSCT regimen and reported 100% progression-free survival and 81% showing an improvement of neurological function after a median of 3 years of follow-up (Burt et al., 2009b). However, 25% of patients had a relapse post-therapy; this was higher than what was previously observed in intermediate- and high-intensity regimes.

A group that seems to especially benefit from HSCT are young patients with highly aggressive, rapidly evolving forms of MS (Fagius et al., 2009; Kimiskidis et al., 2008). Case reports have described dramatic clinical improvement, stabilisation of lesion burden, and suppression of relapses among patients with highly active RRMS, experiencing high numbers of relapses (Portaccio et al., 2007).

Unfortunately, the use of HSCT remains limited by its potential severe adverse effects, including death. However, data from EBMT database showed a treatment related mortality that has decreased consistently over time and has dropped to 1.3% in 2001-2007 (Mancardi and Saccardi, 2008).

1.7.8.3 Laquinimod

Laquinimod is a novel oral immunomodulator with both anti-inflammatory and neuroprotective effects (Bruck and Wegner, 2011). It inhibits the leukocytes migration into the CNS and the production of pro-inflammatory cytokines (Wegner et al., 2010). In EAE it was shown to reduce the axonal damage (Thone et al., 2012).

Laquinimod was initially assessed in a phase II trial, comparing two doses (0.1 and 0.3 mg) to placebo, in 209 RR MS patients. There was a significant reduction by 44% of GAD enhancing lesions in the higher dose arm, but no effect on the number of relapses (Polman et al., 2005a). The results of the phase III ALLEGRO (Assessment of Oral Laquinimod in Preventing Progression in MS) study were recently published (Comi et al., 2012). Patients (n = 1106) were randomized for receiving 0.6 mg/day of

Laquinimod or placebo. In the treatment group there was a modest reduction (23%) of the ARR and of the risk of disability progression (23%), compared to placebo. In addition, Laquinimod reduced the number of new T2 lesions and the number of GAD enhancing lesions (Comi et al., 2012) and more importantly, it reduced significantly (p = 0. 004) the rate of grey matter atrophy, indicating a neuro-protective effect (Filippi et al., 2013). Laquinimod is currently being reviewed by the European Medicine Agency (EMA).

1.8 Aims of this thesis

This thesis aimed to analyse the long-term disease evolution and the survival probability of the London Ontario (LO) database patients. We hypothesized that early features of the disease allow estimating the risk, for individual subjects, of developing severe disability. Models predicting the attainment of hard outcomes were developed.

The specific aims of the analyses were:

- **Aim 1**: To carry out a descriptive analysis of the LO database population and to compare the disease progression among patients stratified by the clinical phenotype. To assess the survival, the causes of death and the factors affecting mortality.
- **Aim 2**: To investigate the prognostic value of relapses and to examine their relationship with the long-term disease evolution.
- **Aim 3**: To assess the effect of age on the disease progression and the disability accumulation.
- **Aim 4**: To identify clinical and demographic features predicting the attainment of the SP phase.

Chapter 2

Methods

2.1 The London Ontario database

2.1.1 Methodology of the LO database

2.1.1.1 Data collection 1972-1984 (1st systematic data review)

The LO MS clinic was established in 1972 to provide care for patients in the referral area of southern Ontario. Patients were accrued between 1972 and 1984. Records were reviewed between 1979 and 1984, leading to the creation of the first database. This was the subject of several analyses, published in 4 scientific papers between 1989 and 1991 (Weinshenker et al., 1989b; Weinshenker et al., 1989c; Weinshenker et al., 1991a; Weinshenker et al., 1991b).

The cohort consisted of patients attending the tertiary referral centre for the province of Ontario and the geographically based clinic for residents in the Middlesex County (MC); 90 % of MS patients from MC were followed at the LO clinic (Hader et al., 1988), providing the clinic a geographically based character. The ascertainment of MS patients in the MC area was virtually complete, as all neurologists worked in five hospitals affiliated to the university department and agreed to refer their patients to the MS clinic. All individuals in the community were within 30-60 km distance from the MS clinic. During the period from 1973 to 1984, with the help of the Home care program and of the MS Society, the research team in LO reviewed the medical records from all teaching and non-teaching hospitals, all extended care facilities, community health services and nursing homes in the county. As of January 1984 the prevalence of MS, among residents in MC for at least 1 year, was 91/100000 and the incidence was 3.4/100000 between 1975 and 1979 (Hader et al., 1988). The diagnosis of MS was retrospectively confirmed, according to Poser criteria, using clinical and para-clinical (evoked response studies and CSF analysis) information (Poser et al., 1983).

In the MS clinic patients were evaluated by 4 MS neurologists and never received DMTs, but only steroid for treating relapses, as per standard protocol. Clinical and demographic information were recorded, and the disability outcome was measured using the DSS (Kurtzke, 1955). For those patients with disease onset prior to 1972 data were gathered retrospectively, based on patients' interview and on review of their record, and then longitudinally collected. Clinical evaluations were carried out annually or semi-annually, regardless of the patients' clinical course. Information were stored in research charts, separated from patients' hospital records, and completed at each patient visit. Data were recorded on standardized forms and entered on a mainframe computer. At each visit new information was collected and data previously recorded were confirmed. The frequent clinical assessments and patients' interviews allowed to check the reliability of both the retrospectively and prospectively collected data on the disability outcome. Particularly, those information gathered retrospectively were inserted in the database only when confirmed in serial evaluations and therefore considered indisputable.

At the end of accrual (1984), 1099 patients, with mean disease duration of 11.9 years, were identified. A subgroup of "seen from onset" (SO) patients (n = 197) was seen within 12 months of their first symptoms, and information was collected only prospectively. By comparison against the total population, the MC (n = 196) and the SO subgroups were used as control for assessing the impact of ascertainment and referral biases on data. The disease course was classified as RR in 65.8% (n = 722), or progressive from onset, with (relapsing progressive [RP]: n = 162 [14.8%]) and without (chronically progressive [CP]: n = 205 [18.7%]) superimposed acute episodes. The criterion for progressive disease was continuing deterioration without remission, regardless of the rate of progression.

2.1.1.2 Data collection 1984-1997 (2nd systematic data review)

The original database was extended, as the data collection of surviving patients continued with annual or bi-annual examinations until 1997. After 25 years of follow up, the second systematic data review was carried out; data analyses' results were

published in 5 scientific papers between 1999 and 2006 (Cottrell et al., 1999a; Cottrell et al., 1999b; Ebers et al., 2000; Kremenchutzky et al., 1999a; Kremenchutzky et al., 2006a).

Patients categorized into disease course subgroups (RR, RP and CP) from the original natural history cohort were reclassified according to the internationally accepted, standardized definitions of the clinical phenotype (Lublin and Reingold, 1996). Information on previously recorded DSS scores was revised. For local patients who have become institutionalized, information on the physical status was derived from telephone follow up with physicians, family and, as much as possible, from the patients themselves. This has included visit to patients in local nursing home.

Extensive efforts were made to trace lost to follow up. These included attempts to contact the patients, their family doctors, their relatives and neighbours and local MS society chapters. The assistance from the government of Ontario and its Ministry of Health provided access to the provincial registry of deaths, allowing to identify the final outcome in the majority of patient lost to follow up. From the original 1099 patients cohort 55 were excluded: 41 turned out to have wrong diagnosis and 15 were either duplicates, not originally recognized because of name change, or had follow up shorter than 2 years, or had been lost to follow up. Consequently, the total sample size reduced to 1043 cases (Ebers et al., 2000; Kremenchutzky et al., 2006a).

2.1.1.3 Definitions

2.1.1.3.1 Clinical and demographic features

Information included in the clinical notes concerning the symptoms characterizing the onset of the disease was used to determine the neurological systems involved at onset. For patients recruited when the disease had already started, findings on the initial examination were used to confirm data suggested by the history. Symptoms at onset were grouped into balance impairment, bladder symptoms, gait impairment, diplopia, limbs ataxia, Lhermitte sign, motor symptoms (acute or slow), pain, and

sensory symptoms (face or body) (Weinshenker et al., 1989a; Weinshenker et al., 1989c). Neurological systems involved at onset were categorized into motor, sensory, cerebellar, brainstem, optic and bladder/bowel (Cottrell et al., 1999b). Exacerbations were defined as acute development of new symptoms or worsening of existing symptoms lasting more than 24 hours (Poser et al., 1983).

2.1.1.3.2 Disability outcomes

Among LO database patients, disability was scored using the DSS (Kurtzke, 1955). The analyses presented in this thesis focused on the attainment of hard outcomes:

- DSS 3: ***moderate disability***
- DSS 6: ***ambulatory but unilateral assistance required to walk short distance***
- DSS 8: ***restricted to bed with preserved use of arms***

2.1.1.3.3 The progressive course

In the original natural history series, accrued between 1972 and 1984, progressive disease was defined by a continuous deterioration without remission, regardless the rate of deterioration (Weinshenker et al., 1989b). During the second data collection (1984-1997) the criterion for a progressive course was redefined more strictly. Patients with progressive MS had to show ***at least 1-year of continuous deterioration, without substantial remission, regardless of the rate of worsening.*** In many patients the disease course appeared stable for extended periods however, subjective worsening was often described even if it was not clinically obvious. Therefore, ***transitory plateaus and trivial improvements in the relentlessly progressive course were allowed in the long term, although steady progression was the rule***. The onset of the progressive course was predominantly characterized by a distal central motor dysfunction and the progressive deficit was almost exclusively localized to the distal lower extremity corticospinal tract fibres, and manifested with ambulation impairment (Kremenchutzky et al., 2006a).

During the second systematic data review (1997), researchers in LO focused on information on the onset of SP. Special attention was paid to patients with a single attack before progression (SAP MS) and to those entering the progressive phase when permanent motor disability had not developed yet (Kremenchutzky et al., 2006a). This aimed specifically at addressing the problem of the overlapping between the relapsing and the progressive phase. Those who started to progress when DSS 2 was reached, were identified. Such patients may have had multiple relapses but with complete recovery, making the insidious onset of the SP phase identifiable at an early stage, when they typically complained of exercise-induced impairment of the ambulation. With the advantage of frequent neurological evaluations, in few cases the onset of progression could be even identified at DSS 1, by documenting the asymptomatic emergence of long tract signs. Overall, majority of SP patients attained the progressive course at or before DSS 3 (Figure 4.9), and the great majority (84.8%) of SAP patients at or before DSS 2 (Kremenchutzky et al., 2006a). Among those who had multiple relapses, mostly with partial recovery, and converted to SP MS at higher DSS levels, it was particularly difficult to separate the relapsing phase from the progressive phase (personal communication from George Ebers).

2.1.1.3.4 The disease phenotype

Following the 1996 international consensus on the classification of clinical subtypes (Lublin and Reingold, 1996), patients were reassigned to the new standardized definitions (Cottrell et al., 1999a; Cottrell et al., 1999b; Kremenchutzky et al., 1999a). During the long-term follow up, some PP patients (n = 21), questioned about early symptoms, recalled early exacerbation initially forgotten and were therefore reclassified as SP MS. (Cottrell et al., 1999b). In addition, the second systematic review of data focused on those 367 cases originally identified as progressive patients (Weinshenker et al., 1989b) with or without superimposed relapses (chronically progressive and relapsing progressive MS), attempting to justify the use of this terminology, based on the long term outcome (Kremenchutzky et al., 1999a). The original group of relapsing progressive MS was reclassified as one of the 3

different progressive clinical phenotypes, according to the last consensus (Lublin and Reingold, 1996):

- **PP MS**: disease progression from onset, with occasional plateaux and temporary minor improvements allowed, but not distinct relapses.
- **SP MS**: initial RR disease course, followed by progression with or without occasional relapses, minor remission and plateaux.
- **PR MS**: progressive disease from onset, with clear acute relapses, with or without full recovery.

In view of the identical survival curves, between PP and PR patients, it was concluded that superimposed relapses did not affect the outcome in PP MS and the term "progressive-relapsing" could be abandoned. Accordingly, PR patients were merged in the PP subgroup (Kremenchutzky et al., 1999a).

Eventually, among the total population of 1043 patients, 219 were deemed to have a PP course and 824 a relapsing onset course, of whom 551 had converted to SP MS (Kremenchutzky et al., 2006a).

2.1.2 Data quality check procedures

Following the second systematic review (1997), the data collection continued until 2000, totalling 28 years follow up (1972-2000). In 2006 Prof George Ebers gave to me and to the Sylvia Lawry Centre (Munich, Germany) the database, in order to carry out further analyses on the LO cohort.

The Sylvia Lawry Centre (SLC) (http://www.slcmsr.net/en/about/start.html) was established in 2001 to support the research in MS and to accelerate the development of effective therapies, by using innovative statistical methods and computer technology, combined with medical expertise. To facilitate the accomplishment of these aims the centre was donated 44 data sets from placebo arms of many MS RCTs and many other natural history registries from around the

world. The team of statisticians has gained extensive experience in MS data management and has offered me statistical support for the analyses reported in this thesis. I initially attended the centre in two separate 4-weeks periods, during which I learnt the correct methodology for managing data, planning and carrying out statistical analyses. I then attended the centre in several other short-term periods, when a closer collaboration with the statisticians was needed. Otherwise, we communicated through emails and telephone conferences and we exchanged data and analyses results by uploading word documents on the SLC intranet. In particular, I strictly collaborated with Dr Anneke Neuhaus and Dr Martin Daumer (director of the centre).

The data from the LO database were available on a CD-rom, containing several datasets in SAS, SPSS and Excel format. These were created by the LO research team, following the continuous update of information, correction of data and creation of new variables. Each dataset was named with the date of creation, allowing to trace how data changed over time. Thanks to the information contained the "**Data History doc**" (found in the CD-rom), we could decode meaning of variables in the database. The file also helped us to figure out that the most updated dataset, called **MS0200_1**, had been created by merging the information on SP patients contained in the dataset **FINAL.SPMS.DATABASE.012100,** which was used to carry out the latest analysis of the database (Kremenchutzky et al., 2006a), to the information contained in the dataset called **MS0999_1**.

Before carrying out the statistical analyses we decided to check the reliability of data, by performing independent verifications in parallel, and by comparing the results obtained. For each patient, identified through an "ID number", we checked and compared data contained in **MS0999_1** and in **MS0200_1**. Datasets. This was done manually by me, and using the software R by the SLC. Very few inconsistencies were detected and some arbitrary decisions had to be taken with regard to which information could be trusted. **We concluded that the overall quality of data was highly trustworthy**. In order to solve the few inconsistencies detected, we decided to rely on the two datasets, containing the most updated information: 1)

FINAL.SPMS.DATABASE.012100 for data on SP patients; 2) **MS0200_1** for data on RR and PP patients.

2.1.2.1 Inconsistencies and methods to handle it

1. **Duplicate or wrong diagnosis:**

   Patients (n = 56) identified using the variable **NSTATUS1,** indicating **N** or **DUP,** meaning that they had wrong diagnosis or were duplicates, not initially recognized because of name change (Ebers et al., 2000), were deleted.

2. **Information on times to DSS levels, indicated by the variable TK1 to TK10:**

   A) Type I inconsistency:

   For some patients values of the times to disability endpoints increased and then decreased, creating not continuous patterns: e.g. time to DSS 3 = 5 years; time to DSS 4 = 8 years; time to DSS 6 = 7 years.

Times to DSS 3-6-8-10 (the outcomes analysed in this study)

The DSS (Kurtzke, 1955) is an ordinal scale and the attainment of motor disability (≥ DSS 3) is meant to be irreversible, unless the patient is experiencing an acute exacerbation. Minor improvements on the EDSS can occur. However, they are mostly not sustained for more than 6 months and they are mainly observed among patients with short disease duration and still in the lower intervals of the scale (EDSS 1-3) (Tremlett et al., 2012). In the LO database, with the advantage of the long follow

up, information on previously recorded DSS scores, especially DSS 3-6-8-10, have been repeatedly checked and confirmed. We eventually concluded that, when generating the last dataset (**MS0200_1)**, the research team in LO updated information only on the times to DSS 3-6-8-10, which were the focus of the latest publications and were not subjected to fluctuations (Cottrell et al., 1999a; Cottrell et al., 1999b; Ebers et al., 2000; Kremenchutzky et al., 1999a; Kremenchutzky et al., 2006a). The information on the times to the other DSS levels was not updated, creating the inconsistent **TK** patterns. We therefore choose to trust only the most updated (from **MS0200_1**) information on the times to hard endpoints (DSS 3-6-8-10) and to purge the unreliable information about the times to the other DSS levels.

Times to DSS 1-2-4-5-7-9 (the outcomes NOT analysed in this study)

Subsequently, by checking a previous dataset, named **MS1197**, we identified variables providing information on the DSS levels attained at each year from the disease onset (variable name was **K1-n**). After comparison with the most updated data on the times to DSS 3-6-8-10, from the dataset **MS0200_1,** this information appeared reliable and more accurate than those given by the variables **TK-n**. We therefore decided to trust it and to use it in order to calculate the times to DSS 1-2-4-5-7-9 levels, which nevertheless were not used as outcomes in this study. Information were accepted only when the following conditions were respected:

- **Time to DSS 1 and to DSS 2 < Time to DSS 3**
- **Time to DSS 4 and to DSS 5 < Time to DSS 6**
- **Time to DSS 7 < Time to DSS 8**
- **Time to DSS 9 < Time to DSS 10**

This long process finally led to obtain reliable information on the times to all DSS levels, for the vast majority of patients.

B) Type II inconsistency:

For some patients information on the times to different disability endpoints had the same value (e.g. time to DSS 6 = 31 years; time to DSS 8 = 31 years; duration of disease = 31 years).

In previous datasets, censoring indicators were created (variable **TKXCENS**: **0** = DSS level not reached, **1** = DSS level reached). When the censoring value was 0, the time to the corresponding DSS level was scored equal to the disease duration (procedure required in order to perform survival analysis). By comparing information on times to DSS level, censoring indicators and disease duration, we figured out that, during previous data updates, occasionally, information on the time to disability levels, among patients with the corresponding censoring value equal to 0 (indicating that the level had not been reached), actually indicated the disease duration, thus generating confusion. We decided to accept the value on the times to DSS levels only when the censoring indicator was = 1.

3. Information on censoring levels, indicated by the variable TKXCENS:

Some patients had non-linear censoring values (e.g. censoring for DSS 3 = 1, censoring for DSS 6 = 0, censoring for DSS 8 = 1; or censoring for DSS 3 = 0, censoring for DSS 6 = 1, censoring for DSS 8 = 1).

We concluded that the censoring value = 0 was also incorrectly used for indicating that the specific disability level had been reached, but the time to the DSS level was unknown (left censoring). Subsequent levels of disability were then recorded years later. Therefore, to obtain consistent censoring values we used a backward algorithm. Starting from information whether DSS level 10 was reached or not, along with information on the time to the next lower disability level and the censoring information, we were able to assign consistent censoring values.

When the information on the time to DSS 10 (**TK10**) was available the censoring information for DSS 10 (**TKCENS10**) was scored **1**, otherwise it was scored **0**. In addition, the information on the attainment of DSS 10 (**TKCENS10**) was checked by comparison with the variable **SURVIVAL**, which contained the most updated data on dead patients. In point 8 (below) are provided details on how we assigned the status "death due to MS" (DSS 10 reached). Once the checking was carried out, the following algorithm was applied.

- **Censoring information for DSS 8:**

  A) If information on the time to DSS 8 (**TK8**) was available, the censoring information for DSS 8 (**TKCENS8**) was scored **1**.

  B) If information on the time to DSS 8 (**TK8**) was **NOT** available and the censoring information for DSS 10 (**TKCENS10**) was scored **0**, the censoring information for DSS 8 (**TKCENS8**) was scored **0**.

C) If information on the time to DSS 8 (**TK8**) was **NOT** available and the censoring information for DSS 10 (**TKCENS10**) was scored **1**, the censoring information for DSS 8 (**TKCENS8**) was scored **3**.

- **Censoring information for DSS 6:**

  A) If information on the time to DSS 6 (**TK6**) was available, the censoring information for DSS 6 (**TKCENS6**) was scored **1**.

  B) If information on the time to DSS 6 (**TK6**) was **NOT** available and the censoring information for DSS 8 (**TKCENS8**) was scored **0**, the censoring information for DSS 6 (**TKCENS6**) was scored **0**.

  C) If information on the time to DSS 6 (**TK6**) was **NOT** available and the censoring information for DSS 8 (**TKCENS8**) was scored **1**, the censoring information for DSS 6 (**TKCENS6**) was scored **3**.

- **Censoring information for DSS 3:**

  A) If information on the time to DSS 3 (**TK3**) was available, the censoring information for DSS 3 (**TKCENS3**) was scored **1**.

  B) If information on the time to DSS 3 (**TK3**) was **NOT** available and the censoring information for DSS 6 (**TKCENS6**) was scored **0**, the censoring information for DSS 3 (**TKCENS3**) was scored **0**.

  C) If information on the time to DSS 3 (**TK3**) was **NOT** available and the censoring information for DSS 6 (**TKCENS6**) was scored **1**, the censoring information for DSS 3 (**TKCENS3**) was scored **3**.

When this process was finalized, the censoring information (**TKCENS**) was labelled as follow:

- **1 = disability level was reached and point in time was documented**
- **3 = disability level was reached but point in time was not documented (left censoring).**
- **0 = disability was not reached during the observation time (right censoring).**

4. **Information on the disease duration:**

Two variables indicated the disease duration: **DUR** and **DUR1**. The variable **DUR** appeared inconsistent, as it often indicated different values between the two most update datasets (**MS0200_1 and MS0999_1).** However, **DUR1** seemed more consistent. In order to check its accuracy we decided to apply the following rules:

- **DUR1** had to be equal to time to DSS 10 (when DSS 10 was reached).
- **DUR1** had always to be ≥ the time to the last DSS level recorded.

Six patients did not meet the second condition and therefore the variable was deemed to be unreliable. For the remaining patients, the information on the length of the disease course was considered reliable.

5. **Information on the year of last visit:**

The year of the last visit was indicated by the variable **YRLV**. This turned out to be unreliable, as some patients seemed to have been examined after the year of death. We decided to calculate the year of last visit by adding the value of the disease duration (**DUR1**) to the value of the year of the onset of the disease, creating a new variable (**YRLVcalc**).

6. **Information on the type of the disease course:**

The type of the disease course was indicated by the variable **NSTATUS.** According to **Data History doc**, during the last data update, 20 patients

turned out to be wrongly labelled as SP and were reclassified as RR. There were identified, by using the dataset **FINAL.SPMS.DATABASE.012100** (containing the final list of SP patients), and labelled correctly.

7. **Information on number of relapses:**

   The number of relapses was indicated by the variables **RELY1** (number of attacks in the first year), **RELY12** (number of attacks during the first two years) and **PREREL** (number of attacks during the RR phase, before the onset of progression). The accuracy of the three variables was checked by applying the following rules:

- **RELY12** had to be ≥ to **RELY1**
- **RELY12** had be ≤ **PREREL**

Two patients were found to have **RELY12** > **PREREL;** therefore the variable **PREREL** was deemed to be unreliable. For the remaining patients, information of number of relapses during the first two years and during the RR phase was deemed to be reliable.

8. **Information on survival, causes of death and death due to MS (DSS 10):**

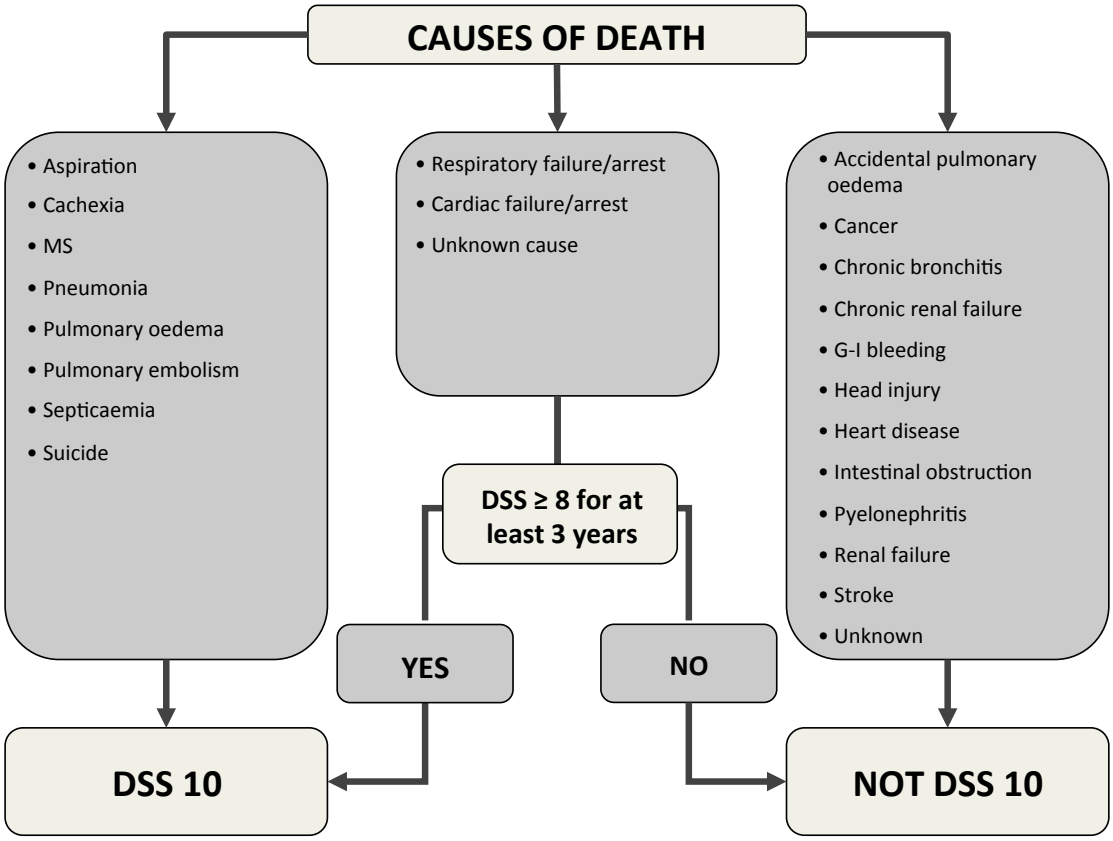
According to **Data History doc,** the variable **SURVIVAL** contained the most updated information on those patients who had died, by the end of the observation period. Information on causes of death were indicated by two variables: **IMMCAUSE** (primary cause of death) and **SECAUSE** (secondary cause of death). Among dead patients, as indicated by the variable **SURVIVAL**, the patients who died from MS and therefore reached DSS 10 were identified by applying the following rules (Figure 2.1):

A) Primary causes of death recorded as aspiration, cachexia, dehydration, "Multiple Sclerosis", pneumonia, pulmonary oedema, pulmonary embolism, septicaemia and suicide, were considered **attributable to MS.**

B) Primary causes of death recorded as G-I bleeding, accidental pulmonary oedema, accident, cancer, heart disease, chronic bronchitis, chronic renal failure, head injury, intestinal obstruction, pyelonephritis, renal failure and stroke, were considered **NOT attributable to MS.**

C) For those patients who died from unknown causes, respiratory failure/arrest or cardiac failure/arrest, an arbitrary decision had to be taken, regarding whether the death was attributable to MS or not. We applied a composite criterion, which was based on the information about the secondary cause of death and on whether a bedridden status (DSS 8) had been reached and sustained for at least 3 years, before dying.

Figure 2.1 Algorithm developed and utilized in order to define DSS 10 (death due to MS).



9. **Information on clinical and demographic features:**

   Information on clinical and demographic features was checked by comparison among the 3 datasets. When inconsistencies were detected it was agreed to trust only information contained in the most updated dataset (**MS0200_1**).

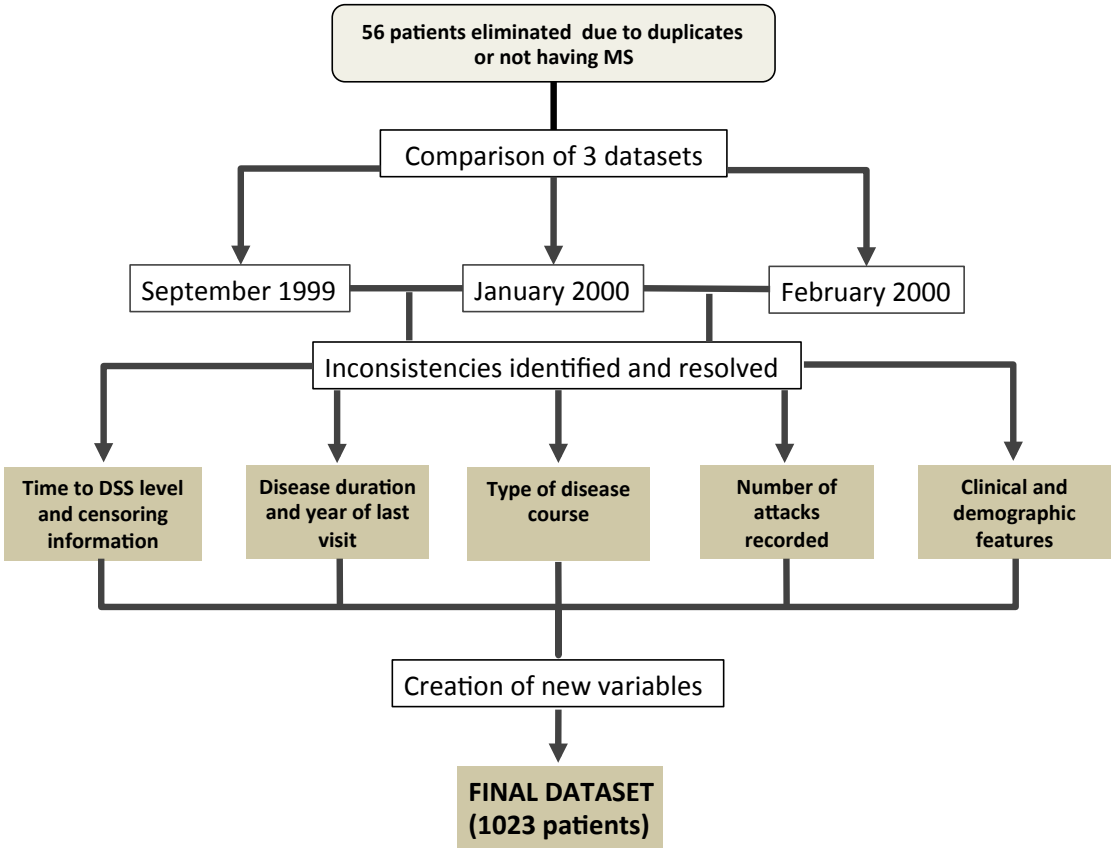
2.1.2.2 The creation of variables

Variables for carrying out the analyses were created from the raw data or computed from existing variables. In addition, when needed, categorical variables (grouping) were created from ordinal variables.

2.1.2.3 The final dataset

Utilization of specific and objective rules has prevented the statistical bias that occurs when data are manually corrected or censored. The systematic data clean-up and the quality check resolved the inconsistencies, which were caused by the stratification of data updates over a number of years, and led to the creation of a final dataset, containing the most reliable information (Figure 2.2).

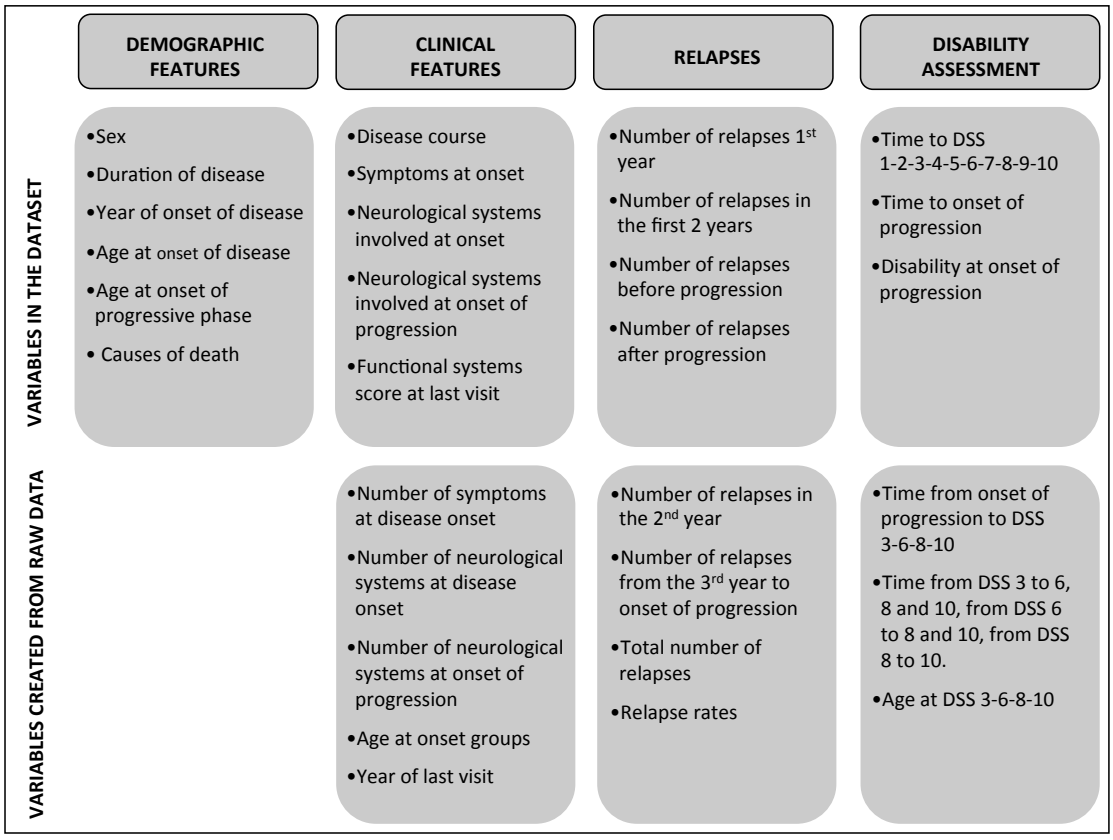
Figure 2.2 Schematic representation of the data quality check procedures carried out on the database.



The dataset now contains, for each patient, the following information: ID, gender, time (years) to DSS 1-2-3-4-5-6-7-8-9-10 and corresponding censoring indicators, time (years) from DSS 3 to 6, 8 and 10; times from DSS 6 to 8 and 10; time from DSS 8 to 10 and corresponding censoring indicators, duration of the disease (years), age at disease onset, year of onset of the disease, clinical subtype of the disease, time (years) to the onset of the progressive phase, age at DSS 3, 6, 8 and 10, age at onset

of the progressive phase, DSS at onset of the progressive phase, time (years) from the onset of the progressive phase to DSS 3-6-8-10 and corresponding censoring indicators, symptoms at the disease onset, neurological systems involved at the disease onset, neurological systems involved at the onset of progressive phase, the number of relapses in the 1st and 2nd year of the disease and corresponding relapse rate, the number of relapses before and after the onset of the progressive phase and corresponding relapse rate, number of relapses after the second year of the disease up to the onset of progressive phase and corresponding relapse rate, total number of relapses and corresponding relapse rate, functional systems score at last DSS assessment, diagnostic certainty according to Poser criteria (Poser et al., 1983), primary and secondary cause of death, year of last visit (Figure 2.3).

Figure 2.3 Information contained in the LO database. The boxes list the groups of variables that passed the quality check procedures and constitute the final data set. In the top row the variables already present in the dataset are listed. In the bottom row the variables created from the raw data are listed.



2.2 Statistical analyses

A standardized approach for carrying out analyses was mutually agreed with the SLC. This consisted, firstly of setting up a statistical plan for each aim of the project. Subsequently, statistical analyses were performed by me, using the SPSS software, and independently by the SLC statisticians, using R software. Eventually, results from the two analyses were compared. If differences were detected, the analyses were repeated after revising together the statistical methods, until results matched. This approach guaranteed high reliability to our conclusions.

2.2.1 Survival analysis

Due to the lack of information on the disease evolution, in the entire natural history cohort, from the onset of the disease until death, we need to rely on estimates. With respect to a given endpoint (e.g. the attainment of a level of disability, the onset of progression) for every patient, at the end of the study, fit in one of the following categories:

- The endpoint was reached.
- The endpoint was not reached and the patient was observed until the end of the study.
- The endpoint was not reached and the patient was lost to follow up during the study period.

The last two categories form the group of censored patients. Analysing only observed data, without taking in account censored information, is easier but invariably leads to underestimate the time to reach the endpoint, and consequently to overestimate the disease severity.

Survival analyses (Cox Regression and Kaplan Meier) allow me to assess a dichotomized outcome (that has or has not happened) as a function of the time until the event (endpoint) occurs (Singh and Mukhopadhyay, 2011). By calculating the

number and the proportion of subjects failing to attain the endpoint, at certain time intervals, survival analyses assess the estimated time to and the probability of experiencing the event in specific subgroups. In addition, when estimating the cumulative probability, these techniques include in the analysis each subject who did not experience the event for the period, up to the time he/she became censored. The biggest limitation is related to the proportion of censored patients, which tends to increase with the time needed to attain the endpoint and affects the accuracy of the estimated time intervals. The larger the number of censored information, the longer will be the estimated time to the endpoint. However, long follow-up of natural history cohorts guarantees lower proportion of censored data (Clark et al., 2003). Therefore, although they might provide longer and not necessarily more accurate estimates, these techniques are preferable to analyses strictly based on observational data.

The survival analyses can be potentially affected by bias from the immortal time. This is the period of follow up, during which, by design, the outcome cannot occur (Suissa, 2008). For instance, when assessing the effect of a variable occurring during the first five year of the disease (e.g. relapses), the endpoint must necessarily happen at least after five years from onset.

2.2.1.1 Kaplan Meier analysis

The **Kaplan Meier** technique maximizes the use of information available. Each patient included in the analysis contributes to calculate the estimated probability of an event, for as long as he/she is known to be event-free. With the increasing number of subjects experiencing the event, at certain time points, the cumulative risk of attaining the endpoint becomes lower or, in other words, the probability of surviving (remaining event free) becomes greater (Clark et al., 2003). The Kaplan Meier survival curve represents a plot of the survival probability against the time (disease duration) and it is used to estimate the median survival time to the event (time at which, in 50% of cases, the event occurred).

Survival curves of two or more groups of patients can be compared using the **log-rank test.** This allows one to assessing the null hypothesis, where there is no difference among groups, in the probability of an event (Peto et al., 1977). The log-rank test is based on the assumption that the survival probabilities are the same for subjects early and late in the study. It calculates, in each group, at each time point, the number of events observed and those expected since the occurrence of the last event. The number of expected events is obtained by multiplying the proportion of subjects at risk by the number of events observed at specific time point. The values are used for computing a total expected number of events in each group, and for making the comparison (Singh and Mukhopadhyay, 2011). P-values express the statistical significance of the difference observed between the groups. The log-rank test can also be used for assessing the prognostic value of specific patient related features, potentially affecting the survival time.

However, Kaplan Meier analysis remains of limited value when assessing the influence of multiple factors occurring simultaneously (multivariate analysis) (Clark et al., 2003).

2.2.1.2 Cox regression analysis

The **Cox (proportional hazard [PH]) linear regression** model is widely used for performing multivariate survival analyses (Bradburn et al., 2003a). By stratifying the cohort into smaller subgroups, the Cox regression analysis allows to assess the relationship between a set of covariates and the time to a particular event.

Covariates in the model can be either categorical or numerical. The size of the risk of reaching the endpoint, at specific times, for each covariate, is expressed by the hazard ratio (HR). This is calculated from the regression coefficient, which can be either negative (associated with a reduced risk) or positive (associated with an increased risk). Therefore, an HR above 1 indicates that the covariate associates positively with the event, and increases the risk proportionally. When the covariate correlates with the event conversely, so that higher values reduce the risk, the HR

value is below 1. When analysing numerical covariates, HR expresses the risk of the event due to a single unit value (e.g. 1 year of age). By using the regression coefficient, the HR indicating the risk due to specific values (e.g. the risk when aged 30) can be calculated.

2.2.1.3 Cox regression analysis: multivariate models

The great strength of the Cox regression analysis is the possibility to weight the effect of each covariate on the outcome over time, and to assess the degree of interdependency with the other variables. A covariate exerting a statistically significant effect on the risk of experiencing an event, in a univariate analysis, can become statistically NOT significant when tested along with other covariates, in a multivariate analysis. For this reason, multivariate models are useful tools when needing to estimate the risk of an event according to the concomitant effect of several variables. A standard approach for building up multivariate models implies to first test all variables of interest in the multivariate model. Subsequently, those covariates found to have no statistically significant relevance ($p > 0.05$) can be excluded stepwise, until the model contains only variable impacting significantly ($p < 0.05$) on the risk.

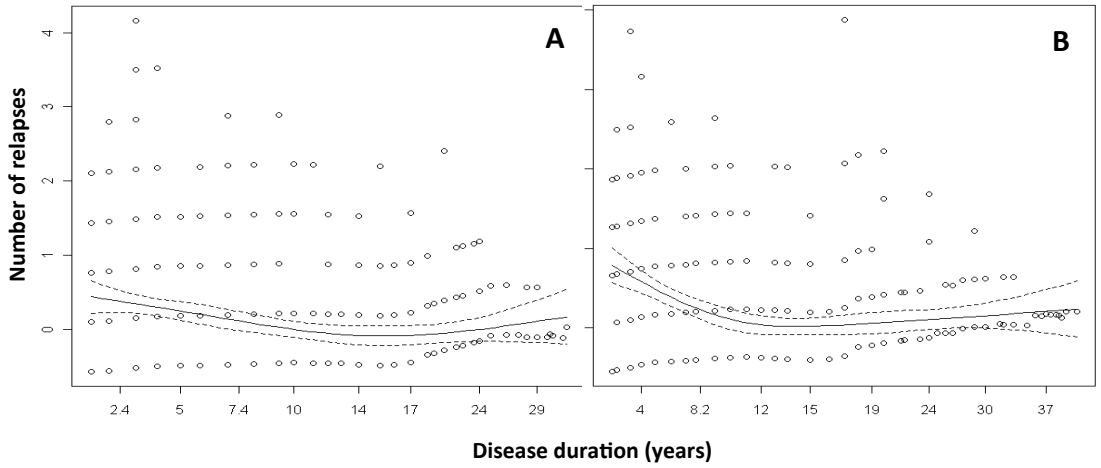
Finally, by combining the HRs from each covariate, impairing survival to varying degree, we can obtain a single HR value, expressing the overall risk of attaining the endpoint in a specific model. By combining the effect of all variables, specific clinical scenarios can be simulated.

2.2.1.4 Cox regression analysis: adequacy of the model (goodness to fit)

It is important to evaluate how well the model represent the data. The adequacy of a survival model depends on whether it represents the survival patterns in the data to an acceptable degree (Bradburn et al., 2003b). The key assumption of the PH model is that the hazards of the event in any group are proportional (constant over time)

and not overlapping at any time point. Adequate models do not violate the PH assumption. To verify the PH assumption, the hazard in each group can be plotted (Schoenfeld residual plot). If the model is valid, the hazard curves for the groups should be parallel and should not cross, as shown in the plots below (Figure 2.4).

Figure 2.4 Cox regression analysis. Schoenfeld residual plots for checking the assumption of the PH model. Hazard of attaining SP (A) and DSS 6 (B) over time (x-axis) according to the number of early relapses (y-axis). Analysis of the LO database.



2.2.2 Binary logistic regression analysis

The binary logistic regression analysis can be used when assessing the effect of covariates on the probability of a dichotomized event, occurring during some fixed interval of follow up (Abbott, 1985). The odds ratio (OR) expresses the size of the effect exerted by the covariate. Similarly to Cox regression analysis, OR is calculated from the regression coefficient, which can be negative (associated with a reduced risk) or positive (associated with an increased risk). An OR of 1 indicates that the odds of an outcome are equally likely for both groups under comparison. The higher the value above 1, the stronger is the relationship between the covariate and the outcome. OR below 1 indicates a converse relationship with the outcome (the covariate associates with a lower risk).

Although the logistic regression often leads to similar conclusions, compared to survival analyses, especially in studies with short follow up and a slow rate of disease

progression (Green and Symons, 1983), the Cox regression analysis remains more attractive because of its ability of considering the time to the event. However, the binary the logistic regression analysis can be useful when the Cox regression model violates the PH assumption. In addition, the logistic regression analysis, unlike the survival analyses, allows to assess the effect of time-dependent covariates (e.g. disease duration) on the outcome.

2.2.2.1 Binary logistic regression analysis: multivariate models

Similarly to the Cox regression analysis, the binary logistic regression analysis can be used for building up models, taking into account the concomitant effect of several covariates. The ORs from each covariate, generated in the model, can then be used for computing a single OR, expressing the overall probability of the event to occur, due to the combined effect of all variables.

Chapter 3

The long-term disability accumulation and mortality in the London Ontario database

3.1 Introduction

Over the past 60 years, a great wealth of information on the natural history of the disease has been collected. Despite this, MS prognosis keeps puzzling clinicians and remains largely unpredictable, especially at individual level. Clinical features are heterogeneous and the disease severity ranges from benign forms to malignant cases (Lublin and Reingold, 1996).

Familiarity with the natural history of MS is crucial to the understanding of the health, social and economic impact of the disease on the patients, their family and careers as a whole. A detailed knowledge of the overall disease course helps physicians managing patients and it also gives reasonable expectations for the long term outcome to the individual patient, facing decisions on his/her personal and professional life. MS registries are a valuable source of information for improving our understanding of the disease prognosis. They can serve as important guide for clinical and therapeutic decisions, and ultimately for optimizing the use of currently available DMTs. By Identifying risk factors, we can rationalize the therapeutic approach, in order to treat timely and aggressively those at higher risk of developing severe disability, and to avoid exposing non disabled young adults to potentially toxic drugs. In addition, information on the disease course enables to improve the RCTs methodology, by refining inclusion criteria and by calculating, within defined limits, the sample size and the follow up required for detecting a beneficial effect of therapies. Finally, natural history studies can offer important clues on possible biological mechanisms driving the disease progression and affecting the prognosis.

3.1.1 The methodology of natural history studies

To obtain reliable information on the natural course, data should be gathered from a population that can be representative of the disease as a whole. This can be achieved in geographically based studies, including all cases of MS living in a well-defined geographical area (Confavreux et al., 1980; Runmarker and Andersen, 1993; Tremlett et al., 2006; Weinshenker et al., 1989b). A large number of patients is needed and, as the disease course normally spans over 40-50 years, decades of follow up are necessary. Collecting data over the long term is extremely challenging and requires huge efforts, especially for systematically following up each patients and minimizing missing information (Ebers, 2001).

Unfortunately, longitudinal studies are often affected by different sources of bias (Table 3.1). Ideally, all cases should be identified at the disease onset and data should be collected prospectively until death (inception cohort). However, inception cohorts of MS patients are virtually impossible to obtain, as the clinical onset does not correspond to the biological onset of the disease. In addition, the long disease course often prevents physicians from maintaining longitudinal assessments of all patients. When high levels of disability are reached extra efforts should be made to follow up home bound or institutionalized patients, in order to maximize the data collection. When gathered retrospectively, data might be inaccurate, owing to limited information in medical records and to imprecision of patients recall ("***recall bias***").

Furthermore, the accessibility to neurologists, the varying medical care standards and the cultural differences, with regards to seeking for medical attention, represent potential sources of "***ascertainment bias***". Hospital-based and clinic-based studies are often biased by a predominance of more severe cases. Consequently, the number of patients with benign forms, who tend not to attend the specialized clinics, might be artificially low, and the study might ultimately overestimate the disease severity. However, some tertiary centres can see virtually all cases in their

area, especially when they benefit from epidemiological studies, which minimize the ascertainment bias (Runmarker and Andersen, 1993; Weinshenker et al., 1989b), other see only the majority of cases (Confavreux et al., 1980; Tremlett et al., 2006). In population-based cohorts, patients' migration can also influence the quality of data ("***migration bias***") (Miller et al., 1992; Sumelahti et al., 2002). In addition, during the study, the frequency of assessments and the "***compliance bias***" (when patients expectations alter the examination findings) can affect the accuracy of the disability scores. This can be minimized by sufficiently long follow ups and frequent clinical evaluations, carried out at fixed intervals (Degenhardt et al., 2009).

Table 3.1 Sources of bias potentially affecting the quality of natural history data, and ideal features of a natural history cohort.

Sources of bias	Ideal features
• Recall bias (retrospective data collection)	• Longitudinal data collection (patients seen from onset)
• Ascertainment bias (Hospital/Clinic based studies)	• Geographic ascertainment (inception cohort)
• Missing information (high rate of drop off)	• Maximize data collection (low rate of drop off)
• Migration bias	• No use of DMTs

3.1.2 MS registries

Muller was the first to describe the evolution of MS over time (Muller, 1949). Since then, our knowledge on the natural history of the disease has been mainly based on long-term cohort studies, spanning over the past 60 years. Table 3.2 summarizes the methodological features of the most important MS registries from **US veterans** (Kurtzke et al., 1977; Kurtzke et al., 1979), **Los Angeles** (US) (Clark et al., 1982; Detels et al., 1982; Visscher et al., 1984), **Europe** (multicentre) (Riise et al., 1992), **Denmark** (Bronnum-Hansen et al., 1994; Bronnum-Hansen et al., 2004), **Sweden** (Eriksson et al., 2003; Runmarker and Andersen, 1993; Skoog et al., 2012), **Norway** (Myhr et al., 2001), **Germany** (Poser et al., 1982a), **Groningen** (The Netherland) (Minderhoud et al., 1988b), **Turkey** (Kantarci et al., 1998), **Scotland** (UK) (Phadke, 1987; Phadke, 1990), **Lyon** (France) (Confavreux et al., 1980; Confavreux et al., 2000; Confavreux et al., 2003; Confavreux and Vukusic, 2006a; Confavreux and Vukusic, 2006b), **Rennes**

(France) (Leray et al., 2010), **British Columbia** (Canada) (Kingwell et al., 2012; Koch et al., 2009; Koch et al., 2010; Sayao et al., 2007; Tremlett et al., 2005; Tremlett and Devonshire, 2006; Tremlett et al., 2006; Tremlett et al., 2008a; Tremlett et al., 2008b; Tremlett et al., 2009a; Tremlett et al., 2009b) and **London Ontario** (Canada) (Cottrell et al., 1999a; Cottrell et al., 1999b; Ebers et al., 2000; Kremenchutzky et al., 1999a; Kremenchutzky et al., 2006a; Weinshenker et al., 1989b; Weinshenker et al., 1989c; Weinshenker et al., 1991a; Weinshenker et al., 1991b).

General findings from natural history studies allow to draw meaningful and often cohering overall conclusions, however methodologies, demographics and choice of endpoints largely vary, making comparison not always simple (Degenhardt et al., 2009). The majority of studies gathered information retrospectively and partially prospectively and few studies collected data only longitudinally. Although the completeness of data acquisition is a cardinal feature, the percentage of cases lost to follow up is often not clearly addressed. In addition, ascertainment might remain incomplete, despite the use of prevalence material (geographically based studies).

Each study has contributed to our understanding of the disease course and the prognosis. However, here I analysed the methodology of those databases advantaged by a large sample size, long duration of follow up and high accuracy and frequency of clinical assessments (Table 3.3).

Table 3.2 methodological features of natural history studies.

Study	Location	Study period	Ascertainment	Follow up	Sample size (n)
Kurtzke et al. 1977, 1979	United States	1942-1963		Retrospective and longitudinal	527
Clark et al. 1982; Detels et al. 1982	Los Angeles (US)	1970-1979	Geographic	Retrospective	886
Visscher et al. 1984	Los Angeles (US)	1970-1980	Geographic	Retrospective	941
Riise et al. 1992	Europe	1953-1983	Clinic	Retrospective	598
Bronnum-Hansen et al. 1994, 2004	Denmark	1949-2000	Geographic	Retrospective and longitudinal	9881
Runmaker et al. 1993; Eriksson et al. 2003; Skooget al. 2012	Sweden	1950-2009	Geographic	Longitudinal	255
Myhr et al. 2001	Norway	1976-1987	Geographic	Retrospective and longitudinal	220
Poser at al. 1982	Germany	1961-1977	Geographic	Retrospective and longitudinal	221
Minderhoud et al. 1988	Groningen (The Netherland)	1962-1987	Clinic	Retrospective and longitudinal	342
Kantarci et al. 1998	Turkey	1994-1997	Clinic	Retrospective	1259
Phadke et al. 1987, 1990	Scotland	1970-1981	Geographic	Retrospective and longitudinal	1055
Confavreux et al. 1980, 2000, 2003, 2006	Lyon (France)	1957-1997	Clinic and Geographic	Longitudinal	1844
Leray et al. 2010	Rennes (France)	1976-2004	Clinic and Geographic	Retrospective and longitudinal	2054
Tremlett et al. 2005, 2006, 2007, 2008, 2009; Sayao et al. 2007; Koch et al.2009, 2010; Kingwell et al. 2012	British Columbia (Canada)	1980-2003	Clinic and Geographic	Retrospective and longitudinal	2837
Weinshenker et al. 1989, 1991; Cottrell et al. 1999; Ebers et al. 2000; Kremenchutzky et al. 1999, 2006;	London Ontario (Canada)	1972-2000	Clinic and Geographic	Retrospective and longitudinal	1023

Table 3.3 clinical and demographic features of the largest natural history cohorts.

MS registries	Sample size (n)	Mean age at onset (years)	F/M ratio	Mean disease duration (years)	Number (%) of RR	Number (%) of SP	Number (%) of PP	Endpoints	Number (%) reaching endpoints
Lyon	**1844**	31	1.8	11	1066 (57.8%)	496 (27%)	282 (15.2%)	DSS 4, 6, 7	DSS 4 = 1026 (56%) DSS 6 = 595 (32%) DSS 7 = 380 (21%)
Rennes	**2054**	31.4	2.3	12.8	991 (48.2%)	618 (30%)	445 (21.8%)	DSS 3, 6	DSS 3 = 1415 (68.9%) DSS 6 = 718 (35%)
Gothenborg	**255**	32.5	1.9	> 25	40 (15.6%)	162 (63.5%)	44 (17.2%)	DSS 6, 7, 10	
British Columbia	**2837**	30.6	2.3	20.1	1039 (36.6%)	1445 (50.9%)	353 (12.4%)	EDSS 3, 6, 8	DSS 3 = 1287 (45%) DSS 6 = 518 (18%) DSS 8 = 100 (3.5%)
London Ontario	**1023**	30	1.9	24	273 (26.1%)	551 (52.8%)	219 (20.9%)	DSS 3, 6, 8,, 10	

3.1.2.1 The Lyon database

Confavreux has been one of the pioneers of modern natural history studies (Confavreux et al., 1980; Confavreux et al., 2000; Confavreux et al., 2003; Confavreux and Vukusic, 2006a; Confavreux and Vukusic, 2006b). The Lyon cohort was established in 1957 and included all patients with a diagnosis of MS, according to Poser's classification (Poser et al., 1983), attending the only referral centre in the city of Lyon and the region of Rhone-Alpes. Data were computerized in 1990, using the EDMUS software (Confavreux et al., 1992a). This helped to standardize clinical evaluations, as the regional network of neurologists received regular EDMUS training.

The database is therefore advantaged by a geographically based ascertainment, which led to the recruitment of 1844 patients. These were followed up longitudinally, with yearly assessments, until 1997, when the database was closed for research purpose. By the end of the observation period, the total population had a mean disease duration of 11 years: 1066 (57.8%) were still in the RR phase, 496 (26.8%) had converted to SP MS and 282 (15.2%) had PP course. Disability was assessed using the DSS (Kurtzke, 1955), and focused was placed on irreversible (for at least 6 months) scores of 4 (***limited walking ability, but without aid or rest for more than 500 meters***), which was attained by 56% (n = 1026), score of 6 (***ability to walk with unilateral support no more than 100 meters without rest***), which was attained by 32% (595) and score of 7 (***ability to walk no more than 10 meters without rest while leaning against a wall or holding onto furniture for support***), which was attained by 21% (n = 380). Almost half (n = 804) of the population received Azathioprine at some point during the disease course.

3.1.2.2 The Rennes database

Patients in the Rennes database were identified through the Rennes MS Clinic, a regional centre for MS in west France, serving Britanny, Pays de Loire and borders regions (Leray et al., 2010). The ascertainment was therefore mainly geographically

based and started in 1976. Data were collected retrospectively for those patients with disease onset prior to this, and then longitudinally collected up to 2004. Information were computerized in 1996 using the EDMUS software (Confavreux et al., 1992a).

By the end of the study (2004), 2054 patients diagnosed with MS, according to Poser's criteria (Poser et al., 1983), were recruited. In the total population the mean disease duration was 12 years and the disability was scored using the EDSS (Kurtzke, 1983). However, for data analysis authors preferred to use the 10 step scale (Kurtzke, 1955) and focused on irreversible (sustained for at least 6 months) score of 3 (***moderate disability***) and score of 6 (***unilateral assistance required to walk 100 meters***). In the total population, 445 patients (21.7%) had progressive onset (PP MS) and 1609 patients (78.3%) had relapsing onset. Among those, 618 (38.4%) had converted to SP MS. During the follow up period, 68.9% reached DSS 3 and 35% DSS 6. A total of 1154 (56.2%) received DMTs for at least 6 months.

3.1.2.3 The Gothenburg database

The Gothenburg MS clinic served all patients diagnosed with MS, according to Poser's criteria (Poser et al., 1983), living in Gothenburg from 1950 to 1964 at the time of the disease onset (Eriksson et al., 2003; Runmarker and Andersen, 1993; Skoog et al., 2012). Data were collected longitudinally for more than 25 years and follow up was extended up to 2009 (Skoog et al., 2012). At that point the database comprised 255 patients; 44 (17.2%) had PP course, 40 (15.6%) had remained in the RR phase and 162 (63.5%) had converted to SP MS. For 9 patients, the type of the disease course could not be established (Skoog et al., 2012). The disability was scored using the DSS (Kurtzke, 1955), and the analyses focused on DSS 6 (***unilateral support needed for short distance to walk***), DSS 7 (***essentially wheelchair dependent***) and DSS 10 (***death due to MS***). No patient received any DMTs. Despite the small sample size, the database is advantaged by high geographic ascertainment, by the minimal number of patients (n = 3) loss of follow up and by the extended longitudinal assessments.

3.1.2.4 The British Columbia database

The university of British Columbia's MS clinic was established in 1980 and incudes 4 clinics, covering 80% of the MS population in British Columbia (Sweeney et al., 1986). Data were collected retrospectively for those patients with disease onset prior to this, and then longitudinally, up to 2003. By the end of observation period, 5727 patients with definite MS according to Poser's criteria (Poser et al., 1983), were identified. However, analyses included only patients with minimum 15 years follow up. Therefore, the cohort consisted of 2837 patients with a mean disease duration of 20 years; 353 (12.4 %) had PP MS and 2484 (87.6%) had initial RR course, of whom 1445 (50.9% of the total population) had entered the SP phase (Tremlett et al., 2005; Tremlett and Devonshire, 2006; Tremlett et al., 2006; Tremlett et al., 2008a; Tremlett et al., 2009a). Disability was assessed using the EDSS (Kurtzke, 1983) and analyses focused on irreversible (sustained for > 150 days) score of 3, (***moderate disability***), of 6 (***cane requirement***) and of 8 (***wheelchair bound***). A total of 439 patients received DMTs.

3.1.3 Clinical and demographic features of MS

Natural history cohorts provide a comprehensive amount of data about demographic and clinical characteristics of the disease. Information, to large extent, appears consistent among studies (Table 3.4), however some discrepancies exist and are probably due to methodological differences.

Table 3.4 Female/male ratio in natural history cohorts.

Study	Location	Sample size (n)	Feale/Male %	F/M ratio
Riise et al. 1992	Europe (multicentre)	598	64/36	2.1
Bronnum-Hansen et al. 2004	Denmark	9881	60/40	1.5
Runmaker et al. 1993	Sweden	308	60/40	1.5
Myhr et al. 2001	Norway	220	62/38	1.7
Poser at al. 1982	Germany	221	65/35	1.9
Minderhoud et al. 1988	Netherland	342	60/40	1.5
Kantarci et al. 1998	Turkey	1259	64/36	n/a
Phadke et al. 1990	Scotland	1055	65/35	1.9
Confavreux et al. 2003	France	1844	64/36	1.8
Leray et al. 2010	France	2054	69/31	2.3
Debouverie et al. 2008	France	2871	72/28	2.6
Tremlett et al. 2005	Canada	2837	70/30	2.3
Sumelahtiet al. 2002	Finland	1614	66/34	1.9

3.1.3.1 Sex ratio

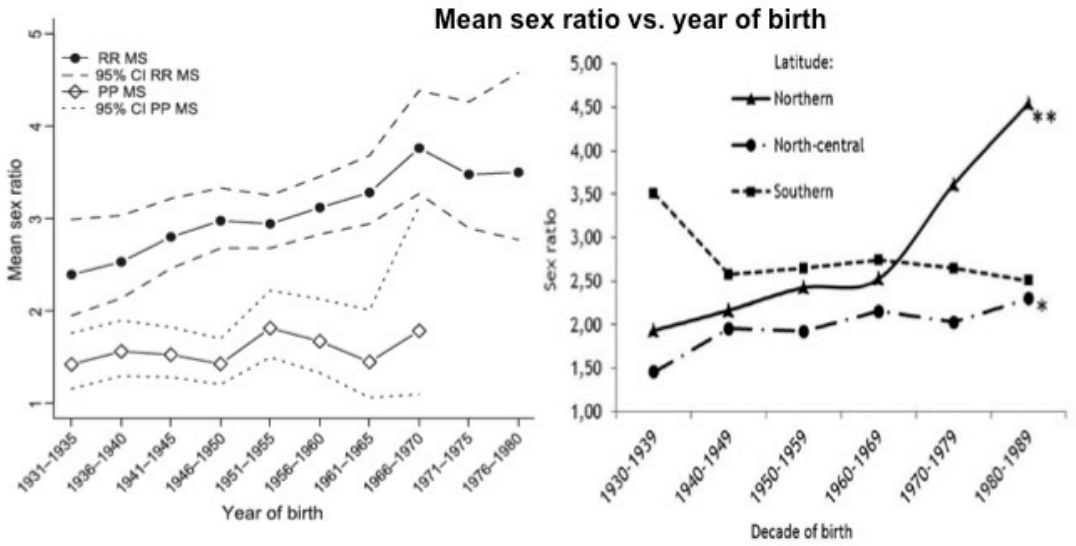
The Female/Male (F/M) ratio has gained increasing interest during recent years. It is considered a robust epidemiological marker and can be used as internal control, within the same group, for testing the potential impact of ascertainment bias (Orton et al., 2006; Trojano et al., 2012).

Interestingly, at the beginning of last century MS was thought to be a predominantly male disease (Brain, 1930), and in the late 70s it was first described a female

preponderance (Acheson, 1977). There is now consensus that women are twice as likely than men to be affected by MS (Compston and Coles, 2008). However, the F/M ratio largely varies among natural history cohorts (Table 3.4). The highest proportion of MS women was reported in the Lorraine cohort (72%; F/M ratio = 2.6) from France (Debouverie et al., 2008), and the smallest proportion (60%; F/M ratio = 1.5) was observed in Danish and Swedish studies, advantaged by a large sample size (Bronnum-Hansen et al., 2004) and by a geographically based ascertainment (Runmarker and Andersen, 1993).

It is now well established that the proportion of women affected by the disease has increased over time. This was observed in Canada (Sadovnick, 2009), Japan (Houzen et al., 2003; Osoegawa et al., 2009), Denmark (Bentzen et al., 2010), Iran (Maghzi et al., 2010), France (Debouverie et al., 2007), Norway (Celius and Smestad, 2009), US (Wallin et al., 2004) and Australia (Barnett et al., 2003). A more sophisticated approach, using stratification by year of birth, which minimizes the artefact related to the ascertainment, confirmed this trend. In a sample of more than 27000 cases from Canada, the F/M ratio for the entire cohort increased over 50 years, from 1.33 to 3.96 (Orton et al., 2006). Similar results were reported in two smaller studies from Iran (about 8000 cases) and from the MSbase registry (about 15000 cases), both using the year of birth approach (Sahraian et al., 2010; Trojano et al., 2012). Interestingly, among patients from the Msbase registry, the increased F/M ratio (from 2.35 to 2.73 over 60 years) was paralleled by an increased proportion of women with RR disease (Trojano et al., 2012). Furthermore, a recent study from the Canadian Collaborative Project demonstrated a gradual increase of the F/M ratio over time in RR MS only, and no significant changes in the PP MS group (Ramagopalan et al., 2010) (Figure 3.1). The magnitude of the increasing F/M ratio was also shown to be highest in northern latitudes and lowest in southern latitudes (Figure 3.1). The authors concluded that the global increase of the MS incidence and the "gradient effect" might be partially driven by females with RR course (Trojano et al., 2012). However, a recent Norwegian study could not replicate these results, and found no association between sex ratio changes and the latitude (Kampman et al., 2013).

Figure 3.1 Mean sex ratio change (y-axis) over time, in patients grouped by year of birth (x-axis) and stratified by disease phenotype (Ramagopalan et al., 2010) and by geographic area (Trojano et al., 2012).



The factors accounting for the increasing number of women affected by MS remain unclear. Given the relatively short time over which this was observed, this trend cannot be attributed to genetic changes but more likely to gene-environment interactions (Orton et al., 2006). Other possible candidates are smoking, known to affect the risk of MS (Hernan et al., 2001) and to have increased in women over time, and changes in lifestyle (Sellner et al., 2011).

3.1.3.2 Age at onset

It is not always easy to determine when the first clinical presentation occurred, especially if the onset is characterized by vague, sensory symptoms. The oldest age disease onset (35 years) was reported among patients from the Danish database (Bronnum-Hansen et al., 2004), and the youngest age (27 years) among Turkish patients (Kantarci et al., 1998). This variability among studies probably reflects differences in the proportion of retrospectively collected information. Nevertheless, there is a general consensus that disease starts on average at around the age of 30 (Compston and Coles, 2008).

3.1.3.3 Presenting symptoms

Table 3.5 Symptoms at the disease onset in natural history cohorts. **Patients can experience more than one symptom at onset.**

Study	Location	Sample size (n)	Initial symptoms (%)
Riise et al. 1992	Europe	598	- Visual (25) - Brainstem (22) - Pyramidal (35) - Cerebellar (17) - Sensory (46)
Myhr et al. 2001	Norway	220	- Visual (16) -Brainstem/cerebellar (34) -Motor (32) - Sensory (34)
Kantarci et al. 1998	Turkey	1259	- Visual (20) - Brainstem/cerebellar (30) - Motor (40) - Sensory (43)
Phadke et al. 1990	Scotland	1055	- Visual (11) - Brainstem (24) - Cerebellar (4) - Spinal cord (42)
Confavreux et al. 2003	France	1844	- Visual (18) - Brainstem (9) - Long tract dysfunction (52)
Leray et al. 2010	France	2054	- Visual (21) - Brainstem (11) - Long tract dysfunction (51)
Debouverie et al. 2008	France	2871	- Visual (19) - Brainstem (14) - Long tract dysfunction (38)
Tremlett et al. 2005	Canada	2837	- Visual (18.5) - Motor (17.4) - Sensory (40.7) - Brainstem/cerebellar (16.8)
Sumelathi et al. 2002	Finland	1614	- Corticospinal (26) - Infratentorial (22) - Sensory (33)

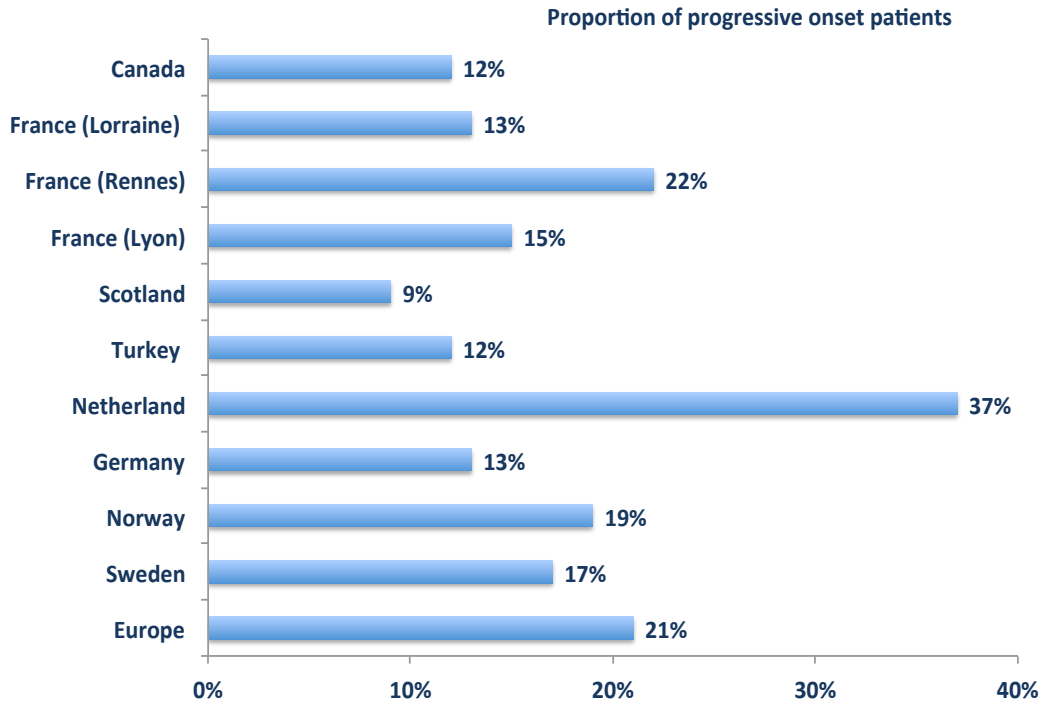
Similarly to age, the retrospective ascertainment of symptoms occurring at the disease onset might be difficult. In addition, attributing symptoms to specific anatomic locations it is not always straightforward. Therefore, the categorization of neurological disturbances varies among studies (Table 3.5). For instance, in the cohorts from the EDMUS databases (Confavreux et al., 2003; Debouverie et al., 2008; Leray et al., 2010), motor, sensory, cerebellar and sphincter symptoms are pooled together, and categorized as “long tracts dysfunction”. However, there is

general agreement, among MS registries, that sensory disturbances are the commonest presenting symptoms, followed by motor dysfunctions (Table 3.5).

3.1.4 The type of the disease course

It is well established that the majority of MS patients present with an initial RR course, and only a minority experience a progressive course from onset. However, in some cases, categorizing correctly the disease course might be complicated. The diagnosis of PP MS has intrinsic difficulties, as it can only be made in retrospect (Lublin and Reingold, 1996). In addition, especially when not seen at onset, patients might forget previous attacks, which occurred years before the assessment. This can result in an overestimation of the number of cases with a true PP course (Kremenchutzky et al., 1999a). These factors probably account for the variable proportion of PP MS, among natural history cohorts (Figure 3.2), ranging from the excessively high 37% in Netherland (Minderhoud et al., 1988a), which probably reflects the study methodology focused on the assessment of the onset of progression, to the very low 9% reported in Scotland (Phadke, 1987). On average, 15% of cases with a PP course is considered a reasonable estimate (Compston and Coles, 2008).

Figure 3.2 Percentage of patients (x-axis) with PP MS, among natural history cohorts: the exact value is indicated next to each bar. On the y-axis are listed the geographic locations of natural history studies.



3.1.4.1 Clinical and demographic features according to the type of the disease course

The disease phenotypes are distinguished by different clinical and demographic features. Since the early studies it has been described a higher incidence of a PP course among men, compared to women (Muller, 1951). The F/M ratio remains shifted towards a female's preponderance in RR MS, but it becomes much closer to unity in PP MS: 1.3 (Confavreux and Vukusic, 2006a), 1.4 (Leray et al., 2010) and 1.06 (Tremlett et al., 2005).

In addition, initial symptoms vary in relation to the type of the disease course. Motor disturbances are the most common symptoms at the onset of the PP and of the SP courses (Cottrell et al., 1999b; Kremenchutzky et al., 2006a), whereas optic neuritis and sensory problems are more likely to occur as presenting symptoms of the RR course (Confavreux and Vukusic, 2006a; Leray et al., 2010; Riise et al., 1992; Tremlett et al., 2009b). Relapsing onset and progressive onset patients are also strikingly distinguished by the age at first symptom (Table 3.6). On average, PP MS starts 10

years later than RR MS, however the age at the onset of progression has been reported very similar, between PP and SP patients (Confavreux and Vukusic, 2006a; Koch et al., 2007; Leray et al., 2010; Tutuncu et al., 2013). The only study that failed to replicate this observation was from the British Columbia database (Tremlett et al., 2009b).

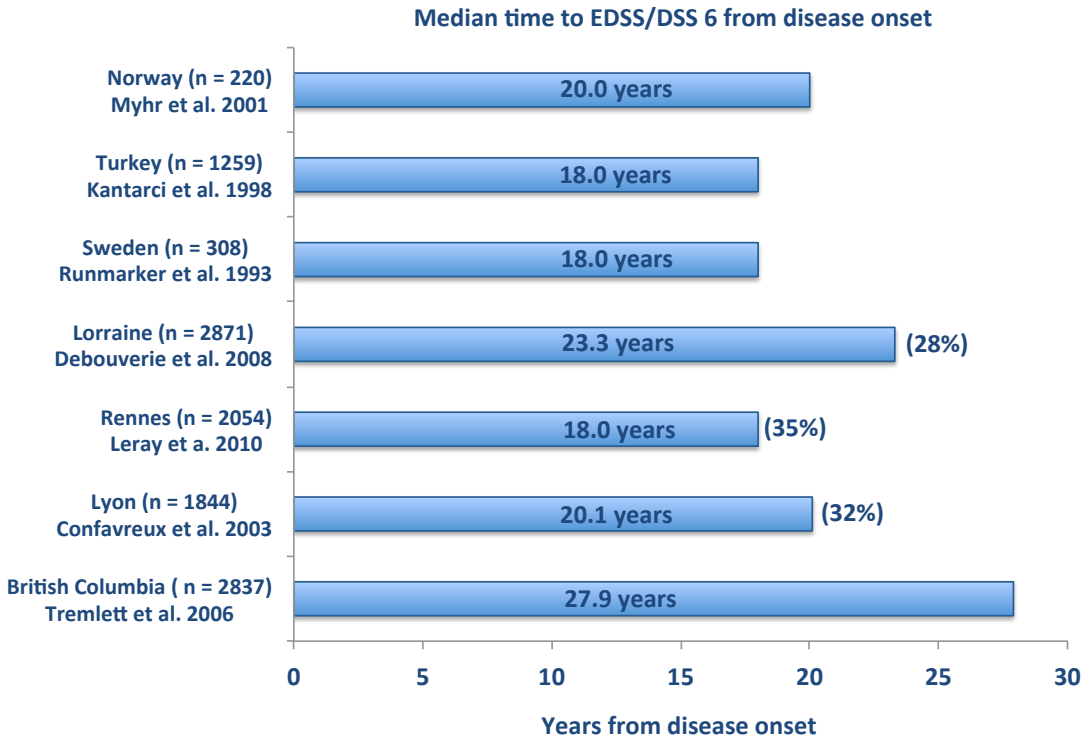
Table 3.6 Mean age at onset of RR, SP and PP MS reported in natural history cohorts.

	Mean age at onset (years)		
Database	**RR MS**	**SP MS**	**PP MS**
Lyon	29	39	40
Rennes	29	n/a	38
Groningen	30	39	39
Mayo clinic	29	45	45
British Columbia	29	49	41

3.1.5 Accumulation of disability

Among natural history cohorts, the accumulation of disability has been assessed with the Kaplan Meier survival analysis, estimating the mean and median times to DSS/EDSS landmarks levels. As discussed in methods (Chapter 2), the Kaplan Meier estimate is affected by the number of patients reaching the endpoint (censored information), which is a direct function of the duration of the follow up. This probably accounts for the differences observed among studies (Figure 3.3). I choose the attainment of DSS/EDSS levels of 6 for comparing the outcome among databases, as it is the most commonly used endpoint and its definition (***need for unilateral support to walk ≤ 100 meters without rest***) does not vary among cohorts (Confavreux et al., 2003; Debouverie et al., 2008; Kantarci et al., 1998; Leray et al., 2010; Myhr et al., 2001; Runmarker and Andersen, 1993; Tremlett et al., 2006).

Figure 3.3 Kaplan Meier median times (x-axis) to EDSS/DSS 6 in natural history cohorts. Percentages of patients reaching the endpoint, when available, are indicated in brackets next to each bar. On the y-axis are listed the geographic location of natural history studies, the sample size (in bracket) and the first author of the articles where the estimate was taken from.



Unfortunately, the number of lost to follow up and the censored information remain unmentioned in most of studies. The estimated median time to EDSS/DSS 6 mainly ranged between 18 and 20 years. The British Columbia cohort again stands out as an outlier, showing a remarkably slow rate of disability accumulation (27.9 years) (Tremlett et al., 2006), which might be partially accounted for by the relatively small percentages of patients with PP course (Figure 3.2).

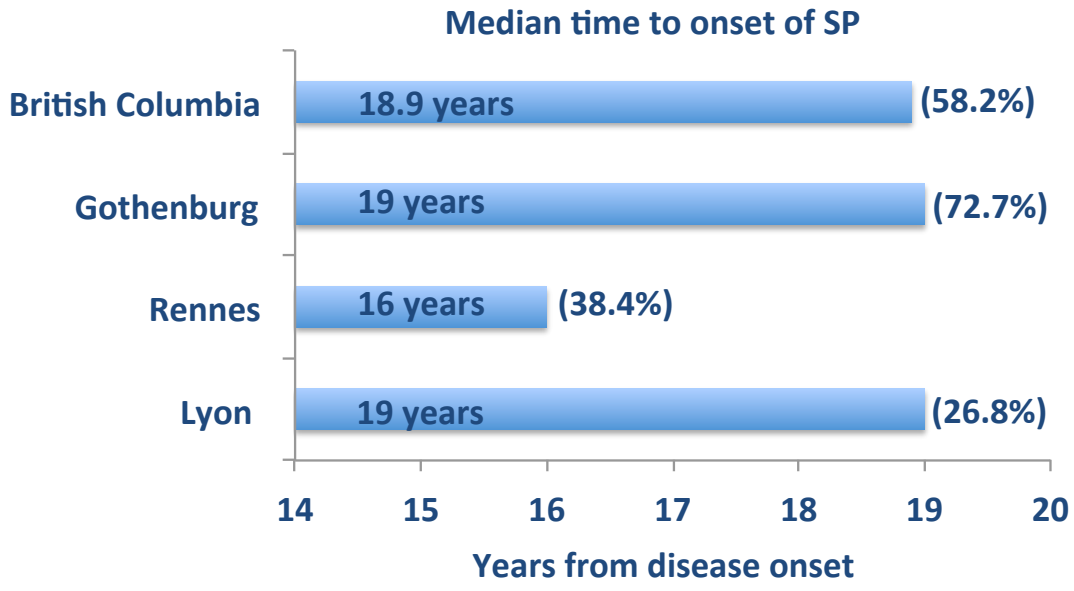
3.1.5.1 The onset of secondary progression

As largely discussed in chapter 1 (paragraph 1.2), defining the onset of the SP phase remains difficult, especially when it is clouded by concomitant relapses (Kremenchutzky et al., 2006a). For this reason, among natural history studies, the onset of progression has not been explored as often as the attainment of severe disability. In early studies the time to SP was calculated without using the Kaplan

Meier analysis, and varied between 9 (Runmarker and Andersen, 1993) to 11 years (Confavreux et al., 1980).

However, the rate of conversion to SP MS is known to increase over time (Confavreux et al., 1980; Mc and Compston, 1952; Weinshenker et al., 1989b). Therefore, the survival analysis allows a more reliable estimate of the risk of developing a progressive course and of the latency to progression, among patients with an initial RR course (Eriksson et al., 2003; Leray et al., 2010; Tremlett et al., 2008a; Vukusic and Confavreux, 2003). Although the proportion of censored information varied, data from natural history cohorts appear consistent. Overall, studies converge on 19 median years, as an estimated time to enter the SP phase (Figure 3.4). It is important to note that data from British Columbia have been lately reported differently. A second analysis, including a larger sample (n = 5162) and a lower percentage of patients converting to SP MS (35%), showed a median time to SP of 21.4 years (Koch et al., 2010). The differences between the two studies were not addressed by the authors.

Figure 3.4 Kaplan Meier median times (x-axis) to SP in natural history cohorts. Percentages of patients reaching the endpoint are indicated in brackets next to each bar. On the y-axis are listed the geographic location of natural history studies.



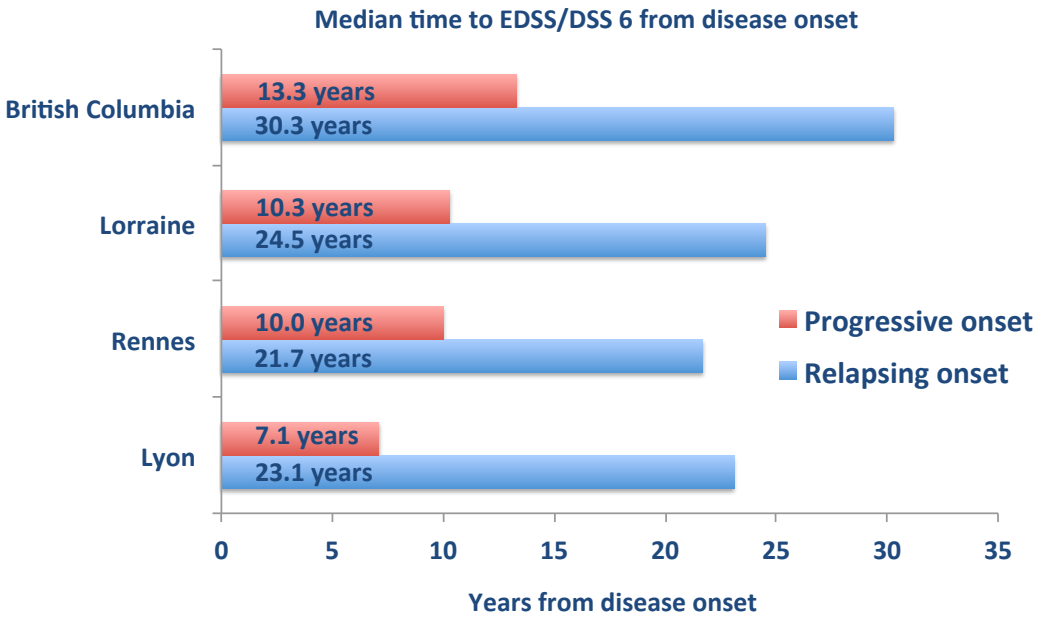
3.1.5.2 The evolution of secondary progression

The evolution of the SP phase was described only in the Lyon and in the British Columbia cohorts. The two studies reported discordant results; median time to DSS 6 from onset of SP was 4 years and 8.2 years, respectively (Tremlett et al., 2008a; Vukusic and Confavreux, 2003). In addition, median times to DSS 7 (Vukusic and Confavreux, 2003) and to DSS 8 (Tremlett et al., 2008a), from conversion to SP MS were 8 and 15.9 years, respectively.

3.1.5.3 Relapsing onset versus progressive onset

The rate of disability accumulation is affected by the clinical phenotype. It is well established that a PP course associates with a significantly faster attainment of hard outcomes, from the disease onset (Figure 3.5) (Confavreux and Vukusic, 2006a; Debouverie et al., 2008; Kantarci et al., 1998; Leray et al., 2010; Myhr et al., 2001; Phadke, 1990; Runmarker and Andersen, 1993; Tremlett et al., 2006). However, once established disability occurs, further disease progression is largely unaffected by the type of the disease course. In the French cohorts, times from DSS 3 (Leray et al., 2010) and from DSS 4 (Confavreux and Vukusic, 2006a; Debouverie et al., 2008) to higher disability levels were shown to be remarkably similar, between progressive onset and relapsing onset patients. In contrast, in the British Columbia database the attainment of DSS 6 and of DSS 8 from the onset of progression was reported significantly shorter in SP MS (8.2 and 15.9 median years, respectively) than in PP MS (13.3 and 25.0 median years, respectively) (Tremlett et al., 2009b).

Figure 3.5 Comparison of Kaplan Meier median times (x-axis) to EDSS/DSS 6 between progressive onset and relapsing onset patients. On the y-axis are listed the geographic location of natural history studies.



3.1.6 Mortality in MS

Survival in MS has been studied in several epidemiological cohorts, gathering a large amount of data. However, the comparison among datasets is difficult, because of the increasing incidence of MS in many countries and because of the variable duration of studies, which affect the mortality estimates (Bronnum-Hansen et al., 2004; Grytten Torkildsen et al., 2008; Hader, 2010; Hirst et al., 2008b; Kingwell et al., 2012; Koch-Henriksen et al., 1998; Leray et al., 2007; Midgard et al., 1995; Phadke, 1987; Ragonese et al., 2010; Redelings et al., 2006; Sadovnick et al., 1991b; Smestad et al., 2009; Sumelahti et al., 2010; Wallin et al., 2000) (Table 3.7).

Table 3.7 Studies assessing the mortality in MS.

Study	Country	Study period	Duration of f/up (years)	Cohort / deceased (n)
Phadke et al. 1987	Scotland	1970-1980	10	216 / 216
Sadovnick et al. 1991	Canada	1972-1988	16	3126 / 145
Midgar et al. 1995	Norway	1950-1984	34	251 / 70
Koch-Henriksen et al. 1998	Denmark	1951-1993	42	6068 / 6068
Wallin et al. 2000	USA	1956-1996	40	2489 / 2059
Bronnum-Hansen et al. 2004	Denmark	1949-2000	51	9881 / 4254
Redelings et al. 2006	USA	1990-2001	11	27.319 / 27.319
Leray et al. 2007	France	1976-2004	28	1879 / 68
Grytten-Torkildsen et al. 2008	Norway	1953-2005	52	878 / 198
Hirst et al. 2008	Wales	1985-2006	21	379 / 221
Smestad et al. 2009	Norway	1940-2006	66	386 / 263
Sumelahti et al. 2010	Finland	1971-2006	36	1595 / 464
Ragonese et al. 2010	Sicily	1981-2001	20	194 / 30
Hader et al. 2010	Canada	1977-2007	30	150 / 105
Kingwell et al. 2012	Canada	1980-2005	25	6917 / 1025

Although the accumulation of severe disability in the long-term is not strictly the immediate cause of death, patients with MS have a statistically significant increase in mortality, compared to the general population (Figure 3.6), with a reduction of life expectancy of 7 to 14 years (Bronnum-Hansen et al., 2004; Grytten Torkildsen et al., 2008; Hader, 2010; Hirst et al., 2008b; Kingwell et al., 2012; Leray et al., 2007; Ragonese et al., 2008; Smestad et al., 2009; Sumelahti et al., 2010).

Figure 3.6 Life expectancy in MS patients: comparison of survival probability between Norwegian RR MS patients and the total population (Hordaland County, Western Norway, 1953-2003). The approximate patients age is shown below x-axis (Grytten Torkildsen et al., 2008).

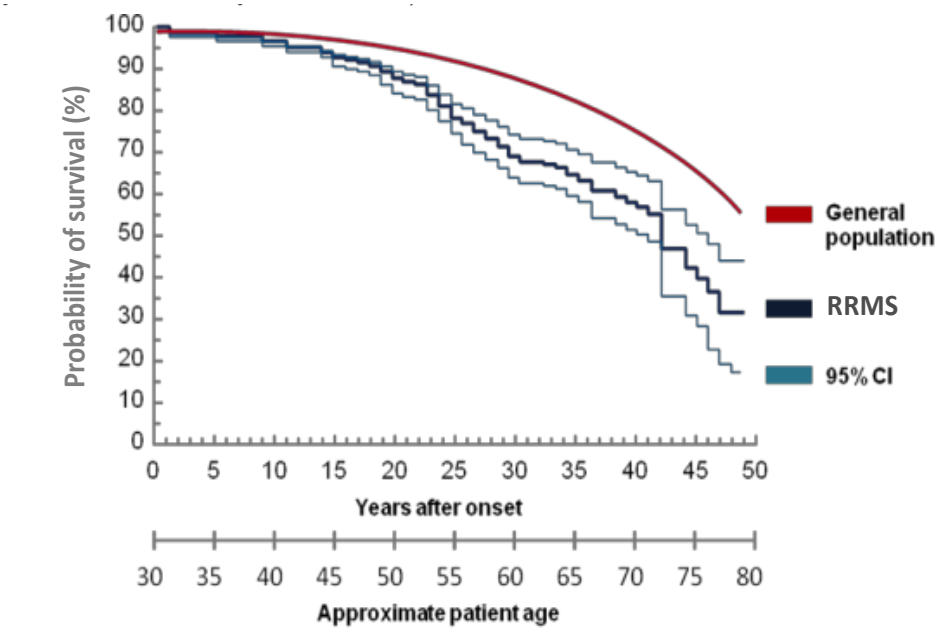


Table 3.8 Mean age at death in MS patients. [1]range.

Sutdy	Location	Mean age at death (years)	Cohort / deceased (n)
Phadke et al. 1987	Scotland	25–80[1]	216 / 216
Koch-Henriksen et al. 1998	Denmark	58.4	6068 / 6068
Ekestern et al. 2004	Austria	57	2469 / 2469
Redelings et al. 2006	USA	60.9	27.319 / 27.319
Leray et al. 2007	France	47	1879/68
Hirst et al. 2008	Wales	65.3	379 / 221
Smestad et al. 2009	Norway	64.0 (RRMS) / 66.0 (PPMS)	386 / 263
Ragonese et al. 2010	Sicily	63	194 / 30
Kingwell et al. 2012	Canada	76.7 (RRMS =76.9 / PPMS = 76.3)	6917 / 1025 (784 / 236)

Survival analyses can be used for calculating the time to death from birth (age at death) (Ekestern and Lebhart, 2004; Hirst et al., 2008b; Kingwell et al., 2012; Koch-Henriksen et al., 1998; Leray et al., 2007; Phadke, 1987; Ragonese et al., 2010; Redelings et al., 2006; Smestad et al., 2009) (Table 3.8). This approach allows to compare the MS related mortality with the expected mortality in matched population-based controls, however it does not take into account the disease duration and severity.

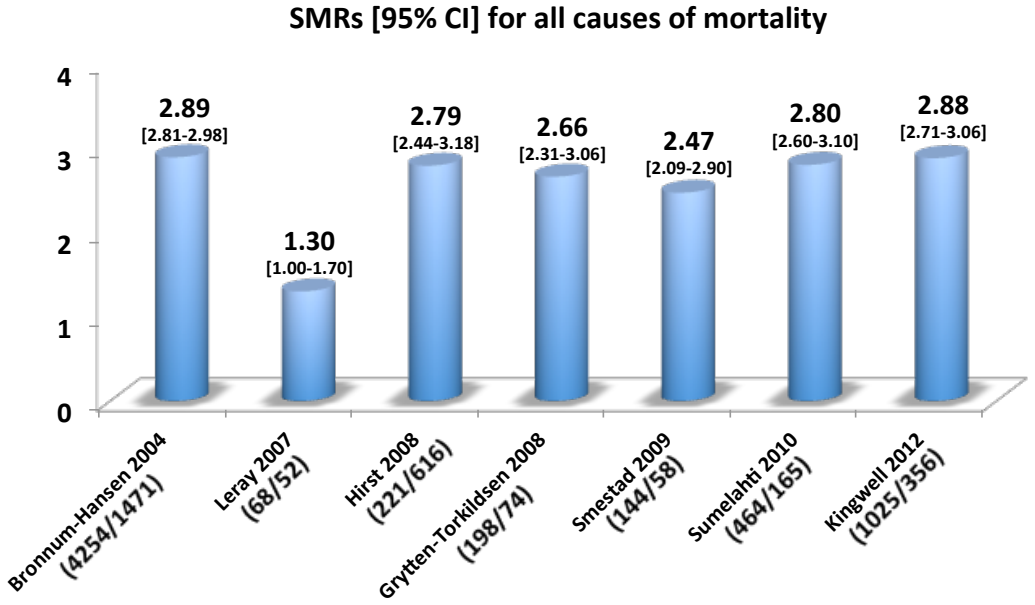
In contrast, when estimating the time from the disease onset to death, the rate of disability accumulation can be better typified (Bronnum-Hansen et al., 2004; Grytten Torkildsen et al., 2008; Hader, 2010; Leibowitz et al., 1969; Midgard et al., 1995; Ragonese et al., 2010; Riise et al., 1988; Smestad et al., 2009; Wallin et al., 2000; Wynn et al., 1990) (Table 3.9). As outlined in table 3.6, survival is extremely variable among cohorts, ranging from the shortest 17 years, reported in a early study from Israel (Leibowitz et al., 1969), to 47.5 years recently described among patients from the British Columbia database (Kingwell et al., 2012). Differences in follow-up duration and sample size, and the improvement of the diagnostic criteria, affect the survival estimates and partially accounted for this large variability. In addition, among recent studies, the reported longer time to death is likely to be explained by the better ascertainment and by the improvement of the care provided to the chronically disabled. An overall average survival from MS onset of 35 years is considered a reasonable estimate (Degenhardt et al., 2009).

Table 3.9 Mean time to death in MS patients reported by epidemiological studies.

Study	Cohort / deceased (n)	Mean age at onset (years)	Survival time from onset or diagnosis of MS (years)
Leibowitz et al. 1969	266 / 52	32.6	17
Phadke et al. 1987	216 / 216		24.5
Riise et al. 1988	598 / 136		Overall 27
		< 35	34.5
		≥ 35	21.7
Midgardet al. 1995	251 / 70	33.6	Men 21.3 (75th percentile)
			Women 24.5 (75th percentile)
Wallin et al. 2000	2489 / 2059		White men 34
			Black men 30
			White women 43
Bronnum-Hansen et al. 2004	9881 / 4254		Overall 31
		34.7	Men 28
		34.1	Women 33
Grytten Torkildsen et al. 2008	878 / 198		Overall 41
			43 (RRMS)
			26 (PPMS)
Hirst et al. 2008	379 / 221		38
Smestad et al. 2009	386 / 263	27.3 (alive at inclusion)	35
		34.8 (deceased at inclusion)	
Hader et al. 2010	150 / 105	30.5	33 (men)
		28.4	38 (women)
Kingwell et al. 2012	6917 / 1025	31	47.5

A more sophisticated approach for assessing mortality in MS is the use of standardised mortality ratios (SMRs) (the ratio of observed to expected number of deaths, using mortality in the general population). The directly age-adjusted SMRs allow the comparison of mortality with respect to the general population, eliminating effects of differing age distributions. Although advantaged by the possibility of adjusting the analysis according to several factors (such as race or sex), directly age-adjusted SMRs are relatively unstable when based on small numbers of deaths. The mortality varies among studies even when using SMRs (Figure 3.7) (Bronnum-Hansen et al., 2004; Grytten Torkildsen et al., 2008; Hirst et al., 2008b; Kingwell et al., 2012; Leray et al., 2007; Smestad et al., 2009; Sumelahti et al., 2010). This is due to the declining pattern of SMR when the duration of follow-up increases, resulting into a higher number of expected and observed deaths (Smestad et al., 2009). A clarifying example is the study from Finland, where the overall mortality rate remained stable during the first 2 years after diagnosis (SMR 0.8), however it became significantly higher (2.4) between 2 and 9.9 years, and increased further (3.1) after 10 years (Sumelahti et al., 2010).

Figure 3.7 Standardized mortality ratios (y-axis) for all cause of mortality: the exact values [95% C.I.] are indicated on top of each column. On the x-axis are listed studies (sample size/number of deceased) from which SMRs were taken.



3.1.6.1 Factors affecting mortality

Several factors have been reported to affect survival. It is well established that a PP course associates with a faster time to death from disease onset (Grytten Torkildsen et al., 2008; Hader, 2010; Kantarci et al., 1998; Kingwell et al., 2012; Leray et al., 2007; Midgard et al., 1995; Phadke, 1987; Smestad et al., 2009; Sumelahti et al., 2002). However, the survival from birth (age at death) is not significantly affected by the type of the disease course. Similar median/mean ages at death have been reported between patients with a PP course and with a RR onset: 68 versus 66 median years in Norway (Smestad et al., 2009), 76.3 versus 76.9 mean years in British Columbia (Kingwell et al., 2012).

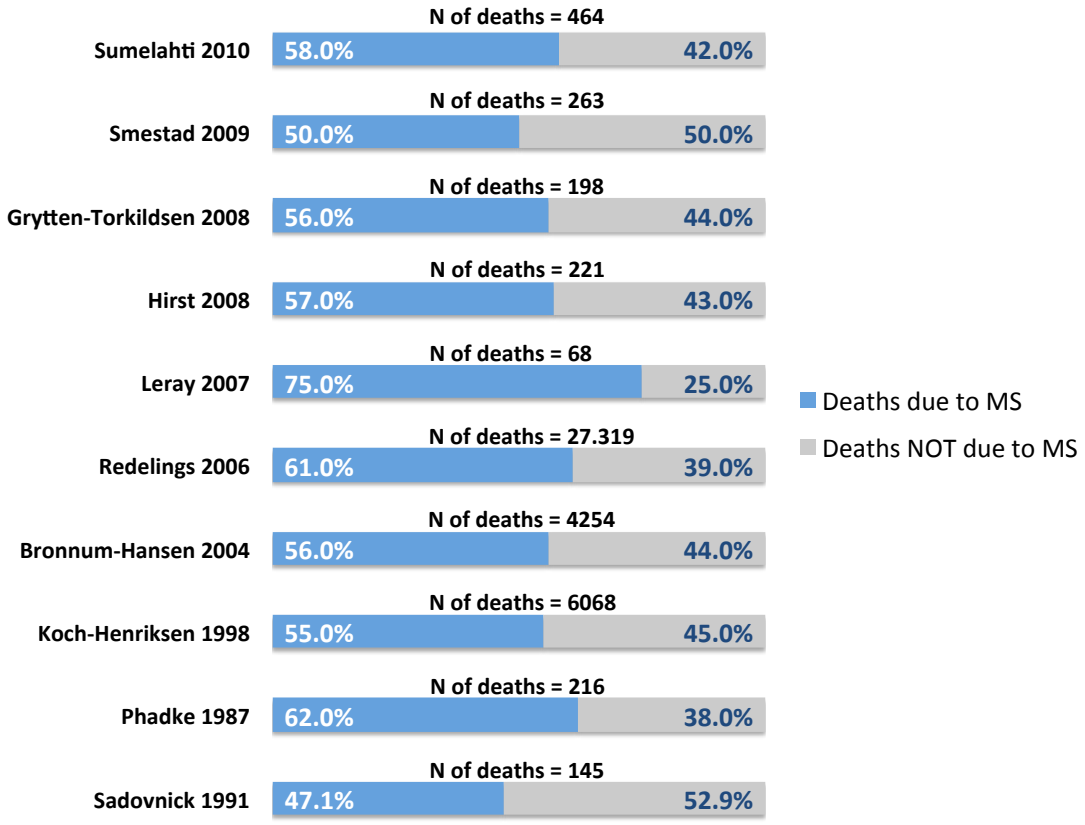
Most of the studies found an association between a younger age at onset and a longer survival or a lower risk of death (Grytten Torkildsen et al., 2008; Hader, 2010; Kingwell et al., 2012; Leray et al., 2007; Smestad et al., 2009; Sumelahti et al., 2002). Data on the prognostic effect of the type of initial symptoms do not seem homogenous. Groups with brainstem/cerebellar involvement at onset have been reported to have shorter (Midgard et al., 1995; Phadke, 1987) or even longer (Smestad et al., 2009) survival. Others found no impact of the initial presentation on the time to and the probability of death (Leray et al., 2007). Data about the relationship between gender and mortality are also contradicting. Although many studies found a longer survival in females groups (Grytten Torkildsen et al., 2008; Hader, 2010; Kingwell et al., 2012; Leray et al., 2007; Midgard et al., 1995; Phadke, 1987), others reported no difference between men and women (Hader, 2010; Smestad et al., 2009; Sumelahti et al., 2002) or even an higher risk of mortality among females (Ekestern and Lebhart, 2004; Ragonese et al., 2010). The lack of homogeneity could be partially attributed to changes of MS sex ratio over time (Orton et al., 2006).

3.1.6.2 Causes of death

Although MS is essentially a chronic and disabling disease, the long-term disability is not necessarily the immediate cause of death. However, MS, in the advanced stages,

does carry the risk of systemic complications, which may lead to death. The record of "MS", as the main cause of death, marks the attainment of the last step of the expanded disability status scale (EDSS 10), This is defined as *"...an acute death due to brainstem involvement or to respiratory failure or death consequent to the chronic bedridden state with terminal pneumonia, sepsis, uremia, cardiorespiratory failure. It excludes intercurrent causes of death. Antemortem, the patient will ordinary be DSS 9, sometimes 8"* (Kurtzke, 1983). This definition can be variably interpreted by doctors and be entirely unknown for those not familiar with MS, leading to a large variation of the proportion of "deaths due to MS", among databases. This ranges from the lowest 50% in the Norwegian cohort (Smestad et al., 2009) and 47% in the Canadian cohort (Sadovnick et al., 1991b) to the highest 75% recorded in the French cohort (Leray et al., 2007) (Figure 3.8).

Figure 3.8 Percentages of deaths due (blue) and NOT due (grey) to MS among studies. On top and on the left of each bar the total number of deaths and studies from which data were taken, are indicated, respectively.



Deaths not related to MS are mainly attributable to the common causes of death in the non-MS population: cardiovascular disease, cancer, infectious and respiratory disease, and accidents or suicide (Bronnum-Hansen et al., 2004; Grytten Torkildsen et al., 2008; Hirst et al., 2008b; Koch-Henriksen et al., 1998; Phadke, 1987; Redelings et al., 2006; Sadovnick et al., 1991b; Smestad et al., 2009; Sumelahti et al., 2002). The incidence of these causes of death varies greatly among studies (Table 3.10).

Table 3.10 Causes of death among MS patients, from epidemiological studies.

	Phadke 1987	Sadovnick 1991	Koch-Henriksen 1998	Sumelahti 2002	Bronnum-Hansen 2004	Redelings 2006	Grytten-Torkildsen 2008	Hirst 2008	Smestad 2009
Number of deaths	**216**	**145**	**6068**	**219**	**4254**	**27319**	**198**	**221**	**263**
Causes of death									
MS	62.0%	47.1%	55.4%	58.0%	56.4%	61.2%	56.5%	57.9%	50.0%
Cardio vascular disease	19.0%	20.6%	17.6%	26.0%	15.5%	10.9%	13.1%	16.0%	14.4%
Cancer	12.0%	30.2%	8.6%	35.0%	10.1%	8.5%	10.6%	9.5%	9.8%
Respiratory disease	3.2%	//	5.1%	//	4.7%	19.7%	5.1%	47.5%	1.5%
Accident/Suicide	//	28.6%	3.8%	5.0%	4.5%	0.3%	2.5%	0.0%	5.3%
Other/unknown	2.7%	//	9.5%	//	13.5%	//	6.6%	8.6%	//

3.1.7 Aim of the study presented in this chapter

This study aimed at describing clinical and demographic features of the LO database following the data quality check procedures (chapter 2). In addition, the disability accumulation over time was estimated in the total population and in patients grouped by clinical phenotypes. Finally survival, factors affecting the time to death and causes of death in the LO population were assessed.

3.2 Methods

The clinical and demographic features of the LO database total population and of the two subpopulations, the highly ascertained subgroup from Middlesex County and the subgroup with information collected only prospectively (from disease onset), were described. Disease features were compared between patients grouped by the initial disease course (progressive onset and relapsing onset). The Chi square test and the Student T-test were used for the comparison of categorical and quantitative data, respectively. The Kaplan Meier survival analysis was used for estimating mean and median times to disability levels from the disease onset and from DSS 3. The Log Rank test investigated differences between survival curves. The Distribution of disability (number of patients at disability levels) at last follow up and the disability progression (percentages of patients attaining disability levels at specific time points) were examined.

Finally, the clinical and demographic features, causes of death and disability accumulation over time, among patients who had died, by the end of the observation period, were assessed. Patients were grouped and compared according to whether death was attributed to MS or not. Deaths due to MS were defined according to a composite criterion, as described in methods (Chapter 2). Kaplan Meier and Cox regression analyses were used to estimate the survival probability and the time to death, among patients grouped by clinical and demographic features.

3.3 Results

3.3.1 Clinical and demographic features

3.3.1.1 Total population

The LO database now comprises 1023 patients. Table 3.11 shows general features of total population (TOT) and the two subgroups: 1) those from Middlesex County (MC): n = 196 (19.1% of TOT population); 2) those exclusively seen from onset (SO); n = 181 (17.6% of TOT population).

Table 3.11 General features of total population and the two MC and SO subgroups.

	Tot population (TOT)	Middlesex County (MC)	Seen from onset (SO)
No of patients	1023	196	181
No of males	345 (33.7%)	62 (31.6%)	55 (30.4%)
No of females	678 (66.3%)	134 (68.4%)	126 (69.6%)
Sex ratio (F/M)	1.96	2.16	2.29
Diagnostic certainty			
Definite	999 (97.7%)	195 (99.5%)	176 (97.2%)
Probable	20 (2.0%)	1 (0.5%)	2 (1.1%)
Possible	4 (0.4%)	0	3 (1.7%)
Disease duration (years)			
Mean years (95% CI)	24.2 (23.6-24.9)	26.3 (25.0-27.5)	16.7 (15.9-17.6)
Median years	23	26	17
Age at onset (years)			
Mean years (95% CI)	30.6 (30.0-31.2)	30.2 (28.9-31.5)	28.1 (26.7-29.4)
Median years	29	28	26
Clinical presentation: n (%)			
Motor	242 (23.7%)	48 (24.6%)	35 (19.3%)
Sensory	518 (50.6%)	95 (48.4%)	104 (57.4%)
Cerebellar	61 (5.9%)	16 (8.1%)	11 (6.0%)
Brainstem	178 (17.3%)	36 (18.3%)	35 (19.3%)
Optic	184 (17.9%)	33 (16.8%)	37 (20.4%)
Bowel/bladder	32 (3.1%)	5 (2.5%)	3 (1.6%)
Clinical subtypes			
PP	217 21.2%	32 16.3%	15 8.3%
RR	272 26.6%	48 24.5%	90 49.7%
SP	534 52.2%	116 59.2%	76 42.0%

During the data quality check (Chapter 2), 20 patients (17 SP; 2 PP; 1 RR) were excluded because their information was found to be unreliable. Consequently, the

sample size has slightly decreased, compared to the last published data (Kremenchutzky et al., 2006a). After 28 years of follow up, the diagnostic accuracy, according to Posers' criteria (Poser et al., 1983), was almost complete (97.7%). Women almost doubled the number of men (F/M ratio = 1.96), the mean disease duration was 24.2 years and the mean age at the disease onset was 30.6 years, with no difference between males and females (31.1 years vs. 30.1 years respectively; p = 0.10). The PP course was reported in 21.7 % and by the end of the observation period, among 806 patients with relapsing remitting course (78.3% of total population), more than half (n = 534; 66.2 % of relapsing onset population) had converted to SP MS. The highly ascertained subgroup from Middlesex County (MC) (Hader et al., 1988) and the subgroup exclusively seen from onset (SO) resemble the total population in the age at onset, the sex ratio, the clinical presentation and the diagnostic certainty, allowing unbiased comparisons. The SO subgroup had obviously a shorter mean disease duration, explaining the larger percentage (49.7%) of patients still in the RR phase. Compared to the total population, the proportion of PP MS was slightly smaller in the MC subgroup, but substantially smaller in the SO subgroup. The number of patients with a PP course increased proportionally with the age at the disease onset and reached its peak among those aged more than 50 at first symptom; similar trend is observed in the two subgroups (Figure 3.9).

Figure 3.9 Percentages of patients with PP course (x-axis) in each age at onset group (y-axis): total population (blue), seen from onset subgroup (yellow) and Middlesex County subgroup (red).

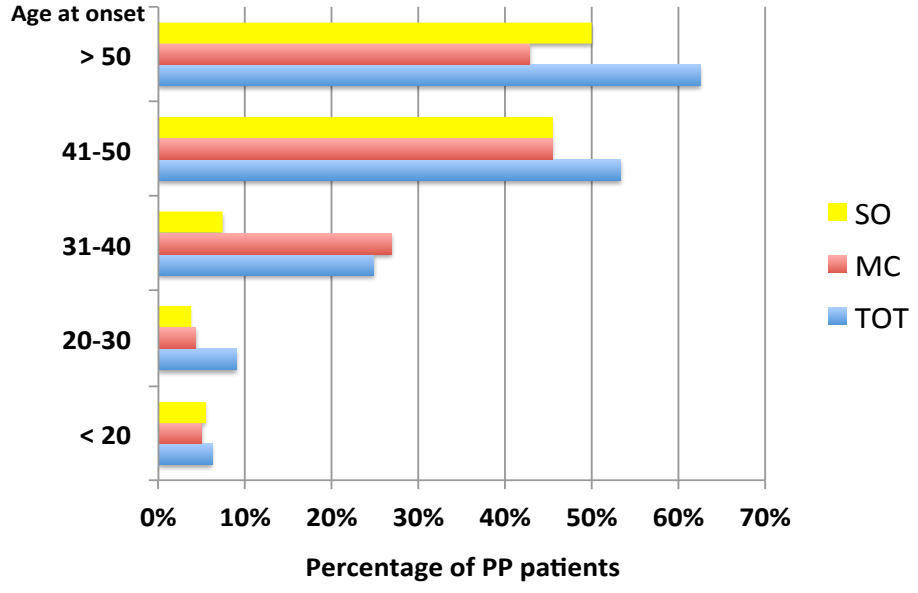
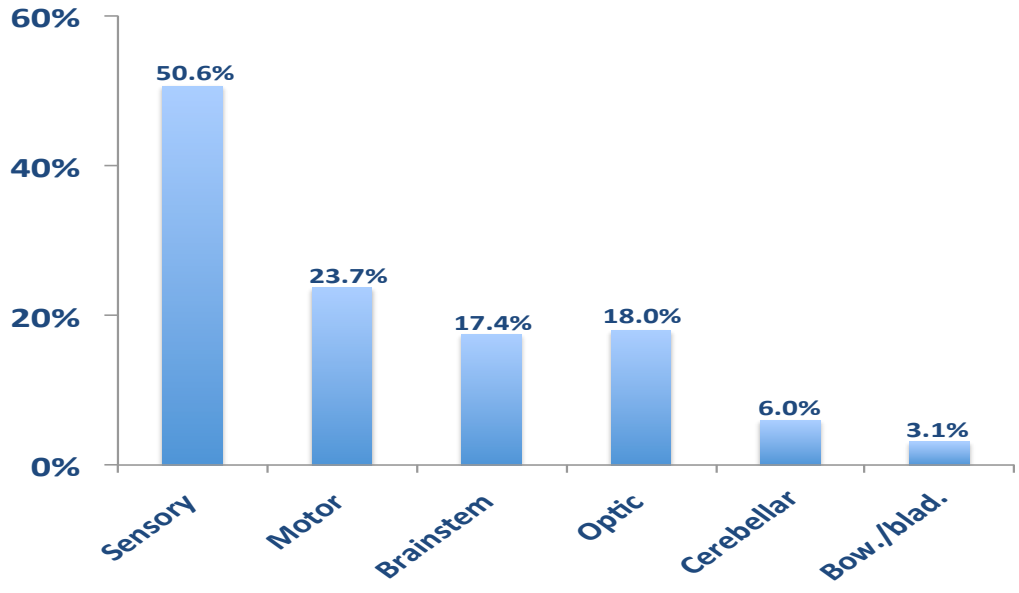


Table 3.12 Symptoms at disease onset in the TOT population

Symptoms at onset of disease	Number of patients (%)
Sensory cord (body)	440 (43%)
Optic neuritis	180 (17.6%)
Gait impairment	138 (13.5%)
Motor slow	135 (13.2%)
Diplopia	114 (11.1%)
Balance impairment	99 (9.7%)
Lhermitte sign	83 (8.1%)
Motor acute	62 (6.1%)
Pain	49 (4.8%)
Sensory face	48 (4.7%)
Limbs ataxia	45 (4.4%)
Vertigo	33 (3.2%)
Bladder symptoms	29 (2.8%)
Myelitis	16 (1.6%)

Figure 3.10 Percentage of patients (y-axis) with different neurological systems involved at disease onset in the total population: on top of each column exact values are indicated.



In Table 3.12 are listed presenting symptoms in the total population, and Figure 3.10 shows percentages of patients grouped by neurological systems involved at onset. Most of patients (43%) presented with sensory impairment, followed by optic neuritis (17.6%), gait impairment (13.5%) and insidious motor deficit (13.2%) (Table 3.12). Accordingly, the commonest neurological system involved at first presentation was the sensory one (50.6%), followed by the motor one (23.7%) (Figure 3.10). More than 60% of patients (n = 677) in the total population had a mono-symptomatic onset, 23% (n = 236) presented with two symptoms and very few (n = 19) with 3 or more symptoms.

3.3.1.2 Relapsing onset and progressive onset patients

Table 3.13 Clinical and demographic features of progressive onset and relapsing onset subgroups.

* Chi-square test; ** Student's T-test

	Progressive onset	**Relapsing-remitting onset**	P values
No of patients	217	806	
No of males	93 (42.9%)	252 (31.2%)	0.001*
No of females	124 (57.1%)	554 (68.8%)	
Sex ratio (F/M)	1.33	2.19	
Disease duration (years)			
Mean (95% CI)	23.7 (22.4-24.9)	24.4 (23.7-25.1)	0.353**
Median	23	23	
Age at onset (years)			
Mean (95% CI)	38.6 (37.2-39.9)	28.5 (27.8-29.1)	< 0.001**
Median	40	27	
Age at onset of progression (years)			
Mean (95% CI)		40.2 (39.3-41.0)	
Median		39	
Systems involved at onset: number of patients (%)			
Motor	97 (44.7%)	145 (17.9%)	< 0.001*
Sensory	80 (36.8%)	438 (54.3%)	< 0.001*
Cerebellar	10(4.6%)	51(6.3%)	0.342*
Brainstem	11(5.0%)	167 (20.7%)	< 0.001*
Optic	10(4.6%)	174 (21.5%)	< 0.001*
Bowel/bladder	7(3.2%)	25 (3.1%)	0.926*

In Table 3.13 patients were grouped according to the initial disease course (PP and RR/SP), and compared. The PP group had larger percentage of males (42.9% versus 31.2%; p = 0.001), bringing the F/M ratio much closer to unity (1.3); this is particularly seen in the younger age at onset groups (Cottrell et al., 1999b). No

differences were observed in the mean disease duration. The two subgroups were distinguished by the different age at first symptom. This occurred between the age of 20 and 40 years for most of the relapsing onset patients (75%), and between the age of 30 and 50 for most of the PP patients (65%) (Figure 3.11). In addition, the clinical onset differed according to the type of the disease course. A motor involvement was more commonly observed within the progressive onset group (44.7% versus 17.9%; $p < 0.001$), whereas relapsing onset patients presented more frequently with sensory symptoms (54.3% versus 36.8%; $p < 0.001$). Most of the patients in both groups had mono-symptomatic onset, (Figure 3.12).

Figure 3.11 Percentages of patients (y-axis) in different age at disease onset categories (x-axis): comparison between progressive onset (red) and relapsing onset (blue) MS.

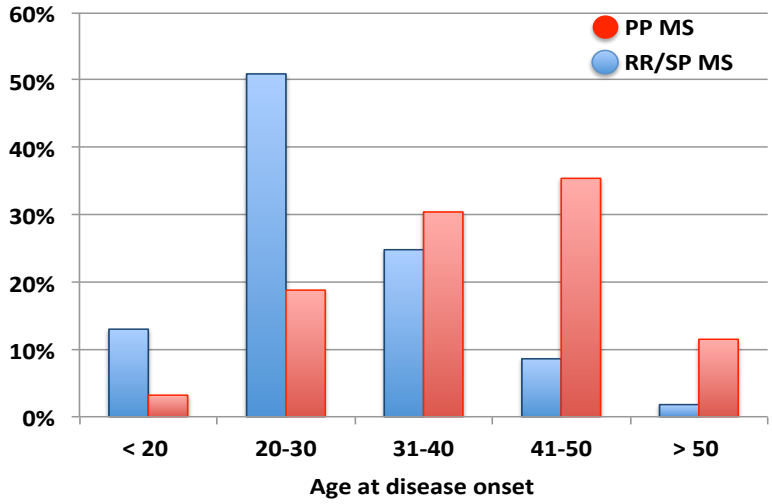
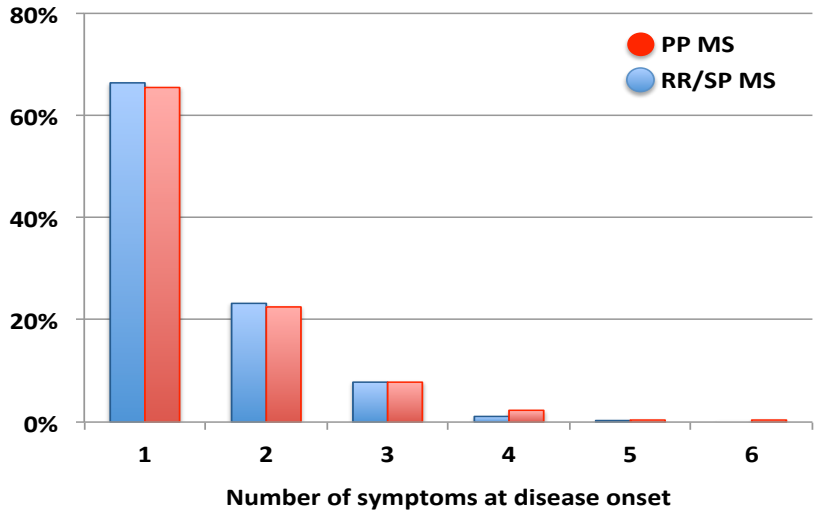


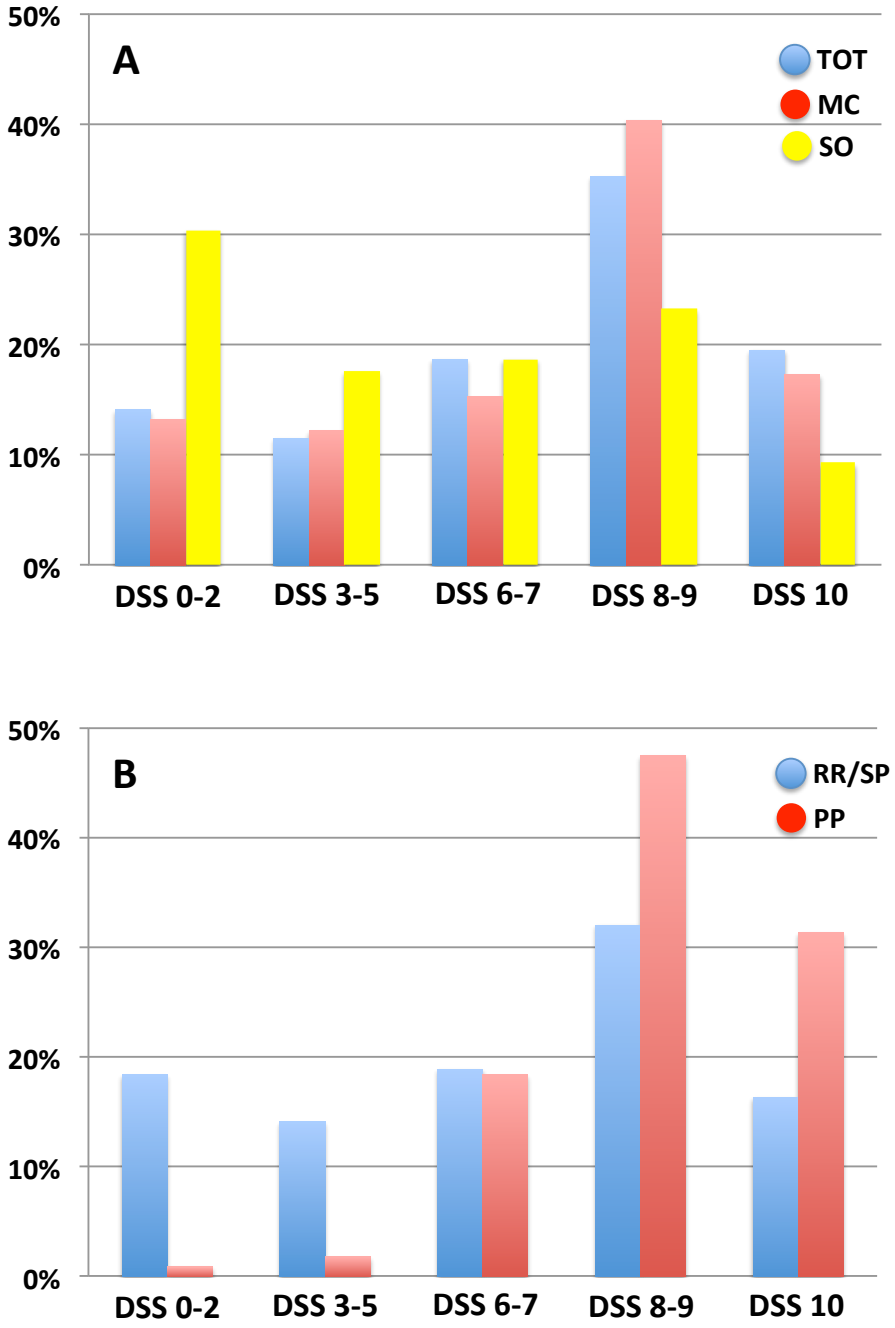
Figure 3.12 Percentages of patients (y-axis) with different numbers of neurological systems involved at disease onset (x-axis): comparison between relapsing onset (blue) and progressive onset (red) MS.



3.3.2 Analysis of disability

3.3.2.1 The distribution of disability

Figure 3.13 Percentages of patients (y-axis) at disability levels (x-axis), by the end of the observation period. A) Total population (TOT = 1023), Middlesex County (MC = 196) and seen from onset (SO = 181). B) relapsing onset (n = 806) and primary progressive (n = 217).



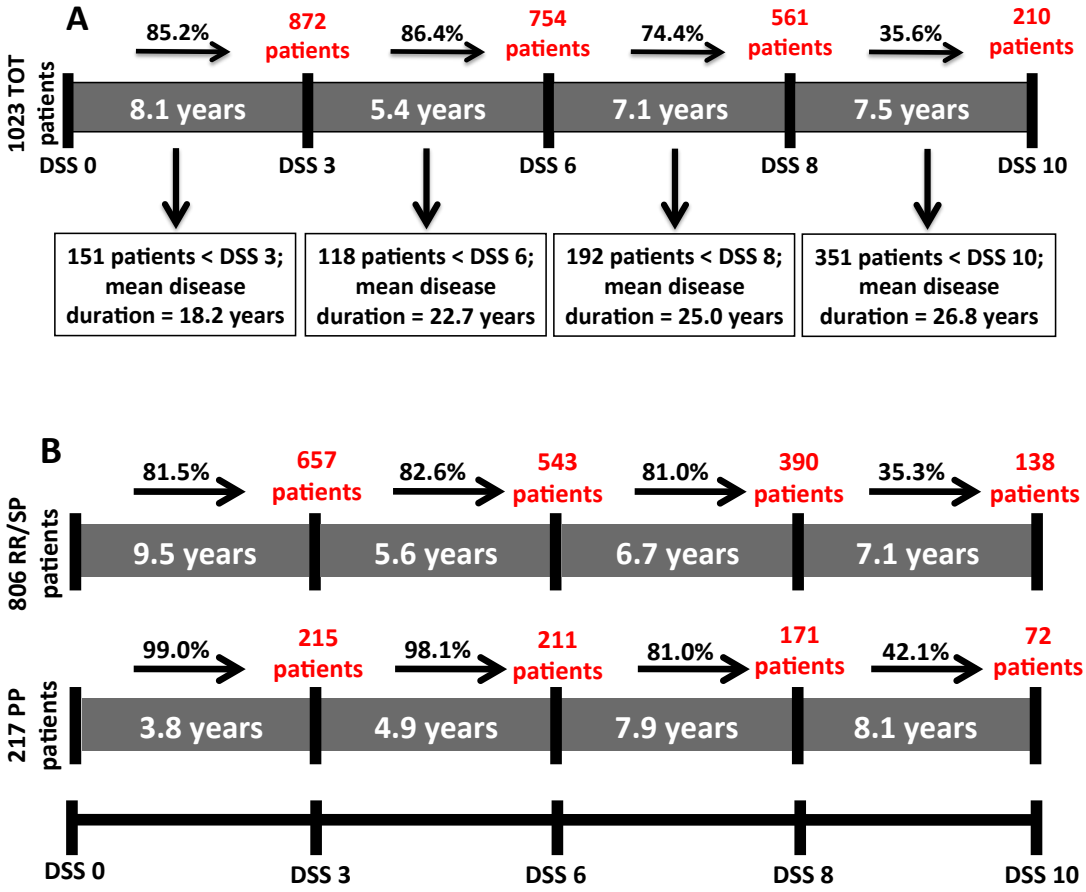
The analysis of disability focused on the attainment of hard outcomes (DSS 3-6-8-10). By the end of the observation period, at last assessment, more than 50% (n = 561) of the total population had disability rated between DSS 8 and DSS 10. Similar distribution was observed in the MC subgroup, whereas a larger percentage of SO patients were still at lower levels of disability, due to the shorter mean disease duration (Figure 3.13 A). Almost half (n = 390; 48.3%) of the relapsing onset patients and most of the PP patients (n = 171, 78.7%) were scored between DSS 8 and 10 (Figure 3.13 B).

3.3.2.2 The progression of disability

Figures 3.14 shows, in the total population (Figure 3.14 A) and in the two disease course subgroups (Figure 3.14 B), the number of patients, reaching each DSS hallmark level during the observation period, the proportion that progressed to the following disability level, and the mean time patients stayed in between DSS levels.

As expected, the percentages advancing to the higher DSS levels slightly decreased along the DSS scale. However, no substantial variations were observed for progressing up to DSS 8, where the number of patients reaching DSS 10 dropped dramatically, as a consequence of the deaths not due to MS (35.1% of total dead patients). In the total population (Figure 3.14 A) and in the relapsing onset subgroup (Figure 3.14 B), the average time in between DSS levels was unevenly distributed, with the highest peak in between DSS 0 and 3, and the lowest peak in between DSS 3 and 6, partially explained by the lack of linearity of the DSS scale. Primary progressive patients took much shorter time to reach DSS 3 than relapsing onset patients, however the average times for progressing to higher disability levels were remarkably similar in the two subgroups (Figure 3.14 B).

Figure 3.14 Number of patients reaching each DSS hallmark level, percentages progressing to the following disability level and the mean time spent in between DSS levels. The number of patients who did not advance to the higher disability level and their mean disease duration are also indicated (for the total population only) in rectangles. **A**) Total population (n = 1023). **B**) Relapsing onset (n = 806) and progressive onset (n = 217) subgroups.



3.3.2.3 Survival analysis of disability

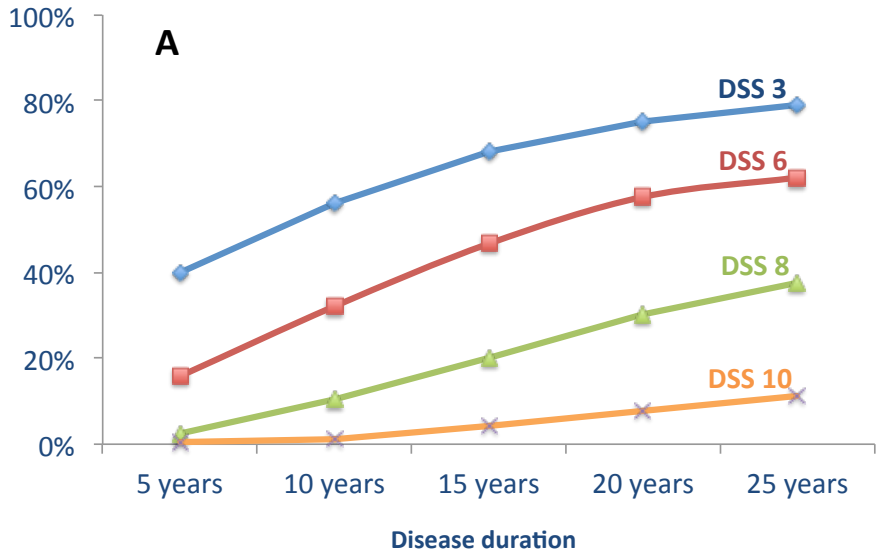
3.3.2.3.1 The disability accumulation over time in the total population

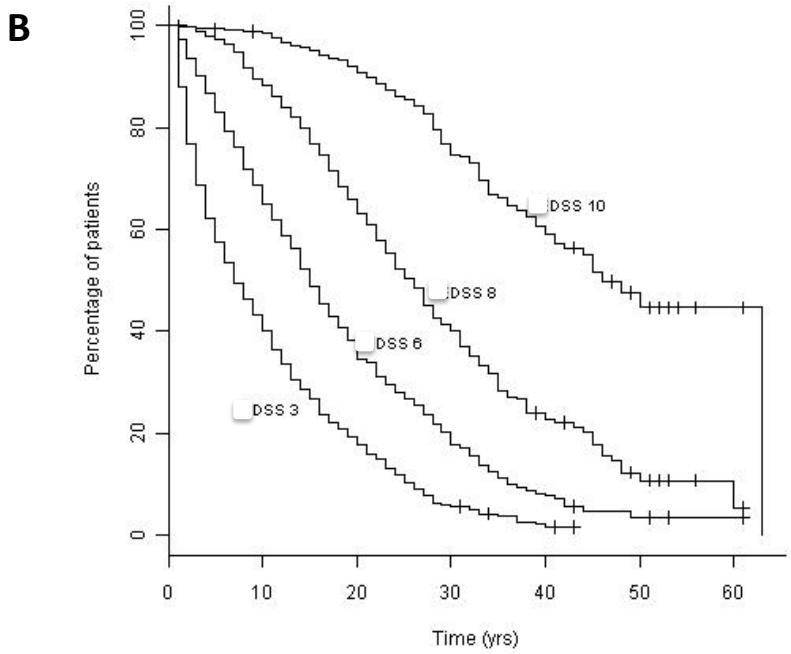
The number of patients reaching each DSS level increased proportionally with the disease duration, and at different rates among patients (Figure 3.15 A). At **5 years** from onset, 2.5% had already reached DSS 8, rising to 10 % at **10 years**. However, after **15 years,** more than 30 % of patients had not reached DSS 3 yet, half of the population had reached DSS 6 and 20% had reached DSS 8. By **25 years** these values increased to 62.1% of patients at DSS 6 and 37.7% at DSS 8. By the end of the observation period, 85.2%, 73.7%, 54.8% and 20.6% had reached DSS 3, 6, 8 and 10, respectively (Figure 3.13 A).

In the total population, the Kaplan Meier survival analysis estimated **7** years (95% C.I. 6.1-7.8), **15** years (95% C.I. 14.0-15.9), **26** years (95% C.I. 24.5-27.4) and **46 years** (95% C.I. 40.0-49.9) as the median time from the disease onset to reach DSS 3, 6, 8 and 10, respectively (Figure 3.15 B).

The comparison of survival curves, between the total population and the MC and SO subgroups, allowed to assess the impact of ascertainment and referral biases on the disability data (Figure 3.16 A and B). The overlapping survival curves demonstrated remarkably similar times to DSS 6 and to DSS 8 (total population versus MC subgroup: p = 0.23, p = 0.59; total population versus SO subgroup p= 0.42, p = 0.37, respectively), indicating that only little bias affected the estimates of the accumulation of disability.

Figure 3.15 Kaplan Meier survival analysis of disability in the total population (TOT = 1023). **A**) Percentage of patients (y-axis) reaching each DSS level at specific time points from the disease onset (x-axis). The analysis of each disability level is independent from the others. **B**) Kaplan Meier survival curves to DSS 3, 6, 8 and 10. The mean and median times from disease onset to DSS levels and the percentages of censored information contributing to the survival estimates are indicated below the graph.

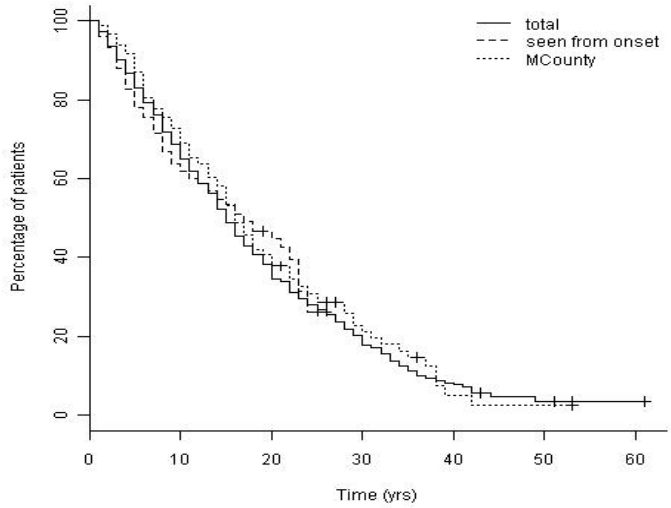




	Number attaining the endpoint	% censored information	Mean years (95% C.I.) to	Median years to
DSS 3	872	14.7%	10.8 (10.1-11.5)	7
DSS 6	709	26.2%	18.5 (17.4-19.6)	15
DSS 8	488	47.6%	28.6 (27.1-30.1)	26
DSS 10	210	79.4%	44.5 (42.2-46.8)	45

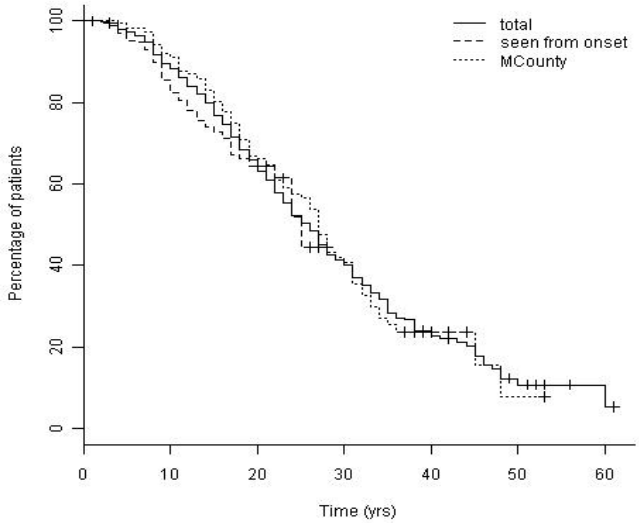
Figure 3.16 Kaplan Meier survival analysis of disability. Comparison of Kaplan Meier survival curves from disease onset to DSS 6 (**A**) and to DSS 8 (**B**) between the total population, the seen from onset (SO n = 181) and the Middlesex County (MC n = 196) subgroups. The mean and median times from disease onset to DSS levels and the percentages of censored information contributing to the survival estimates are indicated below the graph. P values were obtained with Log Rank test.

A



Survival analysis: estimated time to DSS 6					
	Number attaining the endpoint	% censored information	Mean years (95% C.I.) to	Median years to	p value
TOT	709	26.2%	18.5 (17.4-19.6)	15	
MC	135	31.1%	19.1 (17.0-21.2)	16	0.23
SO	91	49.7%	15.6 (14.2-17.1)	17	0.42

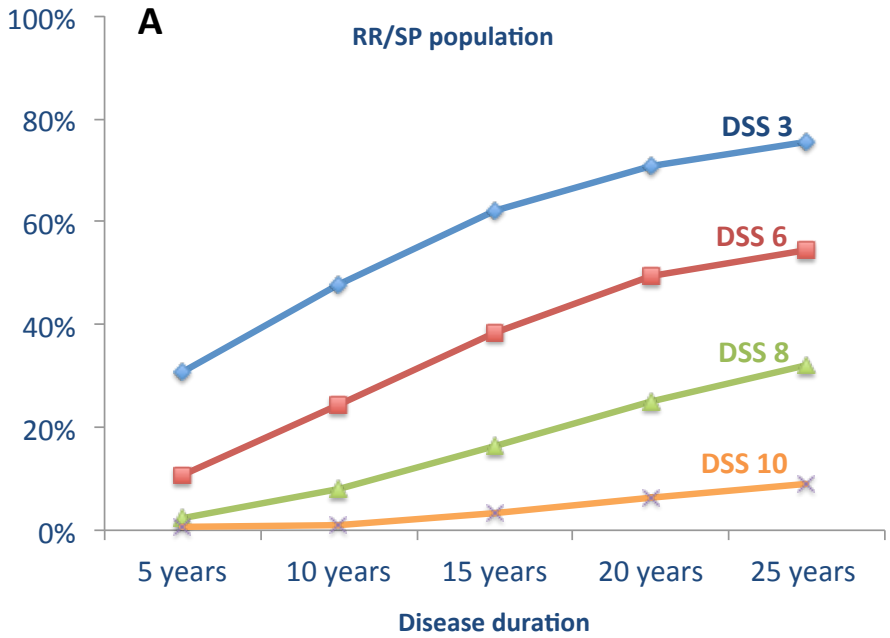
B

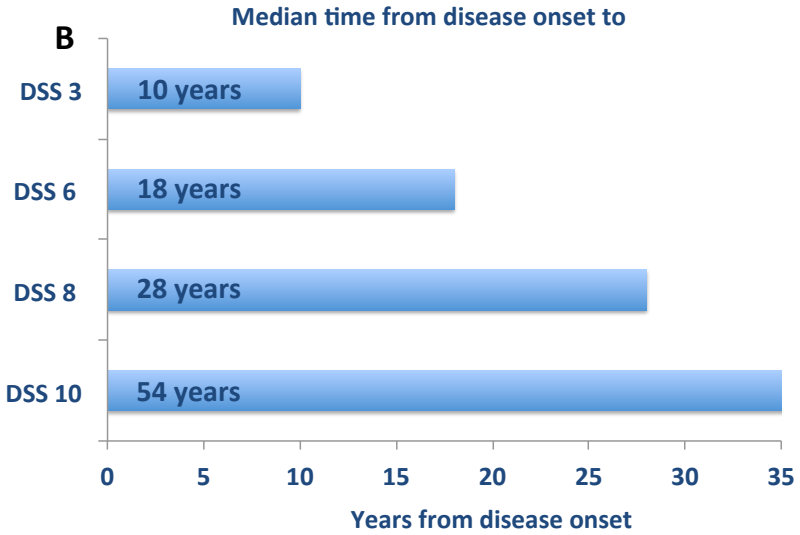


Survival analysis: estimated time to DSS 8					
	Number attaining the endpoint	% censored information	Mean years (95% C.I.) to	Median years to	p value
TOT	488	47.6%	28.6 (27.1-30.1)	26	
MC	98	50.0%	28.2 (25.7-30.8)	27	0.59
SO	56	69.0%	21.2 (19.8-22.6)	25	0.37

3.3.2.3.2 The disability accumulation among relapsing onset patients

Figure 3.17 Kaplan Meier survival analysis of disability in the relapsing onset population (n = 806). **A)** Percentage of patients (y-axis) reaching each DSS level at specific time points from the disease onset (x-axis). The analysis of each disability level is independent from the others. **B**) Estimated median times from onset to DSS 3, 6, 8 and 10. The mean times to endpoints and the percentages of censored information, contributing to the survival estimates, are indicated below the graph.



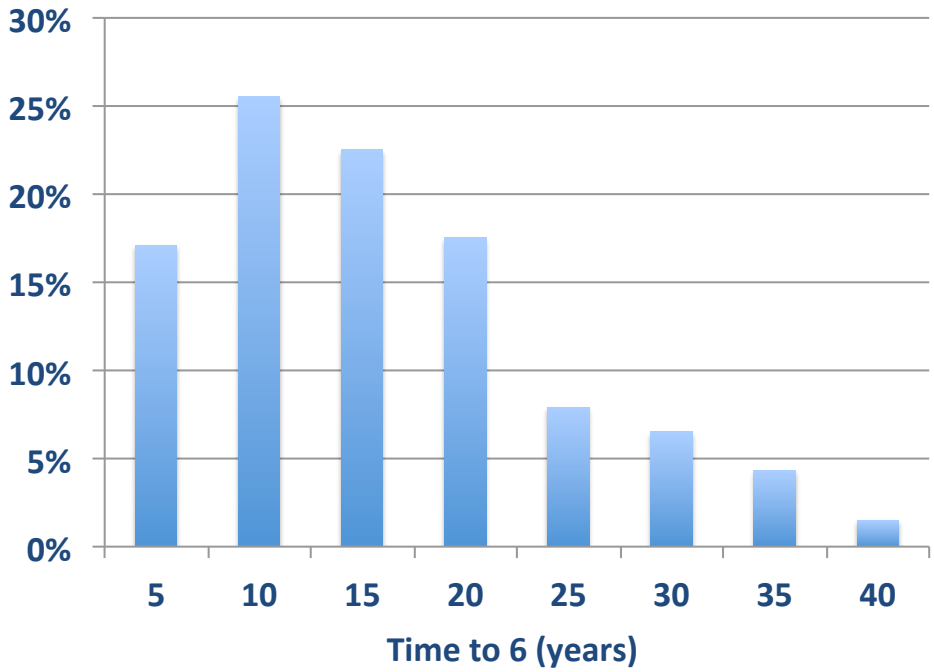


	Number attaining the endpoint	% censored information	Mean years (95% C.I.) to
DSS 3	634	17.2%	12.6 (11.8-13.5)
DSS 6	506	32.6%	21.3 (19.8-22.6)
DSS 8	339	54.0%	30.9 (29.1-32.8)
DSS 10	138	82.8%	46.9 (44.3-49.5)

Within the relapsing onset group (n = 806), at **5 years** from onset, 87 (10.7%) and 18 (2.2%) patients already reached DSS 6 and 8, respectively. These percentages rose to 38.4% (n = 310) and 16.3% (n = 132) **at 15 years**, to 49.5% (n = 399) and 24.9% (n = 201) **at 20 years**, and to 54.4% (n = 439) and 32.1% (n = 259) **at 25 years**, respectively (Figure 3.17 A). However, by **15 years** from the disease onset, 304 patients (37.8%) had not reached DSS 3 yet. By the end of the observation period, 81.5% (n = 657), 67.4% (n = 543), 48.4% (n = 390) and 17.1% (n = 138) were scored at DSS 3, 6, 8 and 10, respectively (Figure 3.13 B). Estimated median times to reach DSS 3, 6, 8 and 10, from disease onset, were **10**, **18**, **28** and **54** years, respectively (Figure 3.17 B).

The time to DSS 6 was known for 506 relapsing onset patients, and it ranged from 1 to 44 years. The quickest 25% required a walking aid within 8 years from onset, and the slowest 25 % in more than 19 years. In Figure 3.18 are presented the percentages of patients grouped by the time interval between the disease onset and DSS 6, highlighting the large variability of the clinical outcome.

Figure 3.18 Distribution of the time to DSS 6 in the relapsing onset group: percentages (y-axis) of patients grouped by the time interval from the disease onset to DSS 6 (x-axis).



3.3.2.3.3 The disability accumulation among progressive onset patients

Progressive onset patients were distinguished by a faster accumulation of disability over time. After **5 years** from disease onset, only a minority (n = 84; 24.9%) was left without moderate disability (DSS 3), and more than 1/3 (n = 75; 35.5%) already reached DSS 6. By **15 years,** the majority (n = 166; 77.4%) had required an aid for walking (DSS 6) and 33.6% (n = 73) had become bedbound (DSS 8). These percentages rose dramatically **25 years** after the disease onset, when only few (n = 20; 9.2%) have not reached DSS 6 yet, and more than half (n = 127; 58.5%) have reached DSS 8 (Figure 3.20 A). By the end of the observation period, 215 (99.1%), 211 (97%), 171 (78.9%) and 72 (33.1%) patients had been scored at DSS 3, 6, 8 and 10, respectively (Figure 3.13 B). Estimated Kaplan Meier median times from the disease onset to DSS 3, 6, 8, and 10 were **3**, **8**, **18** and **34 years**, respectively (Figure 3.20 B).

The time to DSS 6 was known for 208 progressive onset patients, and it ranged from 1 to 49 years. The quickest 25% required a walking aid within 4 years from onset and the slowest 25 % in more than 13 years. In Figure 3.19 are presented the percentages of patients grouped by the time interval between the disease onset and DSS 6.

Figure 3.19 Distribution of time to DSS 6 in the PP group: percentages (y-axis) of patients grouped by the time interval from the disease onset to DSS 6 (x-axis).

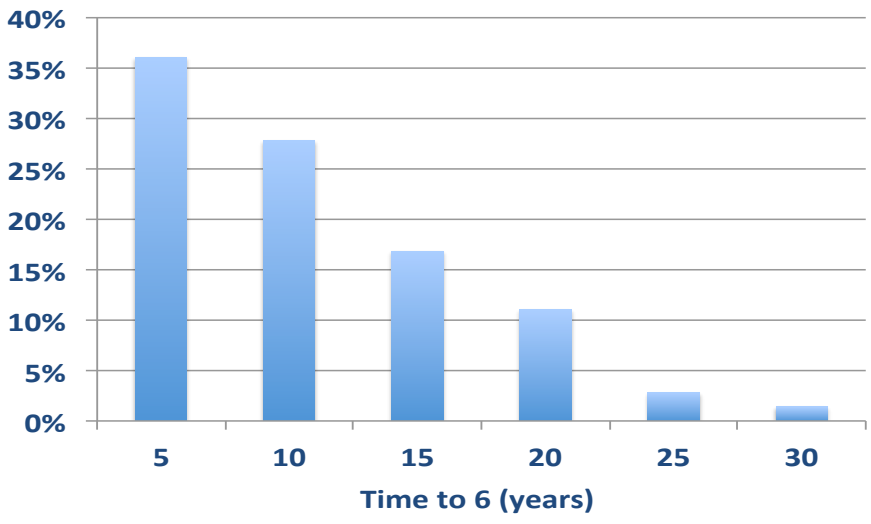
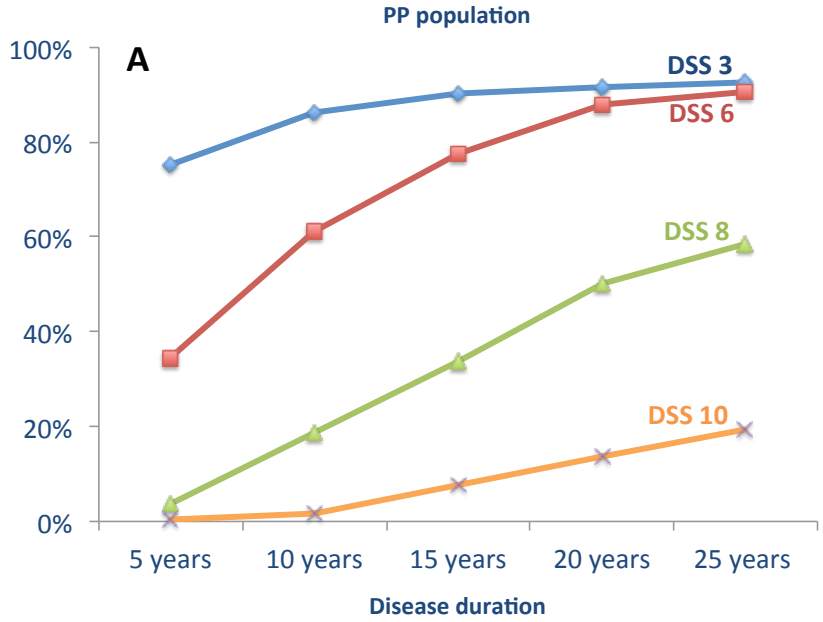
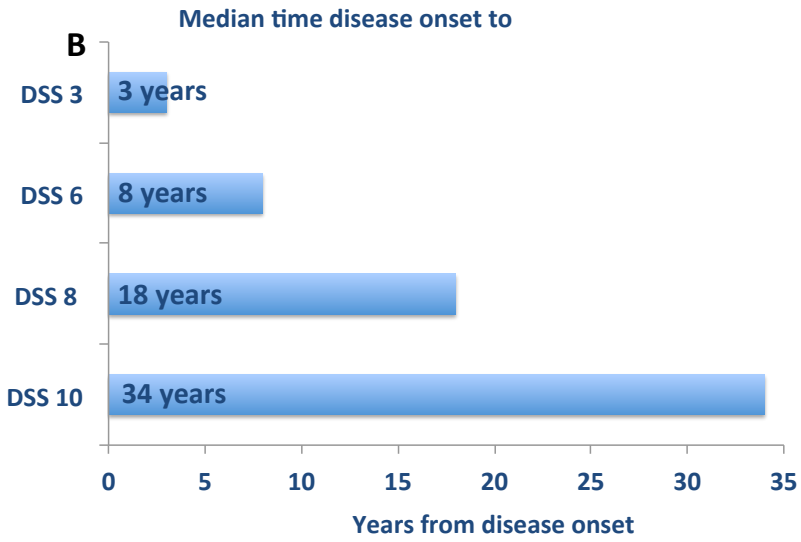


Figure 3.20 Kaplan Meier survival analysis of disability in the progressive onset population (n = 217). **A)** Percentage (y-axis) of patients reaching each DSS level at specific time points from the disease onset (x-axis). The analysis of each disability level is independent from the others. **B**) Estimated median times from onset to DSS 3, 6, 8 and 10. The mean times to endpoints and the percentages of censored information, contributing to the survival estimates, are indicated below the graph.





	Number attaining the endpoint	% censored information	Mean years (95% C.I.) to
DSS 3	201	0.9%	3.9 (3.4-4.5)
DSS 6	208	2.4%	9.5 (8.5-10.6)
DSS 8	149	23.2%	20.5 (18.6-22.3)
DSS 10	72	66.8%	34.6 (32.1-37.0)

3.3.2.3.4 Progressive onset versus relapsing onset MS

The analysis confirmed the well-established notion that a progressive course from onset associates with a worse prognosis. The comparison of the Kaplan Meier survival curves, estimating the disability accumulation from the disease onset, demonstrated significantly shorter times (approximately 10 years earlier) to DSS 6 and to DSS 8, among PP MS patients compared to RR and SP patients pooled together (DSS 6 = 9.5 versus 21.3 years; DSS 8 = 20.5 versus 30.9 years, respectively) (Figure 3.21 A-B). However, the 2 groups matched much more closely when they reached the endpoints from moderate disability. Although still statistically significant, there was less than 3 mean years difference for advancing from DSS 3 to higher disability levels (from DSS 3 to DSS 6 = 5.1 versus 7.9 years, $p < 0.001$; from DSS 3 to DSS 8 = 15.3 versus 17.6 years, $p = 0.03$, respectively) (Figure 3.21 C-D).

The comparison between PP and SP MS yielded similar results. The disease evolution from onset was significantly faster, among PP patients (Figure 3.22). However, the differences between the two groups were much smaller compared to the analysis including also RR patients (Figure 3.21). The PP MS group attained DSS 6 and DSS 8 approximately 6 mean years earlier than the SP MS group (DSS 6 = 9.5 versus 15.7 years, $p < 0.001$; DSS 8 = 20.5 versus 26.5, $p < 0.001$, respectively) (Figure 3.22 A-B). In addition, once again, the disease progression from DSS 3 was largely unaffected by the type of disease course. Times from moderate disability to DSS 6 and to DSS 8 differed little between PP and SP patients (DSS 6 = 5.1 versus 6.4 years, $p = 0.006$; DSS 8 = 15.3 versus 16.4 years, $p = 0.40$, respectively) (Figure 3.22 C-D).

Figure 3.21 Kaplan Meier survival analysis of disability. Comparison of times to DSS 6 and to DSS 8 from disease onset (**A-B**) and from DSS 3 (**C-D**) between relapsing onset (RR/SP n= 806, blue) and progressive onset (PP n = 217, green) patients. P values obtained with Log Rank test.

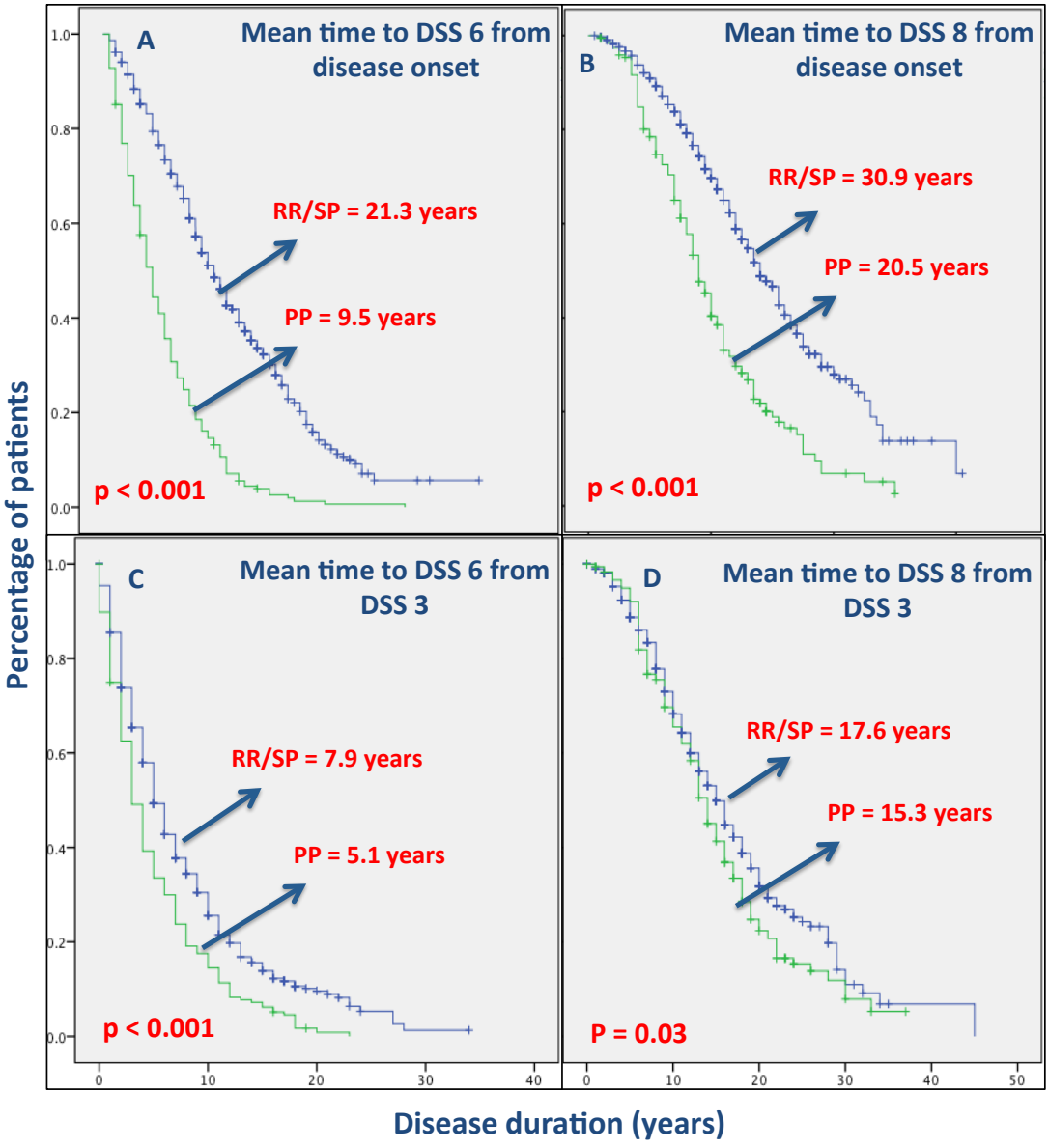
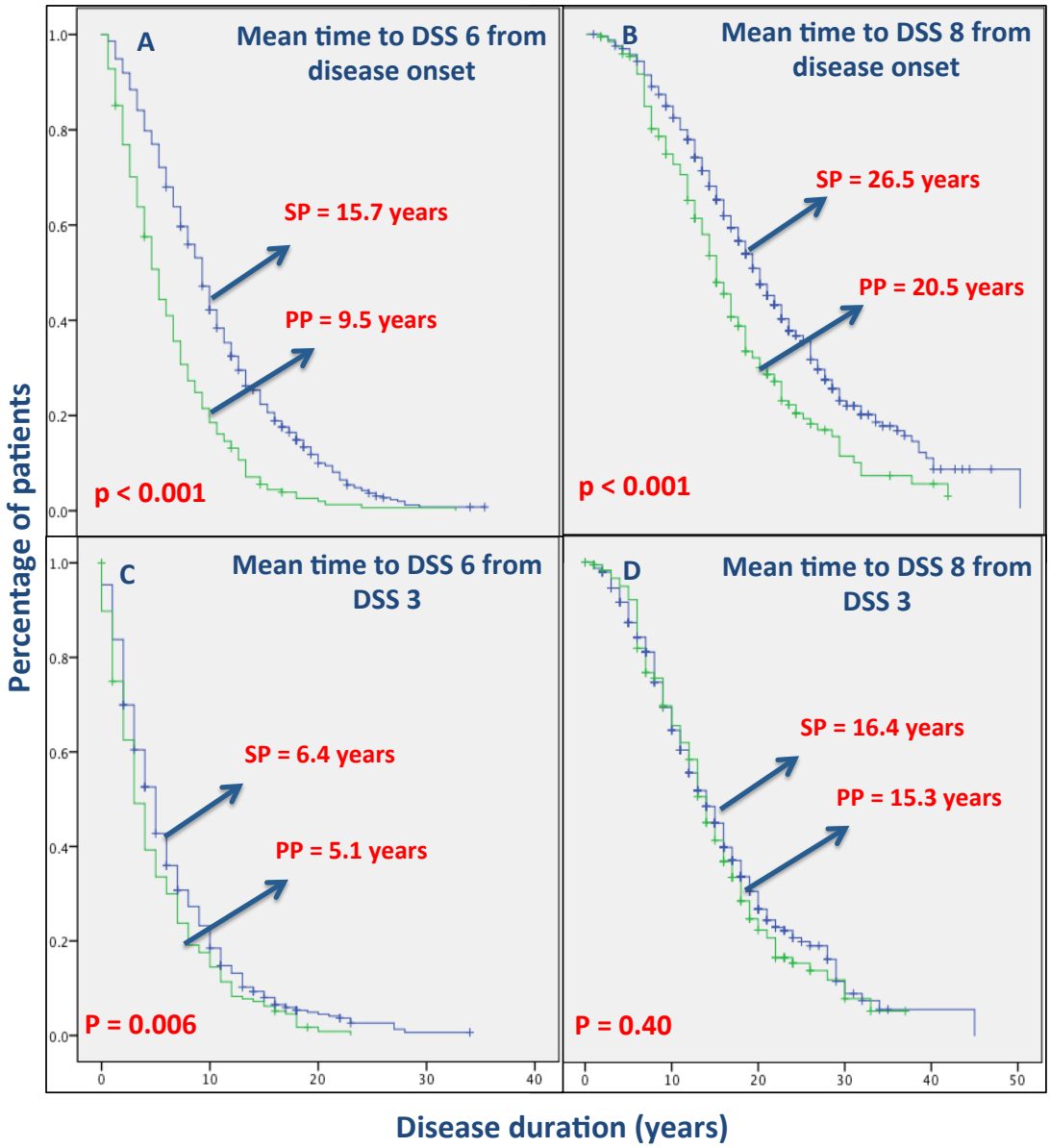


Figure 3.22 Kaplan Meier survival analysis of disability. Comparison of times to DSS 6 and to DSS 8 from disease onset (**A-B**) and from DSS 3 (**C-D**) between secondary progressive (SP n= 534, blue) and primary progressive (PP n = 217, green) patients. P values obtained with Log Rank test.



3.3.3 Mortality and causes of death

During the last review of the LO database (1996), extensive efforts were made to trace patients who had been lost to follow up (Cottrell et al., 1999b). With the help of the Government of Ontario and its Ministry of Health, providing access to the provincial registry of all deaths and to copies of death certificates, the final outcome and the causes of death were ascertained in the majority of patients. This process led to the identification of 286 dead patients (101 PP MS, 185 RR/SP MS). With the extended follow up (2000), the sample increased to 324 (31.6% of the total population). A similar percentage of dead patients (n= 65; 33.1%) was observed within the MC subgroup.

3.3.3.1 Clinical and demographic features of dead patients

By the end of the observation period, 51.5% (n = 111) of the progressive onset group and 26.4% (n = 213) of the relapsing onset group had died. Among the 324 dead patients, 34.2% had a primary progressive course and 75.8% experienced a relapsing onset course; the two subgroups were distinguished by different clinical and demographic features (Table 3.14), resembling what observed in the general population (Table 3.13).

Among the total dead patients, females predominated in the relapsing onset group and the F/M ratio in the PP group was close to unity (F/M ratios: 1.5 versus 0.9; $p < 0.001$). Although an initial RR course associated with a significantly younger age at the disease onset (31.5 versus 39.8 mean years; $p < 0.001$), the age at the onset of progression was remarkably similar between the two subgroups (41.4 versus 39.8 mean years; $p = 0.21$) and not affected by the clinical phenotype. The clinical presentation varied according to the type of the disease course. The progressive onset group more commonly presented with a motor involvement (43.2% versus 23.9%; $p < 0.001$) and the relapsing onset group with sensory symptoms (46.0% versus 34.2%; $p < 0.042$) (Table 3.14).

Table 3.14 Clinical and demographic features of dead patients (n = 324), grouped by the initial disease course (progressive onset and relapsing onset). * Chi-square test; ** Student's T-test

	Progressive onset	**Relapsing-remitting onset**	p values
No of patients	111	213	
No of males	57 (51.3%)	82 (38.5%)	0.027*
No of females	54 (48.7%)	131 (61.5%)	
Sex ratio (F/M)	0.94	1.59	
Age at onset (years)			
Mean (95% CI)	39.8 (37.9-41.7)	31.5 (30.1-32.8)	< 0.001**
Median	41	30	
Time to onset of progression (years)			
Mean (95% CI)		11.4 (9.8-12.1)	
Median		8	
Age at onset of progression (years)			
Mean (95% CI)	39.8 (37.9-41.7)	41.4 (39.8-42.9)	0.21**
Median	41	41	
Systems involved at onset: number of patients (%)			
Motor	48 (43.2%)	51 (23.9%)	< 0.001*
Sensory	38 (34.2%)	98 (46.0%)	0.042*
Cerebellar	8 (7.2%)	18 (8.4%)	0.696 *
Brainstem	8 (7.2%)	49 (23.0%)	< 0.001*
Optic	5 (4.5%)	42 (19.7%)	< 0.001*
Bowel/bladder	4 (3.6%)	7 (3.2%)	0.881*

3.3.3.2 Disease progression and time to death

Among all 324 dead patients, the disease progression from onset to death was assessed. The analysis did not include censored information. The time to death was not known for 2 patients (one with PP and one with SP course), and it widely varied, from 1 to 63 years: the quickest and the slowest 25% (25 and 75 percentiles) reached death within 17 years and in more than 30 years, respectively. Among all dead patients, at **20 years** from onset, 36.7% (n = 119) had died, increasing to 55.5 % (n = 180), 75.3% (n = 244) and 84.8% (n = 275) at **25**, **30** and **35 years**, respectively (Figure 3.26). The median time to death from onset was 24 years and the median age at death was 60 years. Survival was not significantly different between the total population and the MC subgroup (time to death 24.1 versus 26.9 mean years; p = 0.2) (Figure 3.23).

The relapsing and progressive onset dead patients were compared. Patients with a PP course took significantly shorter times to DSS levels, indicating a faster accumulation of disability. However, the proportion dying over time was similar in the two subgroups (Figure 3.24). Among dead patients, by **20 years** from the disease onset, 41.4% (n = 46) with a PP course and 34.2% (n = 73) with a relapsing onset course had already died. At **35 years,** these percentages increased to 91.8% (n = 102) and to 81.2% (n = 173), respectively (Figure 3.24). Indeed, despite the faster attainment of DSS levels, PP patients reached death only 3 mean years earlier (22.7 versus 25.7 mean years; p = 0.005) and the disease progression from DSS 3 was remarkably similar between the two subgroups. In addition, relapsing onset patients died at significantly younger age (57.2 versus 62.4 mean years; p < 0.001) (Table 3.15), secondary to their younger age at onset (31.5 versus 39.8 mean years; p < 0.001) (Table 3.14).

Figure 3.23 Time to death from disease onset: comparison of Kaplan Meier survival curves between the total population (TOT) and he Middlesex County (MC) subgroup. Censored information was not included in the analysis. P value was obtained with Log Rank test.

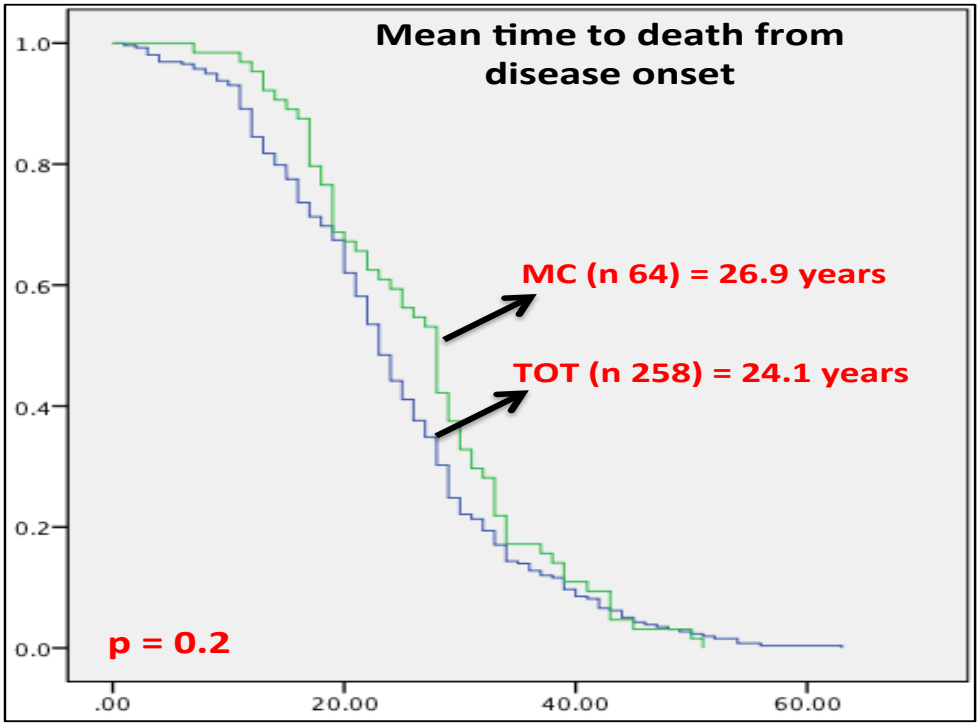


Figure 3.24 Percentages (y-axis), among dead patients (n = 324), dying at specific time points (x-axis) from disease onset in the total population and in the two disease course subgroups: RR/SP (n = 213) and PP (n = 111).

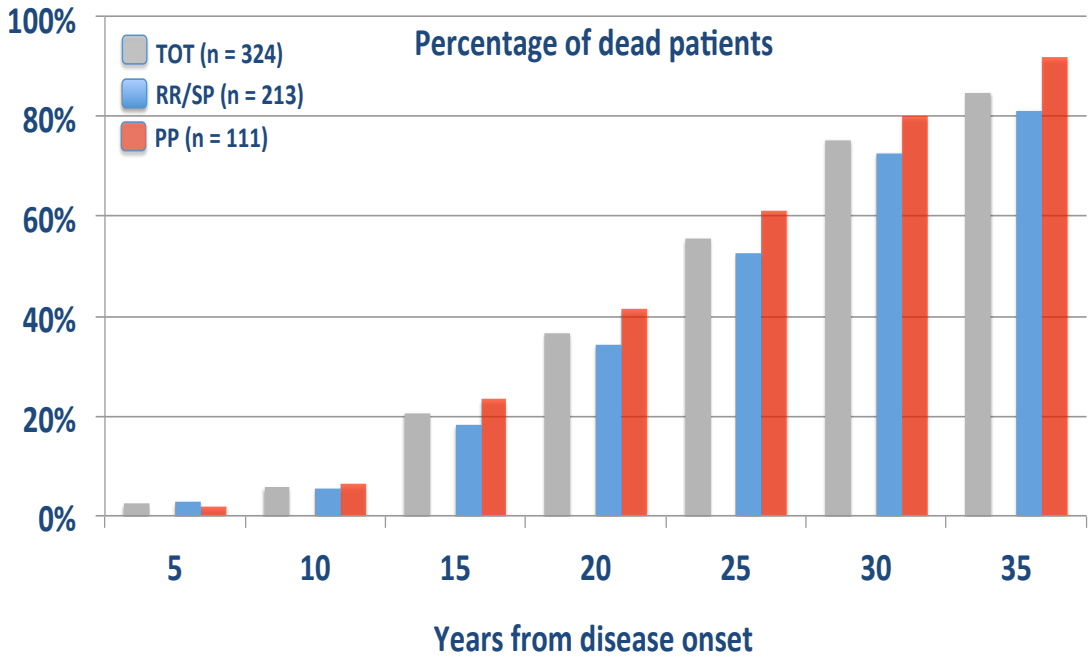


Table 3.15 Disease progression (from onset and from DSS 3) and time to death (from onset and from birth) among dead patients (n = 324) grouped by the initial disease course (progressive onset and relapsing onset). * Log-Rank test.

	Progressive onset (n = 111)	Relapsing-remitting onset (n = 213)	p values
Time to death (years) from disease onset			
Mean (95% CI)	22.7 (20.9-24.4)	25.7 (24.1-27.2)	0.005*
Median	22	24	
Time to death from birth (years); age at death			
Mean (95% CI)	62.4 (60.1-64.8)	57.2 (55.3-59.1)	< 0.001*
Median	63	57	
Mean (95% CI)[median] time from onset to			
DSS 3	3.2 (2.7-3.8) [2]	8.3 (7.4-9.3) [6]	< 0.001*
DSS 6	8.2 (6.8-9.7) [6]	13.4 (12.0-14.8) [11]	< 0.001*
DSS 8	15.4 (13.5-17.3) [14]	18.6 (17.0-20.2) [17]	0.020*
Mean (95% CI)[median] time from DSS 3 to			
DSS 6	4.5 (3.6-5.3) [3]	4.9 (4.1-5.6) [4]	0.503*
DSS 8	11.4 (10.0-12.7) [11]	10.7 (9.6-11.8) [9]	0.441*
death	18.7 (17.2-20.2) [19]	17.4 (16.1-18.6) [16]	0.390*

3.3.3.3 Causes of death

Table 3.16 Causes of death among 324 dead patients. Patients are grouped by the initial disease course and by death attributable to MS or not.

	TOT	RR/SP	PP
Number of deaths	**324**	**213**	**111**
Deaths due to MS: number of patients (% within dead population)	**210 (64.8%)**	**138 (64.7%)**	**72 (64.8%)**
Aspiration	3	2	1
Cachexia	3	3	0
Cardiac arrest/failure (DSS 8 ≥ 3yrs)	10	6	4
Dehydration	2	2	0
"Multiple Sclerosis"	48	33	15
Pneumonia	98	63	35
Pulmonary oedema	1	0	1
Pulmonary embolism	4	2	2
Respiratory arrest/failure (DSS 8 ≥ 3yrs)	10	6	4
Septicaemia	19	13	6
Suicide	6	5	1
Unknown (DSS 8 ≥ 3yrs)	6	3	3
Deaths not due to MS: number of patients (% within dead population)	**114 (35.2%)**	**75 (35.3%)**	**39 (35.2%)**
Gatro-intestinal bleeding	2	0	2
Accidental pulm.oedema	1	1	0
Accident	2	1	1
Bowel infarction	1	1	0
Cardiac arrest/failure (DSS 8 < 3yrs)	4	3	1
Cardiac arrhythmia	1	1	0
Cancer	42	31	11
Chronic bronchitis	1	1	0
Chronic renal failure	1	0	1
Heart disease	26	15	11
Head injury	1	0	1
Intestinal obstruction	1	1	0
Pyelonephritis	1	1	0
Renal failure	1	1	0
Respiratory arrest/failure (DSS 8 < 3yrs)	7	3	4
Stroke	15	9	6
Unknown (DSS 8 < 3 yrs)	7	6	1

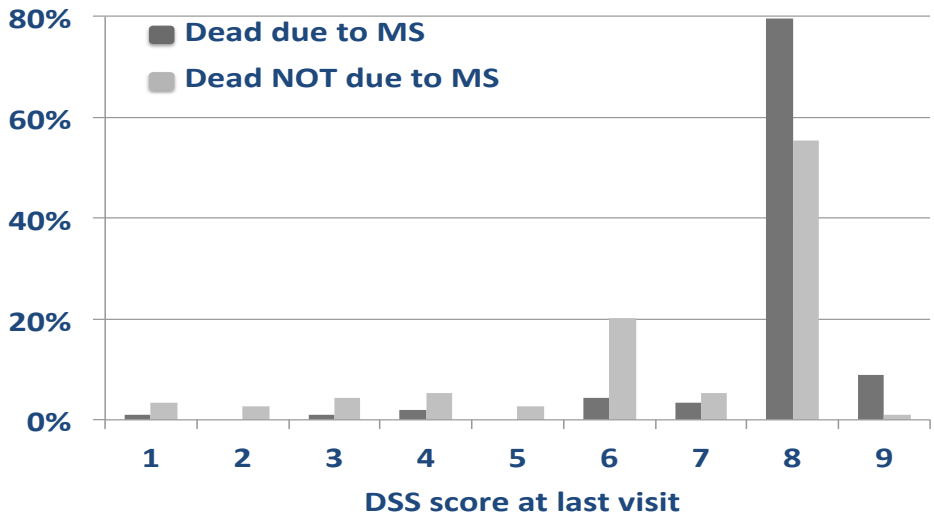
Information on causes of death was available for 311 patients (95.9% of dead patients). The criteria used for defining death due to MS (attainment of DSS 10) were outlined in methods (Chapter 2). Primary causes of death described as aspiration, cachexia, dehydration, MS, pneumonia, pulmonary oedema, pulmonary embolism,

and septicaemia, were considered a direct consequence of MS. For those who died from gastro-intestinal (G-I) bleeding, pulmonary oedema from aspiration, accident, cancer, chronic bronchitis, chronic renal failure, heart disease, head injury, intestinal obstruction, pyelonephritis, renal failure and stroke death was considered unrelated to MS. For 44 patients, who died from respiratory failure/arrest (n = 17), cardiac failure/arrest (n = 14) or unknown causes (n = 13), an arbitrary decision had to be taken, regarding whether the death was attributable to MS or not. By using a composite criterion, based on information on the secondary cause of death and on whether a bedridden status (DSS 8) had been reached and sustained for at least 3 years, 26 patients were deemed to have died from MS and 18 to have died from unrelated causes (Table 3.16).

3.3.3.3.1 Comparative analysis: deaths due to MS versus deaths NOT due to MS

In the total dead population, 210 (64.8%) patients died from causes directly **related** to MS, including 6 who committed suicide, and 114 (35.2%) died from causes **unrelated** to MS (Table 3.16). Comparison of clinical and demographic features, and of the disease progression revealed differences and similarities (Table 3.17). Times to disability endpoints and to death were calculated in the dead population only, without including censored information in the analysis.

Figure 3.25 DSS score at last assessment before death. Percentages (y-axis) of dead due and NOT due to MS at each DSS level (x-axis) recorded before death.



In both subgroups, of dead due and not due to MS, the disease phenotypes were equally distributed (relapsing onset patients: 65.7% versus 65.8%; p = 0.98), and the F/M ratio and the clinical presentation were remarkably similar. However, those who died from causes related to MS were almost 4 mean years younger at the disease onset (33.0 versus 36.7 mean years; p = 0.03). The binary logistic regression analysis confirmed that being older at the disease onset significantly associated with a modestly lower probability of dying from MS (OR = 0.96; p = 0.004). In addition, the dead due to MS were much younger at the onset of progression (38.7 versus 45.0 mean years; p < 0.001), secondary to the shorter duration of the RR phase (8.8 versus 12.3 mean years; p = 0.002).

Interestingly, patients who died from MS took slightly shorter times to all disability endpoints (Table 3.17). Nevertheless, they attained death only 2.1 years earlier than those who died from causes unrelated to MS (23.9 versus 26.0 mean years, respectively; p = 0.075). Consequently, given the similar time to death between the two groups, the younger age at death among the dead due to MS (57.0 versus 62.7 mean years; p < 0.001) was mainly accounted for by their younger age at the disease onset (Table 3.17). The DSS scores, at last assessment, are presented in figure 3.25. Most of the patients in both subgroups were followed up to the latest stage of the disease, before dying; 91.8% of dead due to MS and 61.5% of dead NOT due to MS were scored between DSS 7 and 9 (Figure 3.25).

Causes of death were grouped in categories as follows: cachexia, cardiovascular (heart disease, cardiac arrest/failure/arrhythmia), cerebrovascular (stroke), infections (septicaemia), malignancy, miscellaneous (intestinal obstruction, head injury, internal bleed, bowel infarction, accident), MS, pulmonary (respiratory arrest/failure, pulmonary embolism, pneumonia, chronic bronchitis, aspiration), renal (renal failure, pyelonephritis), suicides and unknown (Table 3.18). Factors affecting the time to death were assessed among the dead due to MS (Table 3.19) and among the dead from causes unrelated to MS (Table 3.20).

Table 3.17 Clinical and demographic features of dead patients (n = 324), grouped by causes of death: due (n = 210) and NOT due (n = 114) to MS. * Chi-square test; ** Student T-test; *** Log-Rank test.

	Deaths due to MS	**Deaths NOT due to MS**	p values
No of patients	210	114	
No of males	89 (42.4%)	50 (43.9%)	0.797*
No of females	121 (57.6%)	64 (56.1%)	
Sex ratio (F/M)	1.35	1.28	
Clinical phenotype			
RR	8 (3.8%)	12 (10.5%)	
SP	130 (61.9%)	63 (55.3%)	
RR/SP	138 (65.7%)	75 (65.8%)	0.989*
PP	72 (34.3%)	39 (34.2%)	
DSS before death			
Mean (95% CI)	7.7 (7.6-7.9)	6.6 (6.2-7.0)	
Median	8	8	
Systems involved at onset: number of patients (%)			
Motor	59 (28.0%)	40 (35.0%)	0.192*
Sensory	93 (44.2%)	43 (37.7%)	0.253*
Cerebellar	15 (7.1%)	11 (9.6%)	0.428 *
Brainstem	37 (17.6%)	20 (17.5%)	0.986*
Optic	30 (14.2%)	17 (14.9%)	0.878*
Bowel/bladder	7 (3.3%)	4 (3.5%)	0.934*
Age at onset (years)			
Mean (95% CI)	33.0 (31.7-34.4)	36.7 (34.6-38.9)	0.036**
Median	32	38	
Time to onset of progression (years) [193 SP patients]			
Mean (95% CI)	8.8 (7.6-9.9)	12.3 (10.2-14.5)	0.002**
Median	7	11	
Age at onset of progression (years) [304 PP/SP patients]			
Mean (95% CI)	38.7 (37.3-40.2)	45.0 (43.2-46.8)	< 0.001**
Median	38	46	
Time to DSS 3 (years) from onset			
Mean (95% CI)	5.8 (5.0-6.6)	8.1 (6.6-9.6)	0.004***
Median	4	5	
Time to DSS 6 (years) from onset			
Mean (95% CI)	10.0 (8.9-11.2)	14.7 (12.6-16.9)	<0.001***
Median	8	12	
Time to DSS 8 (years) from onset			
Mean (95% CI)	16.2 (14.8-17.5)	21.2 (18.6-23.9)	0.004***
Median	15	19	
Time to death (years) from onset			
Mean (95% CI)	23.9 (22.5-25.3)	26.0 (23.8-28.2)	0.075***
Median	23	24	
Time to death (years) from birth (age at death)			
Mean (95% CI)	57.0 (55.1-58.9)	62.7 (60.3-65.1)	< 0.001***
Median	57	64	

Table 3.18 Dead patients (n = 324) grouped by cause of death. For each category clinical and demographic features are indicated.

Deaths due to MS	**No (%)**	**No Females/Males (F/M ratio)**	**Mean (median) age at onset**	**Relapsing/progressive onset**	**Mean (median) DSS before death**	**Mean (median) years to death**	**Mean (median) age at death**
Cachexia	5 (2.3%)	4/1 (4)	25.6 (24)	5/0 (100/0%)	8.0 (8)	25.4 (23)	51.0 (57)
Cardiovascular	10 (4.7%)	5/5 (1)	34.2 (35)	6/4 (60/40%)	8.2 (8)	31.9 (27)	66.1 (63)
Infections	19 (9.0%)	12/7 (1.7)	33.8 (34)	13/6 (68/32%)	7.5 (8)	24.5 (29)	58.3 (58)
Multiple Sclerosis	48 (22.8%)	27/21 (1.2)	32.2 (30)	33/15 (68/32%)	7.7 (8)	23.4 (22)	55.7 (53)
Pulmunary	116 (55.2%)	65/61 (1)	33.9 (33)	73/43 (63/37%)	7.8 (8)	23.2 (23)	57.1 (57)
Suicides	6 (2.8%)	3/3 (1)	26.5 (24)	5/1 (83/17%)	5.3 (6)	21.3 (23)	47.8 (46)
Unknown	6 (2.8%)	5/1 (5)	32.6 (32)	3/3 (50/50%)	8.0 (8)	27.6 (29)	60.3 (64)
Tot.	**210**	**121/89 (1.3)**	**33.0 (32)**	**138/72 (66/34%)**	**7.7 (8)**	**23.9 (23)**	**57.0 (57)**
Deaths NOT due to MS							
Cardiovascular	31 (27.1%)	13/18 (0.7)	37.8 (38)	19/12 (61/39%)	7.0 (8)	29.4 (26)	67.2 (69)
Cerbrovascular	15 (13.1%)	9/6 (1.5)	38.2 (45)	9/6 (60/40%)	7.4 (8)	27.7 (24)	65.9 (68)
Malignancy	42 (36.8%)	29/13 (2.2)	36.7 (38)	31/11 (74/26%)	6.0 (7)	25.7 (24)	62.4 (64)
Miscellanous	8 (7.0%)	4/4 (1)	38.6 (42)	4/4 (50/50%)	6.6 (8)	24.5 (23)	61.7 (62)
Pulmunary	8 (7.0%)	4/4 (1)	37.0 (35)	4/4 (50/50%)	7.3 (8)	21.8 (19)	58.8 (63)
Renal	3 (2.6%)	1/2 (0.5)	38.3 (37)	2/1 (67/33%)	8.0 (8)	25.6 (24)	64.0 (61)
Unknown	7 (6.1%)	4/3 (1.3)	26.2 (25)	6/1 (85/15%)	5.0 (6)	16.0 (12)	42.2 (34)
Tot	**114**	**64/50 (1.2)**	**36.7 (38)**	**75/39 (66/34%)**	**6.6 (8)**	**26.0 (24)**	**62.7 (64)**

3.3.3.3.2 Deaths due to MS

Among the dead due to MS, the time to death was not affected either by the type of the disease course, or by the age at onset or by the type of symptoms at clinical presentation (Table 3.19). Consequently, being younger at onset and having a relapsing onset course predicted a younger age at death and therefore worse prognosis. Those younger at disease onset died at younger age (age ≤ 20 = 42.6 versus age > 30 = 63.2 mean years, $p < 0.001$; age 21-30 = 50.5 versus age > 30 = 63.2 mean years, $p < 0.001$). Similarly, relapsing onset, compared to progressive onset patients, took almost the same time to death (24.3 versus 23.1 mean years, $p = 0.36$), but were significantly younger when they died (RR/SP = 55.0 versus PP = 60.7 mean years; $p = 0.01$), secondary to their younger age at onset (30.7 versus 35.1 mean years, $p < 0.001$). Males compared to females had similar age at first symptoms (32.7 versus 33.1 mean years, $p = 0.71$), took a slightly shorter time to death (22.4 versus 25.0 mean years, $p = 0.10$) and were slightly younger when they died (males = 55.2 versus females = 58.3 mean years; $p = 0.003$) (Table 3.19).

Respiratory diseases (n = 116; 55.2%), MS (n = 48; 22.8%) and infections (n = 19; 9%) were the commonest immediate causes of death (Table 3.18). In the 3 subgroups, the mean age at onset was approximately 33 years, females and relapsing onset disease course predominated, however the F/M ratio, among the dead due to pulmonary diseases, was close to unity. In addition, the mean DSS score before death, the average time to death and the mean age at death were similar.

Six patients (2.8%) committed suicide (3 females/3 males). These were significantly younger at the disease onset (26.5 mean years), and were younger (47.8 years) and less disabled at death (mean DSS = 5.3), secondary to the shorter survival (21.3 mean years) (Table 3.18).

Patients who died from cardiovascular causes (9 from cardiac arrest and 1 from cardiac failure) (n = 10; 4.7%) and spent at least 3 years bedbound, were the oldest at onset (34.2 mean years) and at death (66.1 mean years), were the most disabled

before dying (mean DSS = 8.2), and took the longest time to death (31.9 mean years) (Table 3.18).

For 6 patients (2.8%) the cause of death was unknown, however they were deemed to have died from MS, as they had been bedbound for at least 3 years before dying (3 patients stayed 15 years at DSS 8 and the remaining 3 stayed 6, 7 and 9 years).

Table 3.19 Factors affecting the time to death from the disease onset and from birth (age at death) among patients who died from MS related causes (n = 210). Censored information was not included in the analyses. * Reference category; ** Log-Rank test.

		Kaplan Meier analysis from onset		Kaplan Meier analysis from birth (age at)	
	Number	Mean (95% CI) [median] years to death	p **	Mean (95% CI) [median] years to death	p **
Deaths due to MS	210	23.9 (22.5-25.3) [23]		57.0 (55.1-58.9) [57]	
Gender					
Males	89	22.4 (20.3-24.5) [22]	0.106	55.2 (52.5-57.8) [54]	0.03
* Females	121	25.0 (23.1-26.9) [24]		58.3 (55.7-60.9) [57]	
Type of disease course					
Progressive onset	72	23.1 (20.8-25.3) [22]	0.36	60.7 (57.6-63.9) [60]	0.01
* Relapsing onset	138	24.3 (22.5-26.1) [24]		55.0 (52.7-57.3) [53]	
Age at onset					
≤ 20	17	25.0 (18.9-31.0) [23]	0.54	42.6 (36.4-48.8) [40]	< 0.001
21-30	79	24.8 (22.6-27.0) [25]	0.56	50.5 (48.2-52.9) [50]	< 0.001
* > 30	114	23.1 (21.2-25.0) [22]		63.2 (61.3-65.8) [64]	
Type of symtpoms at disease onset					
Motor					
present	59	22.6 (20.1-25.3) [23]	0.18	57.7 (54.1-61.4) [57]	0.52
* absent	151	24.4 (22.7-26.1) [24]		56.7 (54.5-58.9) [57]	
Sensory					
present	93	22.8 (20.6-24.9) [22]	0.24	55.2 (52.4-57.9) [56]	0.06
* absent	117	24.8 (23.0-26.6) [26]		58.4 (55.8-61.0) [57]	
Cerebellar					
present	15	21.1 (15.3-26.9) [20]	0.44	52.2 (43.7-60.6) [56]	0.32
* absent	195	24.1 (22.7-25.5) [24]		57.3 (55.4-59.3) [57]	
Brainstem					
present	37	23.4 (19.9-26.80 [22]	0.92	55.1 (50.7-59.6) [53]	0.34
* absent	173	24.0 (22.5-25.5) [24]		57.4 (55.3-59.4) [57]	
Optic					
present	30	25.8 (21.8-29.7) [28]	0.27	54.8 (48.6-60.9) [50]	0.96
* absent	180	23.6 (22.1-25.1)[23]		57.3 (55.4-59.3) [57]	

3.3.3.3.3 Deaths NOT due to MS

Among the dead NOT due to MS, males had a shorter survival (males = 23.3 versus females = 28.1 mean years; p = 0.03) and were younger (males = 60.8 versus females = 64.2 mean years; p = 0.06) at death. In contrast to the dead due to MS, a PP course associated with a significantly shorter time to death (PP = 21.9 versus RR/SP = 28.2 mean years; p = 0.02). However, again, a younger age at onset and an initial RR course associated with a younger age at death. Relapsing onset patients died at slightly younger age (RR/SP = 61.2 versus PP = 65.7 mean years; p = 0.53), secondary to their younger age at clinical presentation (33.0 versus 41.5 mean years, p < 0.001). Those older at the disease onset reached death more rapidly (age ≤ 20 = 30.7 versus age > 30 = 23.9 mean years, p = 0.02; age 21-30 = 31.0 versus > 30 = 23.9 mean years, p = 0.06), although at significantly older age (age ≤ 20 = 48.6 versus age > 30 = 67.0 mean years, p < 0.001; age 21-30 = 55.5 versus age > 30 = 67.0 mean years, p < 0.001) (Table 3.20).

Deaths not related to MS were mainly attributed to causes of death normally observed in the general population (Table 3.18). Most of the patients died from malignancies (n = 42; 36.8%), cardiovascular diseases (n = 31; 27.1%) and cerebrovascular diseases (n = 15; 13.1%). The dead due to malignancies were mainly women (F/M ratio = 2.2), with relapsing onset disease course (74%). In this subgroup patients took longer time to die (25.7 mean years) and were older at death (62.4 mean years), compared to most of the dead due to MS. However, few (n = 8) were still in the RR phase when they died, which probably explains the relatively low level of disability (mean DSS = 6.0) at the last assessment. The primary malignancy could be determined for 37 patients (88% of dead from cancer). Lung (n = 9) and breast (n = 8) carcinomas predominated, and were followed by pancreas (n = 4), bowel (n = 3), gastric (n = 2), cervix (n = 2) cancers, Hodgkin's lymphoma (n = 2), sarcoma (n = 2), bladder (n = 1), rectum (n = 1), colon (n = 1), ovary (n = 1) cancers and leukaemia (n = 1).

Table 3.20 Factors affecting the time to death from the disease onset and from birth (age at death) among patients who died from causes NOT related to MS (n = 114). Censored information was not included in the analyses * Reference category; ** Log-Rank test.

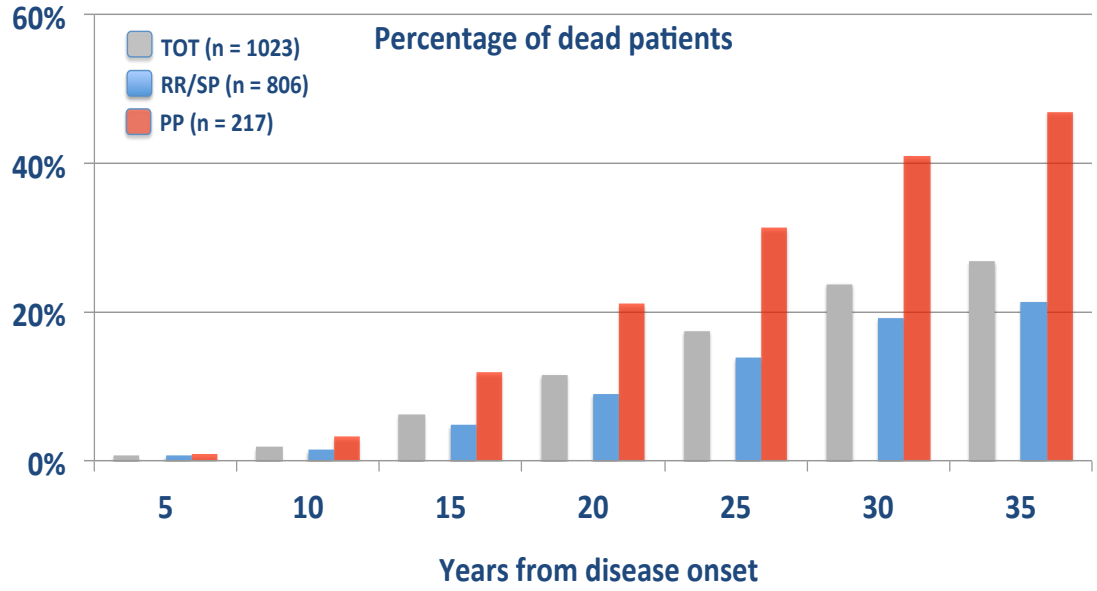
		Kaplan Meier analysis from onset		Kaplan Meier analysis from birth (age at)	
	Number	Mean (95% CI) [median] years to death	p **	Mean (95% CI) [median] years to death	p **
Deaths NOT due to MS	114	26.0 (23.8-28.2) [24]		62.7 (60.3-65.1) [64]	
Gender					
Males	49	23.3 (20.2-26.4) [24]	0.03	60.8 (57.3-64.4) [63]	0.06
* Females	63	28.1 (25.2-31.1) [24]		64.2 (61.0-67.3) [68]	
Type of disease course					
Progressive onset	38	21.9 (19.3-24.5) [22]	0.02	65.7 (62.8-68.7) [65]	0.53
* Relapsing onset	74	28.2 (25.3-31.0) [25]		61.2 (58.0-64.3) [64]	
Age at onset					
≤ 20	13	30.7 (23.5-37.9) [28]	0.02	48.6 (41.6-55.5) [47]	< 0.001
21-30	21	31.0 (24.4-37.6) [31]	0.06	55.5 (48.9-62.2) [59]	< 0.001
* > 30	78	23.9 (21.8-26.0) [23]		67.0 (65.0-69.0) [67]	
Type of symtpoms at disease onset					
Motor					
present	38	26.4 (22.4-30.3) [22]	0.77	67.9 (65.0-70.8) [68]	0.02
* absent	74	25.8 (23.3-28.4) [25]		60.0 (56.9-63.1) [62]	
Sensory					
present	42	29.5 (26.0-33.0) [28]	0.04	64.9 (62.0-67.8) [67]	0.99
* absent	70	23.9 (21.3-26.6) [23]		61.4 (58.0-64.7) [63]	
Cerebellar					
present	11	24.3 (14.9-33.8) [21]	0.91	52.0 (42.6-61.3) [53]	0.02
* absent	101	26.2 (24.0-28.4) [24]		63.9 (61.6-66.2) [65]	
Brainstem					
present	20	25.2 (19.4-31.0) [21]	0.8	61.7 (54.4-68.9) [63]	0.52
* absent	92	26.2 (23.9-28.5) [24]		62.9 (60.5-65.3) [64]	
Optic					
present	17	29.9 (25.6-34.2) [27]	0.23	60.5 (54.8-66.1) [63]	0.26
* absent	95	25.3 (22.9-27.7) [23]		63.1 (60.5-65.7) [65]	

Patients who died from cardiovascular diseases (26 from heart disease, 4 from cardiac arrest, 1 from cardiac arrhythmia) (n = 31; 27.1%) and patients who died from cerebrovascular diseases (stroke) (n = 15; 13.1%) were distinguished by the opposite F/M ratio (0.7 versus 1.5), although the age at the disease onset (37.8 versus 38.2 mean years) and the type of MS phenotype (relapsing onset course = 61% versus 60%) were alike. In addition, both subgroups took similar time to death (29.4 versus 27.7 mean years) and died at similar age (67.2 versus 65.9 mean years).

Miscellaneous causes included: accidents (n = 3), bowel infarction (n = 1), gastro-intestinal bleeding (n = 2), intestinal obstruction (n = 1) and head injury (n = 1). The few (n = 8; 7%) dead from pulmonary diseases (6 from respiratory failure, 1 from respiratory arrest and 1 from chronic bronchitis) who were deemed to have not died from MS, had spent less than 3 years bedbound (n = 4) or were never recorded (n = 4) at DSS 8. Those patients (n = 7; 6.1%), whose cause of death was unknown, stand out for their very young age at onset (26.2 mean years) and at death (42.2 mean years) and the very short time to death (16.0 mean years): two of them stayed less than 3 years bedbound and 5 were never recorded reaching DSS 8.

3.3.3.4 Survival in the total population

Figure 3.26 Proportions of patients (y-axis) in the total population (n = 1023) and in the relapsing onset (n = 806) and progressive onset (n = 217) subgroups dying at specific time points from disease onset (x-axis).



In the total population (n = 1023), the survival analysis allowed to calculate the probability of surviving and to estimate the time to death, taking into account censored information. **Twenty-five years** after onset, 17.5% (n = 180) of the total population had died, rising to 26.8% (n = 275) at **35 years** (Figure 3.26) and to 31.6% (n = 324), by the end of the observation period. The Kaplan Meier estimated mean time to death from onset was 37.5 years (median time = 38 years) and from birth (age at death) was 69.4 years (median age = 71 years). The probability of surviving over time was higher in the relapsing onset group, compared to the PP group: at **25 years** from onset, 86.2% (n = 694) of RR/SP and 68.7% (n = 149) of PP patients were still alive, decreasing to 78.6% (n = 633) and 53% (n = 115) at **35 years** (Figure 3.26) and to 73.5% (n = 593) and 48.8% (n = 106) by the end of the observation period, respectively.

Factors affecting survival in the total population were assessed (Table 3.21). The PP group, compared to relapsing onset group, took a much shorter time to death (30.3 versus 39.6 mean years; $p < 0.001$), and had a much higher risk of dying (PP versus

RR/SP HR = 1.60; p < 0.001) (Table 3.18). However, the estimated age at death was not influenced by the type of the disease course (RR/SP = 69.6 versus PP = 69.3 mean years; p = 0.86). In addition, the probability of surviving was lower among males (time to death: males = 33.0 versus females = 39.5 mean years; risk of death HR = 1.49; p < 0.001) and among those older at disease onset (time to death: age ≤ 20 = 47.2 versus age > 30 = 31.9 mean years; risk of death HR = 0.38, p < 0.001; time to death: age 21-30 = 39.6 versus age > 30 = 31.9 mean years; risk of death HR = 0.58, p < 0.001). Patients presenting with motor and cerebellar symptoms took significantly shorter time to death however, this was not confirmed by the multivariate cox regression analysis (Table 3.21).

Table 3.21 Kaplan Meier survival analysis: estimated time to death. Multiple Cox regression analysis: risk (HR) of death in total population (n = 1023) according to the concomitant effect of clinical and demographic variables. * Reference category.

		Kaplan Meier analysis		**Cox Regression multiple analysis**	
	Number (% censored)	**Mean (95% CI) [median] years to death**	**p**	**Risk of death; HR (95% CI)**	**p**
Total population	1023 (68.3%)	37.5 (35.9-39.0) [38]			
Gender					
Males	337 (59.1%)	33.0 (31.1-34.8) [33]	< 0.001	1.49 (1.19-1.87)	< 0.001
* Females	668 (72.5%)	39.5 (37.5-41.6) [40]			
Type of disease course					
Progressive onset	216 (49.1%)	30.3 (28.2-32.2) [29]	< 0.001	1.60 (1.23-2.09)	< 0.001
* Relapsing onset	789 (73.1%)	39.6 (37.7-41.5) [42]			
Age at onset					
≤ 20	152 (80.3%)	47.2 (42.4-51.9) [56]	< 0.001	0.38 (0.25-0.57)	< 0.001
21-30	403 (75.2%)	39.6 (37.5-41.7)[43]	< 0.001	0.58 (0.44-0.75)	< 0.001
* > 30	449 (57.2%)	31.9 (30.3-33.5) [31]			
Type of symtpoms at disease onset					
Motor					
present	240 (59.6%)	33.9 (31.6-36.2) [34]	0.03	1.14 (0.87-1.50)	0.33
* absent	765 (70.6%)	38.9 (36.9-41.0) [39]			
Sensory					
present	506 (73.3%)	39.6 (37.1-42.0) [43]	0.02	0.91 (0.69-1.19)	0.5
* absent	499 (62.5%)	35.5 (33.5-37.4) [34]			
Cerebellar					
present	58 (55.2%)	32.6 (27.9-37.2) [36]	0.03	1.65 (1.10-2.48)	0.15
* absent	947 (68.7%)	37.9 (36.2-39.6) [38]			
Brainstem					
present	173 (67.1%)	35.3 (32.0-38.6) [35]	0.27	1.23 (0.88-1.72)	0.2
* absent	832 (68.1%)	37.8 (36.0-39.50 [39]			
Optic					
present	183 (74.3%)	40.2 (36.9-43.4) [39]	0.01	0.90 (0.63-1.30)	0.6
* absent	822 (66.5%)	36.6 (34.8-38.3) [37]			

3.3.3.4.1 Dying from MS (the attainment of DSS 10)

Among 210 patients (20.5% of the total population), who had attained DSS 10 by the end of the observation period, 72 (33.1% of PP population) had a progressive onset and 138 (17.1% of RR/SP population) had an initial relapsing course. The number of patients dying from MS over time increased in greater percentages in the PP group (Figure 3.29). **Twenty years** after onset, within the PP group, 13.8% (n = 30) had attained DSS 10 versus only 6.3% (n = 51) of the RR/SP group. These percentages increased to 19.3% (n= 42) and 9.1% (n = 74) at **25 years**, to 26.2% (n = 57) and 13.1% (n = 106) at **30 years**, and to 29.9% (n = 65) and 14.8% (n = 120) at **35 years**, respectively (Figure 3.27).

In the total population, the Kaplan Meier estimated mean and median times for reaching DSS 10 were 44.5 (95% CI 42.2-46.8) and 45 years from the disease onset, and 74.7 (95% CI 72.9-76.5) and 78 years from birth (age at death from MS). Primary progressive patients attained DSS 10 from the disease onset in a significantly shorter time compared to relapsing onset patients (12.3 mean years difference). However, the type of the disease course did not affect the estimated age at DSS 10, which was remarkably similar in the two subgroups (PP = 73.8 versus 76.1 mean years, p = 0.63) (Figure 3.28)

Figure 3.27 Proportions of patients (y-axis) in the total population (n = 1023) and in the relapsing onset (n = 806) and progressive onset (n = 217) subgroups reaching DSS 10 at specific time points from disease onset (x-axis).

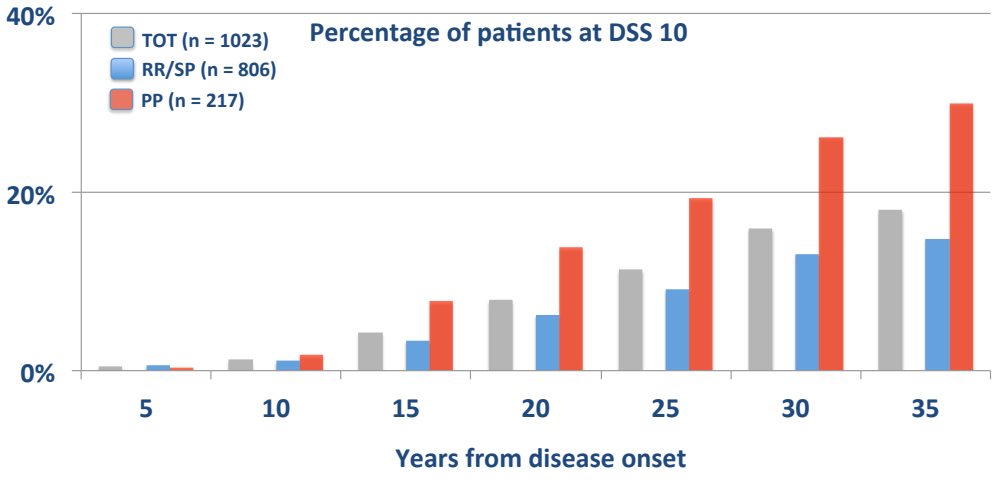
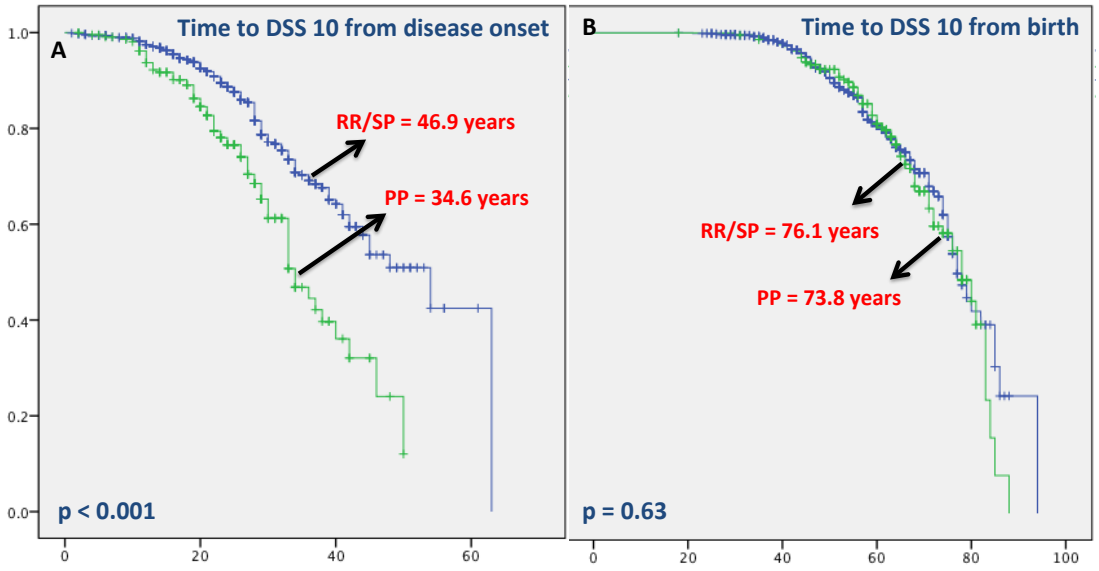


Figure 3.28 Kaplan Meier analysis: comparison of survival curves to DSS 10 from disease onset and from birth (age at DSS 10) between PP and RR/SP patients. P values were obtained with Log Rank test. Estimated mean and median times to DSS 10 are shown below the graph.



	Mean (95% CI) [median] years to DSS 10	Mean (95% CI) [median] age at DSS 10
PP MS	46.9 (44.3-9-49.5) [54]	75.7 (73.3-78.2) [77]
RR/SP MS	34.6 (32.1-37.0) [34]	73.2 (71.0-75.4) [78]

3.3.3.4.2 Factors affecting the attainment of DSS 10.

Within the total population, being male (males = 37.9 versus females = 46.3 mean years, HR = 1.48, p = 0.002) and being older at the disease onset (age ≤ 20 = 30.7 versus age > 30 = 23.9 mean years, HR 0.39, p = 0.02; age 21-30 = 31.0 versus age > 30 = 23.9 mean years, HR 0.71, p = 0.06) associated with a significantly higher risk of dying from MS and with a significantly shorter time to DSS 10 (Table 3.22). The multivariate cox regression model, assessing the effect of all variables simultaneously, confirmed these associations. The type of symptoms at the clinical presentation did not affect the time to and the risk of death due to MS (Table 3.22).

Similarly, within the relapsing onset group, males (males = 40.1 versus females = 48.3 mean years, p = 0.04) and those older at the disease onset (age ≤ 20 = 56.0 versus age > 30 = 39.5 mean years, HR 0.38, p < 0.001; age 21-30 = 44.4 versus age > 30 = 39.5 mean years, HR 0.73, p < 0.001) took significantly shorter times to die from MS. The multivariate model did not confirm the higher risk of reaching DSS 10

among males (Table 3.23). In addition, the type of symptoms at disease onset was shown not to exert any predictive effect (Table 3.23).

In the PP group, beside a modestly increased risk of death due to MS (HR 1.63, p = 0.04) among males, none of the variables analysed significantly affected the probability and the time to DSS 10 (Table 3.24).

Table 3.22 Kaplan Meier survival analysis: **t**ime to DSS 10. Multiple Cox regression analysis: risk of attaining DSS 10 from disease onset in total population (n = 1023). * Reference category.

		Kaplan Meier analysis		**Cox Regression multiple analysis**	
	Number (% censored)	**Mean (95% CI) [median] years to DSS 10**	**p**	**Risk of reaching DSS 10; HR (95% CI)**	**p**
Total population	1023(79.4%)	44.5 (42.2-46.8) [45]			
Gender					
Males	337 (73.6%)	37.9 (35.7-40.1) [41]	0.002	1.48 (1.12-1.95)	0.006
* Females	668 (81.9%)	46.3 (43.6-49.1) [50]			
Age at onset					
≤ 20	152 (88.8%)	54.9 (50.9-58.9) [63]	< 0.001	0.39 (0.19-0.58)	< 0.001
21-30	403 (80.4%)	42.9 (40.5-45.2) [48]	< 0.001	0.71 (0.52-0.95)	0.02
* > 30	449 (74.6%)	37.7 (35.5-40.0) [38]			
No of symptoms at disease onset					
1	667 (80.4%)	43.8 (41.2-46.5) [45]	0.12	0.78 (0.56-1.07)	0.13
* > 1	327 (77.1%)	44.7 (40.9-48.5) [48]			
Type of symtpoms at disease onset					
Motor					
present	240 (75.4%)	39.6 (36.6-42.6) [42]	0.1	0.99 (0.68-1.43)	0.96
* absent	765 (80.3%)	44.9 (42.2-47.5) [46]			
Sensory					
present	506 (81.6%)	46.8 (43.4-50.3) [50]	0.07	0.77 (0.54-1.10)	0.16
* absent	499 (76.6%)	41.7 (39.0-44.5) [40]			
Cerebellar					
present	58 (74.1%)	38.5 (33.6-43.3) [48]	0.27	1.23 (0.70-2.16)	0.45
* absent	947 (79.4%)	44.7 (42.3-47.1) [45]			
Brainstem					
present	173 (78.6%)	42.0 (37.1-46.9) [41]	0.38	0.99 (0.65-1.52)	0.98
* absent	832 (79.2%)	44.8 (42.2-47.4) [45]			
Optic					
present	183 (83.6%)	44.4 (41.0-47.9) [54]	0.7	0.73 (0.46-1.15)	0.18
* absent	822 (78.1%)	44.3 (41.8-46.7) [45]			

Table 3.23 Kaplan Meier survival analysis: **t**ime to DSS 10. Multiple Cox regression analysis: risk of attaining DSS 10 from disease onset. Relapsing onset (n = 806) patients. * Reference category.

		Kaplan Meier analysis		Cox Regression multiple analysis	
	Number (% censored)	Mean (95% CI) [median] years to DSS 10	p	Risk of reaching DSS 10; HR (95% CI)	p
Relapsing onset group	806 (82.8%)	46.9 (44.3-49.5) [54]			
Gender					
Males	244 (78.7%)	40.1 (37.4-42.7) [44]	0.045	1.32 (0.93-1.89)	0.11
* Females	545 (84.2%)	48.3 (45.3-51.3) [54]			
Age at onset					
≤ 20	143 (89.5%)	56.0 (52.4-59.6) [63]	< 0.001	0.38 (0.20-0.71)	0.002
21-30	364 (83.0%)	44.4 (41.9-46.8) [-]	< 0.001	0.73 (0.50-1.05)	0.091
* > 30	282 (78.4%)	39.5 (36.6-42.4) [42]			
No of symptoms at disease onset					
1	526 (83.3%)	46.0 (42.9-49.0) [54]	0.46	0.90 (0.57-1.44)	0.68
* > 1	254 (81.9%)	47.5 (43.3-51.8) [63]			
Type of symtpoms at disease onset					
Motor					
present	144 (78.5%)	42.1 (38.5-45.7) [-]	0.4	0.91 (0.54-1.53)	0.73
* absent	645 (83.4%)	46.9 (43.9-49.8) [54]			
Sensory					
present	426 (84.5%)	49.0 (45.6-52.4) [63]	0.13	1.18 (0.72-1.94)	0.49
* absent	363 (80.2%)	44.3 (41.1-47.5) [44]			
Cerebellar					
present	48 (79.2%)	41.1 (36.1-46.1) [48]	0.46	0.77 (0.37-1.56)	0.47
* absent	741 (82.7%)	47.1 (44.4-49.8) [54]			
Brainstem					
present	162 (80.9%)	44.6 (39.7-49.4) [41]	0.14	0.80 (0.46-1.40)	0.44
* absent	627 (82.9%)	47.3 (44.3-50.3) [54]			
Optic					
present	173 (85.0%)	44.7 (41.0-48.3) [54]	0.31	1.15 (0.66-2.00)	0.61
* absent	616 (81.8%)	47.4 (44.8-50.1) [63]			

Table 3.24 Kaplan Meier survival analysis: **t**ime to DSS 10. Multiple Cox regression analysis: risk of attaining DSS 10 from disease onset. Progressive onset (n = 217) patients. * Reference category.

		Kaplan Meier analysis		Cox Regression multiple analysis	
	Number (% censored)	Mean (95% CI) [median] years to DSS 10	p	Risk of reaching DSS 10; HR (95% CI)	p
Progressive onset group	217 (66.8%)	34.6 (32.1-37.0) [34]			
Gender					
Males	93 (60.2%)	32.4 (28.8-36.0) [33]	0.122	1.63 (1.00-2.64)	0.04
* Females	123 (71.5%)	36.2 (32.9-39.6) [36]			
Age at onset					
≤ 20	9 (77.8%)	43.7 (36.1-51.2) [-]	0.062	0.24 (0.05-1.12)	0.071
21-30	39 (56.4%)	33.3 (27.9-38.7) [34]	0.74	1.02 (0.55-1.89)	0.94
* > 30	167 (68.3%)	33.5 (30.9-36.1) [33]			
No of symptoms at disease onset					
1	141 (69.5%)	35.8 (32.8-38.7) [37]	0.11	0.77 (0.46-1.31)	0.34
* > 1	73 (60.3%)	31.3 (28.0-34.6) [33]			
Type of symtpoms at disease onset					
Motor					
present	96 (70.8%)	32.1 (29.4-34.8) [34]	0.78	0.92 (0.53-1.59)	0.76
* absent	120 (63.3%)	35.0 (31.8-38.1) [33]			
Sensory					
present	80 (66.2%)	36.7 (32.5-40.8) [50]	0.88	0.88 (0.50-1.53)	0.65
* absent	136 (66.9%)	34.0 (31.2-36.8) [33]			
Cerebellar					
present	10 (50.0%)	27.4 (20.9-33.9) [28]	0.2	0.76 (0.27-2.19)	0.62
* absent	206 (67.5%)	34.8 (32.2-37.3) [34]			
Brainstem					
present	11 (45.5%)	30.5 (21.3-39.6) [33]	0.42	0.72 (0.27-1.88)	0.5
* absent	205 (67.8%)	35.1 (32.5-37.6) [34]			
Optic					
present	10 (60.0%)	33.8 (22.5-45.2) [30]	0.72	1.07 (0.33-3.47)	0.89
* absent	206 (67.0%)	34.3 (31.8-36.8) [34]			

3.4 Discussion

3.4.1 The ideal natural history database

The astonishingly high number of studies carried out over the past 60 years proves the natural history of MS as probably one of the most scrutinized, among chronic diseases. Despite the huge efforts for collecting reliable information during the disease course, normally spanning over 40-50 years, none of the MS registries can be considered representative of the whole disease. Indeed, information on prognosis can be only applied to large groups of patients and the long-term outcome remains widely unpredictable at individual level.

The ideal inception cohort, whereby all information are identified at the disease onset and then collected until death, is virtually impossible to obtain. Inevitably, data are affected by several sources of bias (Table 3.1). Although all the studies have valuably contributed to our current knowledge of the natural history of the disease, only few of them were carried out with rigorous methodology. Some strategies should be implemented for minimizing bias (Weinshenker and Ebers, 1987). The accuracy of data collection can be improved when studies benefit from a geographically based survey, ascertaining all MS cases in the community and serving as comparator. This happened in Sweden (Runmarker and Andersen, 1993), Europe (Riise et al., 1988), Germany (Poser et al., 1982a) and Scotland (Phadke, 1987). In principle, ascertainment bias is generally low among cohorts from small geographic areas, where all cases in the area are referred to a single tertiary clinic and few neurologists only examine the patients. This allows a geographically based collection of data and standardized clinical assessments. Good examples are the French databases from the EDMUS network (Confavreux et al., 2003; Debouverie et al., 2008; Leray et al., 2010) and the Swedish database from Gothenburg (Runmarker and Andersen, 1993). In contrast, cohorts of patients from several clinics are advantaged by a large sample size, although they are often affected by incomplete

ascertainment. This is the case of the British Columbia database, including 5727 patients, of which 2837 were followed up for at least 15 years, but covering "only" 80% of the MS population in the area (Tremlett et al., 2006). The use of DMTs, altering the natural history of the disease, can represent an additional drawback and it varies among cohorts: 15% of patients (439 of 2837) in the British Columbia's cohort (Tremlett et al., 2006), 56.2% (1154 of 2054) in the Rennes' cohort (Leray et al., 2010), and an unspecified proportion in the Lorraine's cohort (Debouverie et al., 2008) were treated with IFN or Copaxone. Almost half (n = 804) of the Lyon database population received Azathioprine (Confavreux et al., 2003). This might have had a significant impact when assessing the relationship between inflammatory attacks and late outcomes.

Information collected retrospectively can be affected by recall bias, however very few modern natural history cohorts, such as the Lyon (Confavreux et al., 2003) and the Gothenburg databases (Runmarker and Andersen, 1993), contain only longitudinal data. The proportion of retrospective data largely varies among studies and remains one of the least detailed information provided by the authors. A separate group of patients seen from disease onset only can be used as comparator, in order to assess the potential impact of referral bias (Weinshenker and Ebers, 1987). Equally relevant is the number of lost to follow up, which tends to increase among the most disabled patients, normally not attending the clinics (Weinshenker and Ebers, 1987). This methodological aspect, along with the frequency of the assessments, is often poorly and not clearly addressed in most of the studies. The completeness of data acquisition is a cardinal feature, especially when carrying out survival analyses (Chapter 2), as it strongly affects the estimates of the disability accumulation over time. The large efforts for maintaining long follow up are voided when patients with the most severe outcome are not traced, in order to minimize the rate of drop out. For instance, in the Lyon and Rennes cohorts, despite the observation period lasted 40 (from 1957 to 1997) and 28 (from 1976 to 2004) years, respectively, the mean disease duration was only 11 and 12 years, and the proportion attaining DSS 6 was rather low (32% and 35%), suggesting a high rate of lost to follow up.

3.4.2 The London Ontario database

Among natural history cohorts, the LO database represents one of the most comprehensive and detailed collection of information on the disease course up to its latest stage. Its methodology was advantaged by two subgroups of patients, highly ascertained and with exclusively longitudinal data, which allowed internal comparisons for assessing the potential impact of ascertainment and referral biases. Data collection benefited from annual or semi-annual assessments for most of the patients, 28 years of follow up and special efforts for minimizing the rate of dropouts. In addition, none of the patients was exposed to DMTs. The database went through rigorous data quality check procedures, which further improved the accuracy of the information (Chapter 2).

3.4.2.1 The clinical and demographic features

With the advantage of the long follow up, among the 1023 total patients, diagnostic accuracy, according to Posers' criteria (Poser et al., 1983), was almost complete (97.7%). The long mean disease duration (24.2 years) in the total population reflects the low proportion of lost to follow up, occurring over 28 years of observation period. The mean age at the disease onset (30.6 years) and the clinical presentation, were in line with what observed in most of MS registries (Tables 3.3 and 3.4).

The number of women almost doubled the number of men, confirming the female prevalence in the MS population. The F/M ratio (1.96) in the LO database lays in between the lowest values (1.5) reported in Denmark and Sweden (Bronnum-Hansen et al., 2004; Runmarker and Andersen, 1993) and the highest values reported in France, Lorraine (2.6) (Debouverie et al., 2008) and in British Columbia (2.3) (Tremlett et al., 2006) (Table 3.4). The geographic area was shown to potentially influence MS females' proportions (Figure 3.1), however, it is unlikely to explain these differences, as studies from Canada and north Europe were carried out at similar latitudes. The different methodology and the variable proportion of

relapsing onset disease, more frequently affecting women, might reasonably account for this variation among cohorts. It remains possible that the larger number of females observed in the British Columbia and Lorraine studies, which started data collection more recently, is due to the increased F/M ratio over the past years (Figure 3.1).

The proportion of PP patients in the LO database (21.7%) is similar to the Rennes (Leray et al., 2010) and the European databases (Riise et al., 1992), although it is higher than the average 15% observed among most of the studies (Figure 3.2). The large female preponderance in cohorts reporting low percentages of PP MS cases might partially explain the clinical phenotype distribution: British Columbia (F/M ratio = 2.3), Lorraine (F/M ratio = 2.3) and Germany (F/M ratio = 1.9). Most likely, the completeness of the ascertainment and the recall bias drive these discrepancies. However, it remains unclear if the number of LO patients with PP course was truly above average or the estimates from other studies are less accurate. Among the LO database patients, the clinical phenotypes was redefined (Chapter 2) following the latest consensus on the definitions of the MS disease course (Lublin and Reingold, 1996). Unlike other databases, this reclassification of the clinical phenotype was extensively addressed in detail (Cottrell et al., 1999b; Kremenchutzky et al., 1999a). A primary progressive course had to be characterized by at least 1 year of continuous deterioration, with temporary plateaux allowed, during which worsening occurs, even if not clinically obvious (Cottrell et al., 1999b). Repeated questioning, over yearly assessments, about early symptoms led some patients, initially categorized as PP MS, to recall an early single exacerbation (n = 21) or multiple attacks (= 8), and therefore to be reclassified as single attack progressive (SAP) or RR MS, respectively (Cottrell et al., 1999b; Kremenchutzky et al., 1999a). Given the same long-term outcome, the progressive patients from onset with (PR MS) or without (PP MS) superimposed relapses were merged together and constitute the current PP MS group (Kremenchutzky et al., 1999a).

3.4.2.1.1 The MC and SO subgroups

The total population was compared to the geographically ascertained subgroup from Middlesex County (MC), including 90% of MS patients in the area (Hader et al., 1988), and to the subgroup exclusively seen from disease onset (SO). This allowed to assess the potential impact of ascertainment and referral biases on the LO database. The two subgroups resemble the total population in age at onset, F/M ratio, clinical presentation and diagnostic certainty (Table 3.8).

The lack of retrospective data in the SO subgroup explains the shorter mean disease duration (Table 3.8) and the lower percentages of severely disabled patients (Figure 3.15 A). In addition, among those seen from onset, the proportion of PP MS is much smaller (8.3%). This might be due to the exclusion from this subgroup of those patients presenting with progressive insidious symptoms, seeking for medical attention only years after disease onset. However, this disparity might also have resulted from recall bias, as those first seen at a later point in their disease might have forgotten early remitting symptoms (Weinshenker et al., 1989b).

3.4.2.2 The long term outcome

The survival estimates in the LO database were advantaged by the long follow up and the low rate of dropouts, allowing to observe a large percentage of patients progressing up to the latest stage of the disease. By the end of the observation period, 85.2% (n = 872), 73.7% (n = 754), 54.8% (n = 561) and 20.6% (n = 210) reached DSS 3, 6, 8 and 10, respectively (Figure 3.14 A). More than 50% of the total population was scored between DSS 8 and 10, with similar distribution in the MC subgroup (Figure 3.13 A). This is the largest proportion of patients reaching high disability levels among MS registries, although data available from other cohorts for comparison are very limited.

The analysis of the disability accumulation over time (Figure 3.14 A) highlighted the lack of linearity of the DSS (paragraph 1.6). The average time in between disability

levels was unevenly distributed, with the highest peak (8.1 mean years) in between DSS 0 and 3, and the lowest peak (5.4 mean years) in between DSS 3 and 6. The disability accumulated proportionally with the disease duration but at different rates: at 15 years from onset more than 30% of patients had not reached moderate disability (DSS 3), half (50%) had required walking stick (DSS 6) and 20% had already become bedbound (Figure 3.15 A). The Kaplan Meier estimated median times to DSS 3, DSS 6, DSS 8 and DSS 10 were 7, 15, 26 and 46 years, respectively. The MC and SO subgroups attained disability endpoints in remarkably similar times, compared to the total population, reassuringly demonstrating that only little bias affected the estimates of the disability accumulation (Figure 3.16).

However, among MS registries, the LO population stands out for its faster disease progression. The median time to DSS 6 (15 years) was slightly shorter than the average estimate (18-20 years) from the other cohorts (Figure 3.3). This could be due to the larger proportion of PP MS cases in the LO database. Nevertheless, it seems improbable as progressive onset patients attained disability endpoints (median time to DSS 6 = 8 years) (Figure 3.20) in similar times, compared to other MS populations (Figure 3.5). On the contrary, LO relapsing onset patients had the fastest disease progression (median time to DSS 6 = 18 years) (Figure 3.17), among cohorts (Figure 3.5). Most likely, the variable proportion of censored information accounts for these differences. The Kaplan Meier estimated times to disability endpoints shorten with the increasing number of patients attaining the endpoint. Indeed, in the Lorraine, Rennes and Lyon databases the percentages of censored data, when estimating the time to DSS 6, were 72%, 65% and 68%, respectively (Figure 3.3), much higher than the LO database (26.2%). It can be speculated that, among LO patients, the more severe outcome was driven by genetic and environmental factors. However, this would not explain why there was such a large different outcome between MS patients from LO and from the neighbour area of British Columbia, where MS patients experienced an unusually slow disease evolution (median time to DSS 6 = 27.9 years) (Tremlett et al., 2006) (Figure 3.3).

3.4.2.3 The relapsing onset and the progressive onset patients

The analysis confirmed the well-known clinical and demographic differences between relapsing onset and progressive onset patients (Table 3.13). As seen in other large cohorts from Lyon (Confavreux and Vukusic, 2006a), Rennes (Leray et al., 2010) and British Columbia (Tremlett et al., 2005), men were more likely to experience a progressive course at disease onset. The F/M ratio in the LO PP MS group (1.3) was close to unity, in contrast to the RR group, which was featured by a large female preponderance (2.1). In addition, the two groups were distinguished by strikingly different age at onset, with PP MS staring on average 10 years later than RR MS. However, primary and secondary progressive patients started to progress at remarkably similar age (Table 3.13), confirming that the type of the disease course does not affect the age at the onset of progression (Table 3.6). In line with other studies, the presenting clinical pattern varied according to the disease phenotype. Motor symptoms at onset were observed in most of the PP cases, sensory and visual disturbances were more likely to occur at presentation of the relapsing disease (Table 3.10).

The disability accumulated more rapidly in the progressive onset group: 20 years after disease onset half of PP patients became bed bound (Figure 3.20 A) and half of RR/SP patients required walking assistance (Figure 3.17 A). However, in both groups the clinical outcome largely varied, further confirming the unpredictability of the prognosis at individual level, regardless of the clinical phenotype. The time to DSS 6 widely spanned from 1 to 49 years among progressive onset patients (25 percentiles ≤ 4 years, 75 percentiles > 13 years) (Figure 3.19) and from 1 to 44 years among relapsing onset patients (25 percentiles ≤ 8 years, 75 percentiles > 19 years) (Figure 3.18).

The proportion reaching disability levels increased with the disease duration, and was larger among PP patients. More than 60% of the PP population had already reached DSS 6 at 10 years from disease onset, increasing to 77% at 15 years; 50% had become bedbound (DSS 8), at 20 years (Figure 3.20 A). In contrast, among

relapsing onset patients, at 15 years, 38% and 16% reached DSS 6 and DSS 8, respectively. These percentages increased to 50% and to 25% at 20 years (Figure 3.17 A). By the end of the observation period, the vast majority (79%) of PP patients and almost half (48%) of relapsing onset patients were distributed between DSS 8 and 10 (Figure 3.13 B).

3.4.2.4 The amnestic nature of the disease course.

Among natural history studies, there is large agreement that PP course represents the strongest negative prognostic factor (Figure 3.5). The Kaplan Meier analysis in the LO population confirmed that PP patients took significantly shorter times from the disease onset to disability endpoints, both when compared to relapsing onset (RR/SP) and to SP patients (Figures 3.21 and 3.22). However, controversy remains on whether PP MS is a separate clinical entity (Confavreux and Vukusic, 2006a) and whether, among all MS subtypes, the progressive phase is driven by common mechanisms (Kremenchutzky et al., 2006a; Lassmann, 2010). In the Lyon (Confavreux and Vukusic, 2006a), the Rennes (Leray et al., 2010) and the Lorraine (Debouverie et al., 2008) databases the disease evolution from moderate disability was not influenced by the clinical phenotype. Times from DSS 3 and from DSS 4 to higher disability levels were similar between PP and RR/SP patients. In addition, previous analysis of the LO database demonstrated that the rate of the disability accumulation was homogeneous among progressive subtypes. Those with one (SAP MS), many (SP MS) and no (PP MS) relapses before the onset of progression started to progress at similar age and attained DSS 6, 8 and 10, from the onset of progression, in remarkably similar times (Kremenchutzky et al., 2006a).

The analyses in this study confirmed that progressive and relapsing onset patients share much more than they differ and supported a unifying concept of the disease course (Confavreux and Vukusic, 2006a). Once established disability occurred, further disease progression was only marginally influenced by the type of the disease course. With the exception of the time from DSS 0 to DSS 3, the two subgroups took similar average times to advance from one DSS landmark to the

following (Figure 3.14 B). In addition, the estimated Kaplan Meier times from DSS 3 to DSS 6 and to DSS 8 were only 3 mean years shorter in the PP group versus the relapsing onset group (Figure 3.21), and were almost equal when compared to the SP group (Figure 3.22). The narrower differences between PP and SP MS reflect the larger number of patients attaining the endpoints, following the exclusion of RR patients from the analysis.

In the LO database more than 70% of RR patients converted to SP MS when they were scored at ≤ DSS 3 (Chapter 4), implying that the disease progression from moderate disability heralded the SP phase. Therefore, the similar times from DSS 3 to the endpoints, between RR/SP and PP patients, further proved that the progressive phase is largely unaffected by the type of the initial disease course. It remains unclear and hard to explain why the progressive disease can be preceded or not by the relapsing remitting phase. However, these data, in agreement with previous analyses from the Rennes database (Leray et al., 2010), highlighted DSS 3 as the hallmark status, separating two disease stages apparently independent of each other. Once established disability occurs (DSS 3 or 4), further disability accumulation becomes a self-perpetuating process, amnesic to the previous clinical history (Confavreux and Vukusic, 2006a; Kremenchutzky et al., 2006a; Leray et al., 2010). It is reasonable to conclude that the outcome is largely determined before the attainment of moderate disability. Therefore the most relevant window of therapeutic opportunity probably terminates, once the cascade of events leading to the permanent damage occurs. In addition, the similar disease evolution among all MS progressive subtypes suggests common mechanisms driving the accumulation of severe disability and question the contribution of the inflammatory attacks, featuring the RR phase, to the evolution of the progressive phase. This will be more extensively addressed in the following chapter.

3.4.3 Mortality in MS

Mortality in MS has been largely addressed by several epidemiological studies, most of them advantaged by a very large sample, long follow up (Table 3.7) and the possibility of comparing data with the general population (Figure 3.6). The survival estimates are difficult to compare because of methodological differences and the variable incidence of MS among countries. The LO database, unlike other datasets, offered the possibility of addressing the mortality in a well ascertained MS population, with detailed clinical assessments up to the latest disease stages before death for most of the patients. However, the lack of data on mortality in the general population, at the time the study was undertaken, did not allow assessing survival more sophisticatedly, by calculating SMRs.

3.4.3.1 Dead in the LO database

Following extensive efforts for tracing all patients lost to follow up and for ascertaining their cause of death, 324 dead were identified, representing 31.6% of the total population. Similar percentage (33.1%) of dead patients was observed in the MC subgroup, supporting the accuracy of the estimate.

Among the 324 dead patients, the median time to death was 24 years and the median age at death was 60 years, however survival largely varied. The quickest 25% died within 17 years and the slowest 25% in more than 30 years. Comparison with the MC group showed no significantly different time to death (Figure 3.23), demonstrating that minimal ascertainment bias affected data on mortality. By the end of the observation period, 51% of the progressive onset group and 26% of the relapsing group had died, confirming that PP course associated with a worse outcome. However, among all dead, the type of disease course only marginally affected the time to death. Although they died in larger proportion, progressive onset patients attained death only 3 years earlier than relapsing onset patients (22.7 versus 25.7 mean years, $p = 0.005$) (Table 3.15). Indeed, relapsing onset patients had

worse outcome, as they died younger (6 median years difference), secondary to their younger age at the disease onset (9 median years difference) (Table 3.15).

3.4.3.2 Causes of death

Among MS patients, the severe disability is not necessarily the immediate cause of death. The definition of death due to MS (DSS 10), according to Kurtzke (Kurtzke, 1983), remains somehow ambiguous and can be variably interpreted. The problem of defining causes of death directly attributed to MS has never been addressed in details. Indeed, most of the studies relied exclusively on information from the death certificates, without correlating it with the level of disability reached by the patient before dying.

In the LO database information on causes of death were available for the majority (96%) of dead patients. Deaths from aspiration, cachexia, dehydration, MS, pneumonia, pulmonary oedema, pulmonary embolism, septicemia and suicide were considered attributable to MS (Cottrell et al., 1999b). For those patients (n = 44) who died from respiratory disease (n = 17), cardiac dysfunctions (n = 14) or unknown causes (n= 13), attributing the death to MS was particularly challenging and, to reduce the ambiguity, a composite criterion was applied. This was primarily based on the attainment of DSS 8, as death due to MS is normally a consequence of the chronic bedridden state (Kurtzke, 1983), and was supported by pathophysiological considerations. Respiratory muscle weakness, bulbar function impairment and abnormalities of breathing control represent the respiratory problems most commonly observed in the MS population (Carter et al., 1950; Gosselink et al., 1999). Both inspiratory and expiratory activities and the general pulmonary function were found to be significantly decreased even among MS patients still fully ambulatory, during the early stage of the disease (Mutluay et al., 2005). More importantly, the degree of the respiratory impairment was found to correlate strongly with the DSS score, and to increase proportionally with the level of disability (Gosselink et al., 2000; Mutluay et al., 2005). The bedridden status (DSS 8), which exposes patients to respiratory infections and aspiration pneumonia, plays an important role in the

worsening of the pre-existing respiratory dysfunctions (Gosselink et al., 2000). Similarly, MS patients can loose the parasympathetic and sympathetic control of the cardiovascular system, secondary to brainstem demyelinating lesions (Acevedo et al., 2000; Vita et al., 1993). In addition, the degree of cardiac dysfunctions correlates with the EDSS score, and becomes more severe among the most disabled (Acevedo et al., 2000; Sterman et al., 1985). According to these data, in agreement with my supervisor, I considered a chronic bedridden state sustained for at least 3 years a sufficient criterion for assuming that the respiratory and cardiac dysfunctions, which led to patients' death, were a direct consequence of MS related disability. The same assumption was made for the dead from unknown causes. However, the choice of at least 3 years time frame was purely arbitrary, and it remains possible that, for some patients, being bedridden for such a short time did not actually directly lead to death. The composite criterion was applied to 44 dead patients: 26 patients were deemed to have died from MS and, among these, vast majority had spent much longer than 3 years bedbound (the time varied from 3 to 23 years). For 18 patients DSS 8 was never recorded or was sustained for less than 3 years.

This led to a final estimate, among the total 324 dead, of 210 (64.8%) dead from MS (Table 3.16), representing one of the highest percentages compared to other studies (Figure 3.8). Indeed, the proportion of deaths due to MS ranged widely among cohorts. The differences among recorded causes of death can be attributed to methodological differences, but there are other potential factors. The assessment of causes of death, among MS patients, is inevitably incomplete and rarely confirmed by autopsy. The variable accuracy of the ascertainment depends on the possibility of accessing to death certificates or to death registries and on the quality of the information. Deaths may lack clear documentation as they often occur outside hospital settings. In addition, the interpretation of "death related to MS" can be ambiguous. Therefore, the distribution of causes of death is strongly influenced by the coding practice (Smestad et al., 2009). All these factors are particularly relevant when information on the cause of death is sought years after the last clinical assessment. In the LO database, most of the dead were followed up to the latest clinical stages (median DSS at last assessment = 8), reflecting the low rate of

dropouts (Figure 3.25). Compared to other cohorts, the detailed clinical information probably allowed estimating more reliably the proportion of dead due to MS (Table 3.18).

For the majority of dead due to MS primary causes of death were respiratory diseases (including 10 patients who died from respiratory failure/arrest and had been bedbound for at least 3 years), MS and infections. The 3 subgroups had similar baseline features and similar survival (Table 3.18). Dead from cardiovascular causes, deemed to be MS related (n = 10), took significantly longer time to die and were older at death, compared to the other dead due to MS (Table 3.18). This was expected as the risk of cardiovascular diseases increases with age. Among dead not due to MS, cancers, cardiovascular diseases and cerebrovascular diseases were the 3 most common causes of death (Table 3.18). It is worth noticing that, those who died from unknown causes (n = 7) and did not fulfil the composite criterion, had the shortest survival (16 mean years). This might indicate that their death was attributable to MS, as they experienced a particularly aggressive disease course, with rapid accumulation of disability. Alternatively this small subgroup died from accidental causes or from rapid illness.

The comparative analysis revealed differences and similarities between the dead due to MS or not (Table 3.17). Baseline features were similar and the clinical phenotype was equally distributed (65% of RR/SP MS in both subgroups), demonstrating that the type of the disease course did not influence the probability of dying from causes related to MS. In addition, although those who died from MS had a slightly faster attainment of DSS levels, the two subgroups took similar times to die (23 versus 24 median years, p = 0.07) (Table 3.17), and had accumulated similar disability (median DSS = 8) before death (Table 3.18). This was unexpected and contradicted the notion that the dead due to MS die more rapidly and are more disabled at death. However, the two groups differed by the age at disease onset and at death. Therefore, the dead due to MS, compared to the dead not due to MS, were significantly younger at clinical presentation (33.0 versus 36.7 mean years, p = 0.03), took similar time to death, and consequently died at significantly younger age (57.0 versus 62.7 mean

years, $p < 0.001$) (Table 3.17). In contrast, patients older at clinical presentation reached older age and, therefore, were exposed to a higher risk of pathological conditions such as malignancies, cardiovascular and cerebrovascular diseases.

This indicated that the probability of dying from MS related causes was mainly influenced by the age at the disease onset, which affected the age at death, and not by the severity of the disease course. Indeed, the binary logistic regression analysis confirmed that being older at first symptom modestly but significantly decreased the probability of dying from MS (OR = 0.96; $p = 0.004$). There was no indication that those who died from MS accumulated more severe disability and had a worse outcome, except for a slightly faster disease evolution. These data question the validity of differentiating whether death is directly related to MS or not, and the utility of using DSS 10 as landmark status.

3.4.3.2.1 Suicide

Six patients (1.8% of dead) committed suicide. These were the youngest at clinical onset and at death and had the shortest survival (Table 3.18). The risk of suicide among MS patients has been reported to be increased, compared to the general population: SMR = 2.3 (Fredrikson et al., 2003), SMR = 2.1 (Bronnum-Hansen et al., 2005), SMR = 1.62 (Koch-Henriksen et al., 1998), 7.5x (Sadovnick et al., 1991b). However, assessing its prevalence is particularly challenging, and widely varying incidence rates are found in the literature (Table 3.7), exemplifying cultural biases existing in different populations: 28.6% (Sadovnick et al., 1991b), 5% (Sumelahti et al., 2002), 5.3% (Smestad et al., 2009), 4.5% (Bronnum-Hansen et al., 2004), 3.8% (Koch-Henriksen et al., 1998), 2.5% (Grytten Torkildsen et al., 2008). Part of this variation results from the referral bias, due to the fact that clinic-based cohorts tend to include more severely affected individuals, with higher rates of affective disorders (Hirst et al., 2008b). It is also plausible that the cause of death recorded on the death certificate may be influenced by cultural taboos such as the right to be buried in certain cemeteries (cultural bias), making suicide underreported to avoid social stigma. In Finland, where the suicide rates in the general population are very high,

suicides are not typically disguised or recorded as 'accidents', and there are no obvious cultural or religious factors that would lead to under-reporting (Sumelahti et al., 2010). Indeed, the SMR for suicides (1.7) in Finnish MS patients was not significantly increased compared to the general population (Sumelahti et al., 2010). On the contrary, substantially higher (7.5x) incidence is seen in longitudinally followed patients from Canada where suicide tends to be underreported (Sadovnick et al., 1991b). There is no reasonable doubt that suicide is substantially increased in MS and it should be included as an "MS-related cause of death", unless there is a case-specific reason to think otherwise.

3.4.3.2.2 Malignancies

Those who died from cancer represented 12.9% of dead and were mainly women with relapsing onset disease course. The subgroup was distinguished by a more benign disease evolution (8 patients were still in the RR phase when they died) and less disability at death (median DSS = 7). Compared to the dead due to MS, they were older at death (age at death = 62.4 mean years), secondary to their older age at onset (38 median years), and were therefore exposed to a higher risk of malignancy.

The incidence of cancer in MS largely varies among studies. One of the highest proportion of deaths related to cancer, among MS patients, was reported in Finland (35%) (Sumelahti et al., 2002), compared to cohorts from Denmark (8.6% and 10.1%) (Bronnum-Hansen et al., 2004; Koch-Henriksen et al., 1998), Wales (9.5%) (Hirst et al., 2008b) and Norway (9.8% and 10.6%) (Grytten Torkildsen et al., 2008; Smestad et al., 2009). Indeed, there seems to be contradicting data on the frequency of cancer among MS patients. A lower incidence, compared to the matched general population, was observed in Denmark (SMR = 0.85 and 0.79) (Bronnum-Hansen et al., 2004; Koch-Henriksen et al., 1998) and in Canada (0.67x) (Sadovnick et al., 1991b). In contrast, a higher incidence was observed in Finland (35% vs 20%) (Sumelahti et al., 2002) and in Norway (SMR = 2.25) (Grytten Torkildsen et al., 2008). Other studies, aimed at addressing this specific question, could not find cohering

results, demonstrating similar (Fois et al., 2010; Nielsen et al., 2006) or a lower risk (Achiron et al., 2005; Bahmanyar et al., 2009) of cancer, among MS patients. A recent meta-analysis pooled data from several cohorts, gathering information from more than 45000 patients, and reported a significantly decreased risk (OR = 0.92; p = 0.004) of all cancers in the MS population, relative to controls (Handel and Ramagopalan, 2010). The authors concluded that this could be due to earlier death among MS patients, since the risk of cancer rises with age, further supporting data presented in this thesis.

3.4.3.2.3 Cardiovascular and cerebrovascular diseases

Those who died from cardiovascular and cerebrovascular diseases represented 9.5% and 4.6% of the dead population, respectively. Patients in both subgroups were the oldest at death (69 and 68 median years, respectively) (Table 3.18), therefore exposed to a higher incidence of vascular risk factors. The F/M ratio resembles the general population. As expected, among dead from cardiac diseases, men predominated. The mortality from heart diseases is markedly higher among men, secondary to a higher incidence of cardiovascular risk factors (Jousilahti et al., 1999). In contrast, among dead from cerebrovascular accidents, there was a preponderance of women. Interestingly, although the incidence of cerebrovascular disease is higher among men (Prencipe et al., 1997) women have been reported to be older when experiencing a stroke (Roquer et al., 2003). This might explain why, among patients who died at similar age, there was a larger percentage of women suffering from cerebrovascular events.

The incidence of cardiovascular diseases, observed in the LO database, is lower than what reported in other MS populations. However, deaths due to cardiovascular disease depend heavily on the age distribution of those at risk, and on lifestyle factors. Cardiac problems were responsible for 14.4% (Smestad et al., 2009) and 13.1% (Grytten Torkildsen et al., 2008) of mortality cases in two Norwegian MS cohorts, but for as many as 26% in a Finnish MS population (Sumelahti et al., 2002) and 18.9% in Scotland (Phadke, 1987). Deaths due to cerebrovascular disease are

underreported among studies. These accounted for 5.3% of cases in US (Redelings et al., 2006), however no other data are available for comparison.

3.4.3.2.4 Respiratory diseases

Respiratory diseases not attributed to MS related disability accounted for a low percentage of deaths (2.4% of dead). This is line with results from other studies where the incidence ranged from 1.5% among Norwegian patients (Smestad et al., 2009) to 4.7% in Danish (Bronnum-Hansen et al., 2004) and 5.4% in Finnish (Sumelahti et al., 2010) patients, respectively. In striking contrast with the general trend, 47.5% of deaths in Welsh patients were attributed to respiratory diseases (infections included) (Hirst et al., 2008b).

3.4.3.3 Factors affecting the time to death and the age at death

Factors affecting survival were assessed among dead patients only (not including censored information). Interestingly, among all dead, progressive onset and relapsing onset patients died in similar time (22.7 versus 25.7 mean years; p = 0.005) (Table 3.15), indicating that the time to death was largely unaffected by the clinical phenotype. Same results were observed when the analysis was limited to the dead due to MS (PP group = 23.1 versus RR/SP group = 24.3 mean years, p = 0.36) (Table 3.19). In addition, in both analyses, relapsing onset patients, compared to progressive onset patients, had worse outcome as they died at younger age (Tables 3.15 and 3.19). In striking contrast, among dead not due to MS, the time to death was much shorter in the PP group, compared to the RR/SP group (21.9 versus 28.2 mean years, p = 0.02) (Table 3.20). This was explained by the different survival, in each clinical phenotype group, between dead due to MS or not. In the relapsing onset group, patients who died from causes unrelated to MS had a longer time to death (28.2 mean years), compared to patients who died from MS (24.3 mean years). This was probably accounted for by the dead due to malignancies, cardiovascular and cerebrovascular diseases, who had long disease duration and

large proportion of RR cases. On the contrary, PP dead from MS and from unrelated causes had similar survival (23.1 versus 21.9 mean years).

In addition, males compared to females, took slightly shorter time to die and were younger at death, in both groups of dead due to MS or not (Tables 3.19 and 3.20).

3.4.3.4 Survival analysis

The Kaplan Meier analysis allowed estimating the probability of surviving among all MS patients. The survival probability reduced proportionally with the disease duration, from 82.5% of patients still alive at 25 years from onset, to 68.4% 10 years later (Figure 3.26). The number of patients dying over time was larger in the PP group, compared to relapsing onset group, reflecting their faster disease progression. At 35 years from the disease onset only half (53%) of PP patients and majority (78.6%) of RR/SP patients were still alive (Figure 3.26).

Among the total population (n = 1023 patients) the estimated mean survival time was 37.5 years from disease onset and 69.4 years from birth (age at death), which cohere with results from other large registries (Tables 3.8 and 3.9). Patients with a PP course had an increased risk of death (HR = 1.60; $p < 0.001$) and took on average 10 years less to die, compared to the relapsing onset group (30.3 versus 39.6 mean years). However, age at death was remarkably similar in the two subgroups (RR/SP = 69.6 versus PP = 69.3 mean years; $p = 0.86$) and therefore not influenced by the type of disease course, as already reported in other studies (Kingwell et al., 2012; Smestad et al., 2009). In addition, the probability of surviving was lower among males and among those older at disease onset (Table 3.21), confirming the trend observed among dead patients without including censored information.

3.4.3.4.1 The attainment of DSS 10

The analysis restricted to patients reaching DSS 10 (dead due to MS) yielded similar results. By the end of the observation period, among the total population, 20.5% had

attained DSS 10 in an estimated mean time from onset of 44 years and at an estimated mean age of 74 years. The proportion of dead from MS was larger in the progressive onset group (33.1% of PP population) than in the relapsing onset group (17.1% of RR/SP population). In line with previous studies (Grytten Torkildsen et al., 2008; Hader, 2010; Kantarci et al., 1998; Kingwell et al., 2012; Leray et al., 2007; Midgard et al., 1995; Phadke, 1987; Smestad et al., 2009; Sumelahti et al., 2002), the analysis confirmed that PP patients took significantly shorter time from onset to death (12 mean years difference), however the estimated age at death was not affected by the type of disease course (PP = 73.8 versus RR/SP = 76.1 mean years, p = 0.63) (Figure 3.30). The type of symptoms at clinical presentation did not affect the time to and the risk of death due to MS (Table 3.19). These associations were also seen among the relapsing onset patients (Table 3.20). In contrast, among PP patient none of the clinical and demographic baseline variables affected the probability of surviving (Table 3.21).

3.4.4 Conclusions

The clinical and demographic features of the LO database population resemble the other MS cohorts. Survival estimates were advantaged by the large number of patients reaching the disability levels analysed. It can be reliably concluded that half of relapsing onset patients and half of progressive onset patients are expected to require a walking aid by 18 and 8 years from onset, respectively. The slightly faster disease progression in the LO database, compared to other cohorts, is most likely explained by the smaller proportion of censored information, shortening the survival estimates. The comparison with the highly ascertained subgroup from Middlesex County and with the subgroup seen from onset demonstrated that data are affected by little bias.

The results from the comparative analyses, between relapsing onset and progressive onset patients, lent support to the amnestic nature of disease evolution and highlighted the attainment of moderate disability (DSS 3), heralding for most of the

patients the SP phase, as the clinical watershed separating two independent stages. Once established disability occurred, disease evolution was similar between relapsing onset and progressive onset groups and therefore largely unaffected by type of disease course, implying common mechanisms driving progression among patients. The outcome is largely determined before entering this common pathway, leading to the accumulation of severe disability.

The analysis of mortality confirmed that life expectancy in the MS population is slightly shortened. Half of the patients were dead by the age of 71. Among those who died from MS related causes, 50% were dead within 45 years from onset and by the age of 78. The PP course associated with a higher risk of death and with shorter survival however, the type of the disease course did not affect the estimated age at death. This was observed both when analysing all dead patients or only those who died from MS. The comparative analysis demonstrated that survival is not influenced by causes of death. The probability of dying from MS related causes is primarily driven by the age at the disease onset, affecting the age at death. These data question the validity of differentiating whether the death resulted from the disability related to MS or not, and of using EDSS 10 as landmark status.

Chapter 4

Relapses and the long-term disability accumulation

4.1 Introduction

Relapses are the most florid clinical feature of the RR phase and they can produce temporary or even permanent disability (Hirst et al., 2008a; Lublin et al., 2003). Typical inflammatory attacks in appropriate anatomic locations are sufficient for experienced clinicians to diagnose MS with high reliability. However, their relationship with the progressive unremitting disability, which is the heart of the medical, social and economic impact of the disease, remains largely unclear. This represents one of the most controversial aspects of the disease (Edan, 2012; Hutchinson, 2011; Lublin, 2011) and it is highly relevant to the clinical practice, to the patients' therapeutic management, and to the RCTs design. The relapse number is used for monitoring the disease activity (Cohen et al., 2004) and as indicator of the response to treatments (Sormani et al., 2012). In addition, the ARR is the most common primary clinical endpoint in RCTs (paragraph 1.7, Chapter 1) and it is considered a valid surrogate marker for the short-term disease evolution (Sormani et al., 2011).

Among relapsing onset patients, the attainment of the SP phase and the accumulation of long-term disability might result from serial, cumulative exacerbations over time, each contributing to the permanent deficit. However, evidence from radiological, pathological and clinical studies, on the relationship between relapses and late outcomes, remains ambiguous. The baseline MRI inflammatory lesions load, which is a surrogate of the relapses frequency, was shown to predict the conversion from CIS to clinically defined MS, but to correlate poorly with the long term disease evolution (2008; Calabrese et al., 2013; Fisniku et al., 2008a). Mechanisms determining the axonal loss, which is considered the essential cause of the permanent disability (Trapp and Nave, 2008), are unclear (Dutta and Trapp, 2011; Lassmann et al., 2012). The axonal damage is known to occur in the early stage of the disease, within active demyelinating lesions (Kuhlmann et al., 2002; Trapp et al., 1998), but it was also found in the NAWM (Bitsch et al., 2000; Bjartmar et al., 2001; DeLuca et al., 2006; Evangelou et al., 2000;

Frischer et al., 2009; Trapp et al., 1998). It has been suggested that the inflammation is the essential driver of the neurodegenerative process, up to the latest stages of the disease (Frischer et al., 2009). Nevertheless, the inflammatory lesions load in the spinal cord, was found to correlate poorly with the extent of the axonal damage (DeLuca et al., 2006; Lovas et al., 2000). Indeed, DMTs seem to have only limited effect on the disability accumulation during the progressive phase (paragraph 1.7, Chapter 1). This suggests that biological mechanisms underlying acute attacks might be different from those driving the disease evolution (Chaudhuri and Behan, 2004; Trapp and Nave, 2008).

Natural history studies offer the possibility to better define the role of the inflammatory attacks on the development of severe disability. This allows to test the validity of prognostic models, predicting late disability based on the number of relapses, to assess the reliability of the attacks frequency as indicator of the disease activity and to elucidate the complex interaction between mechanisms underlying the inflammatory attacks and those driving the disease progression.

4.1.1 The natural history of relapses

4.1.1.1 The type of symptoms

Inflammatory attacks can virtually present with any kind of neurological disturbance. The clinical features of the onset attack have been widely described (Table 3.2, Chapter 3) and are known to influence the long-term disease evolution. Analyses from Lyon, Lorraine, British Columbia, and Gothenburg cohorts demonstrated a faster disease progression, among patients with multiple symptoms and with motor involvement at clinical onset, and better prognosis, among patients presenting with optic neuritis (Confavreux et al., 2003; Debouverie et al., 2008; Eriksson et al., 2003; Tremlett et al., 2006).

4.1.1.2 The recovery from relapses

The percentage of patients experiencing residual deficit from the onset attack varies among databases, probably due to the different definitions of incomplete remission (Table 4.1). A partial recovery from the first attack was shown to associate with a significantly shorter times from the disease onset to EDSS/DSS levels (Table 4.1), but not to affect the disease progression from established disability (DSS3 or DSS 4) (Confavreux et al., 2003; Debouverie et al., 2008; Leray et al., 2010). In addition, in the LO database, the time to SP was similar, among SAP patients with full, partial and no recovery from the first attack (Kremenchutzky et al., 2006a) (Table 4.1).

Table 4.1 The percentage of patients with different degree of recovery from the onset attack, and the predictive effect of incomplete recovery from the first relapse: data from MS registries. * Single attack progressive (SAP) patients only.

Database	Remission from 1st attack	Effect on disease evolution
Lyon	**Complete** (≤ EDSS 2) = 82%	
	Incomplete (at least minimal neurological symptoms,≥ EDSS 3) = 18%	Shorter times to DSS 4, 6, 7
Rennes	**Complete** (≤ EDSS 2) = 82%	
	Incomplete (at least minimal neurological symptoms,≥ EDSS 3) = 18%	Shorter times to DSS 3, 6
Lorraine	**Complete** (≤ EDSS 1) = 84%	
	Incomplete (at least minimal neurological symptoms,≥ EDSS 2) = 16%	Shorter times to SP and to DSS 3, 4, 6
Gotenborg	**Complete** (absence of any new additional symptoms) = 68%	
	Partial (20% improvement) = 32%	Shorter times to SP and to DSS 7
London Onatrio*	**Full** = 65%	
	Partial (funcion abnormal) = 28%	
	Absent = 7%	No effect on time to SP

The degree of recovery from inflammatory attacks after the clinical onset and its contribution to the development of permanent disability was assessed by only few

studies, which were limited by their relatively short duration. Lublin et al. examined 224 patients from placebo arms, with a mean EDSS score of 2.5, and reported, in 42% of cases, a residual deficit (0.5 or ≥ 1 EDSS point increase), occurring after a mean time of 64 days from exacerbations (Lublin et al., 2003). Similarly, Hirst et al. described, among 182 patients (mean EDSS score = 3.7), attending an MS clinic and experiencing 279 relapses, in 49.4% a residual increase of ≥ 0.5 EDSS point, 127 mean days after an inflammatory attack (Hirst et al., 2008a).

However, these results could not be replicated in a separate study assessing patients from 2 placebo arms (n = 256 and n = 320 both with mean EDSS of 2.8) (Young et al., 2006). The analysis showed no consistent effect of relapses, after 200 days, on the development of sustained EDSS score (Young et al., 2006). In addition, among 1078 patients, experiencing 2578 relapses over a period of 15 years, only 7 attacks were reported to cause permanent severe disability (EDSS 6 or more, sustained for at least 6 months) (Bejaoui and Rolak, 2010).

4.1.1.3 The frequency of relapses

The frequency of relapses varies among MS cohorts. The recall bias most likely explains the lower estimates reported by retrospective studies (Leibowitz et al., 1964; Mc and Compston, 1952; Myhr et al., 2001; Panelius, 1969), compared to longitudinal studies (Confavreux et al., 1980; Fog and Linnemann, 1970; Goodkin and Hertsgaard, 1989; Patzold and Pocklington, 1982) (Figure 4.1). The number of attacks is known to decrease over time (Mc and Compston, 1952; Patzold and Pocklington, 1982; Tremlett et al., 2008b), partially due to the regression to the mean (Martinez-Yelamos et al., 2006). This is the tendency for a group to return to the average, rather than sustaining an increase above average (Goodin, 2004; Weinshenker et al., 1996). In the British Columbia database the annualized relapse rate reduced by 17% every 5 years of disease duration (Figure 4.2 A).

Figure 4.1 Relapse rate reported by MS registries**.**

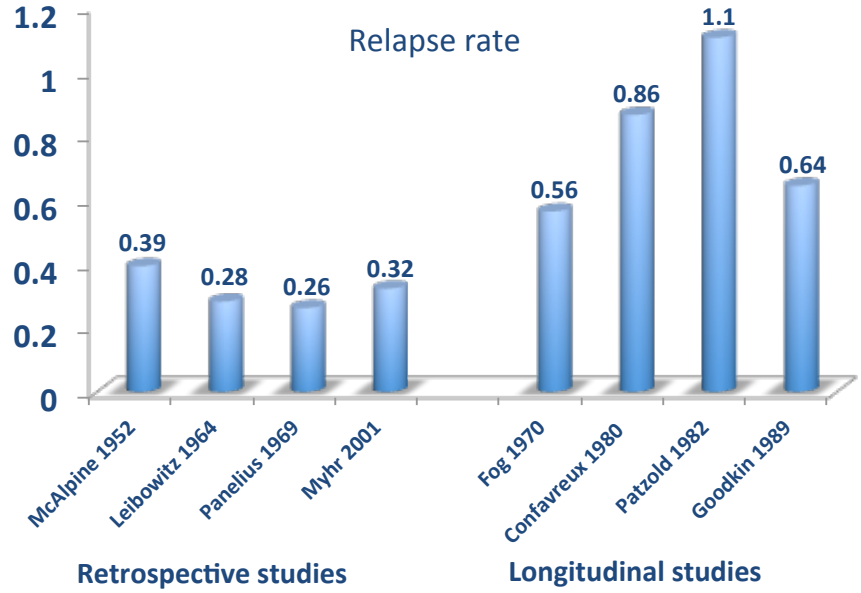
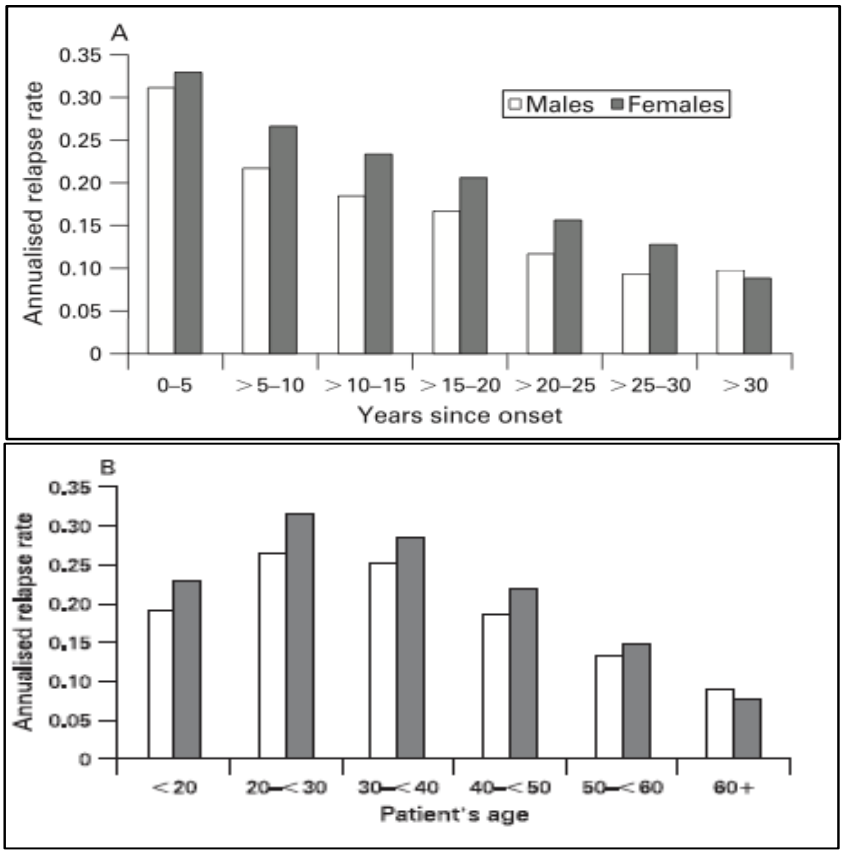


Figure 4.2 Annualized relapse rate according to disease duration (A) and according to patients' age (B), among patients from the British Columbia database (Tremlett et al., 2008b).



The decrease of the attacks frequency was also shown to be proportional to the patients' age and to become more evident by growing older (Figure 4.2 B). In addition, more than 70% of patients experienced at least 5 years relapse-free period, highlighting the largely variable presenting pattern of inflammatory attacks (Tremlett et al., 2008b).

This is particularly relevant to RCTs using relapse related outcome measures for testing the efficacy of therapies. At least 40% of the relapse reduction observed in the placebo groups might be actually due to the regression to the mean (Martinez-Yelamos et al., 2006). In addition, the ARR in trials populations has reduced over time, making the comparison of data from different studies problematic (Nicholas et al., 2011). Two large meta-analyses, pooling data from several trials, estimated, over a 10 years period, a decline of 0.37 relapses per year, in both the treatment and the placebo group (Inusah et al., 2010; Nicholas et al., 2011). This might be due to the inclusion in early studies of more active patients, or to the exclusion from recent studies of patients requiring treatment because of a more aggressive disease course (Inusah et al., 2010). Finally, the changes of the ARR during trials, observed over the last 30 years, have also been accompanied by a 2%/year reduction of the pre-trial ARR (Steinvorth et al., 2013).

4.1.2 The predictive effect of relapses

4.1.2.1 Early relapses and the disease evolution

It is widely agreed that early relapses are predictors of the long-term disease evolution and affect adversely the prognosis. This information has been strongly validated by several analyses, which were carried out with different methodologies and in databases from different geographic areas. A large number of attacks during the first two (Leray et al., 2010; Weinshenker et al., 1989c) and five (Confavreux et al., 2003; Debouverie et al., 2008; Eriksson et al., 2003; Kantarci et al., 1998; Tremlett et al., 2009a) years, was shown to be associated with a faster disease evolution (Table 4.2).

In the Lyon database, patients with a higher number of relapses occurring during the first 5 years attained DSS 4, DSS 6 and DSS 7, from the disease onset, in significantly shorter time. Nevertheless, results were affected by the large proportion of censored information (68% did not reach DSS 6). For instance, the time for requiring a walking aid was only slightly different among those grouped by early attacks frequency (1 attack = 25.3 years, 2 attacks = 21.9 years, ≥ 3 attacks = 24.7 mean years), however differences reached statistical significance (p = 0.001) (Confavreux et al., 2003).

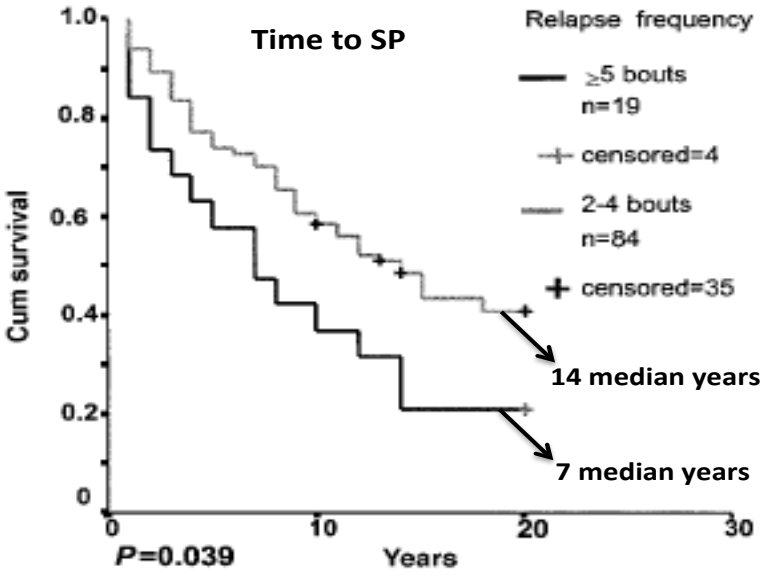
Table 4.2 The relationship between early relapses and late outcomes: results from the analyses carried out in MS registries.

Database	Early relapses grouping	Endpoint	Association with early relapses
Lyon	1, 2, ≥ 3 attacks during the first **5 years**	DSS 4, DSS 6, DSS 7	Larger number = shorter time to endpoints
Lorraine	1, 2, 3, > 3 attacks during the first **5 years**	SP, EDSS 3, EDSS 4, EDSS 6	Larger number = shorter time to endpoints
Goteborg	2-4, ≥ 5 attacks during the first **5 years**	SP, DSS 7	Larger number = shorter time to endpoints
British Columbia	< 0.2, 0.2-- < 0.4, ≥ 0.4 relapse rate during the first **5 years**	SP, EDSS 6	Larger number = shorter time to endpoints
London Ontario	1, 2-4, ≥ 5 attacks during the first **2 years**	DSS 6	Larger number = shorter time to endpoints
Rennes	1, ≥ 2 attacks during the first **2 years**	DSS 3, DSS 6	Larger number = shorter time to endpoints

In the Lorraine database, patients with 2, 3 and > 3 attacks, during the first 5 years, compared to those with 1 attack, took significantly shorter times to SP and to disability endpoints (EDSS 3, EDSS 4, and EDSS 6) (Debouverie et al., 2008). However, the differences between those with > 3 and 2 relapses were relatively small (mean difference = 4.8 years for reaching EDSS 6 and 1 year for reaching SP) and, again, the large percentage of censored information (72% did not reach EDSS 6) represented an important limitation (Debouverie et al., 2008). In both the French cohorts, the number of inflammatory attacks experienced during the first 5 years did not influence times for advancing from DSS 4 to DSS 6 (Confavreux et al., 2003; Debouverie et al., 2008) and to DSS 7 (Confavreux et al., 2003).

The analysis of the relatively small population from the Goteborg database confirmed the association between a larger number of attacks during the first 5 years and a worse outcome (Eriksson et al., 2003). The group with 5 or more attacks, compared to those with 2-4 attacks, had a higher risk of and a shorter time to SP (7 median years difference) (Figure 4.3) and to EDSS 7. However, the comparison of patients with 2 versus 3 or more attacks did not demonstrate any significant difference (Eriksson et al., 2003). In addition, early relapses did not influence the attainment of EDSS 7 from the onset of SP.

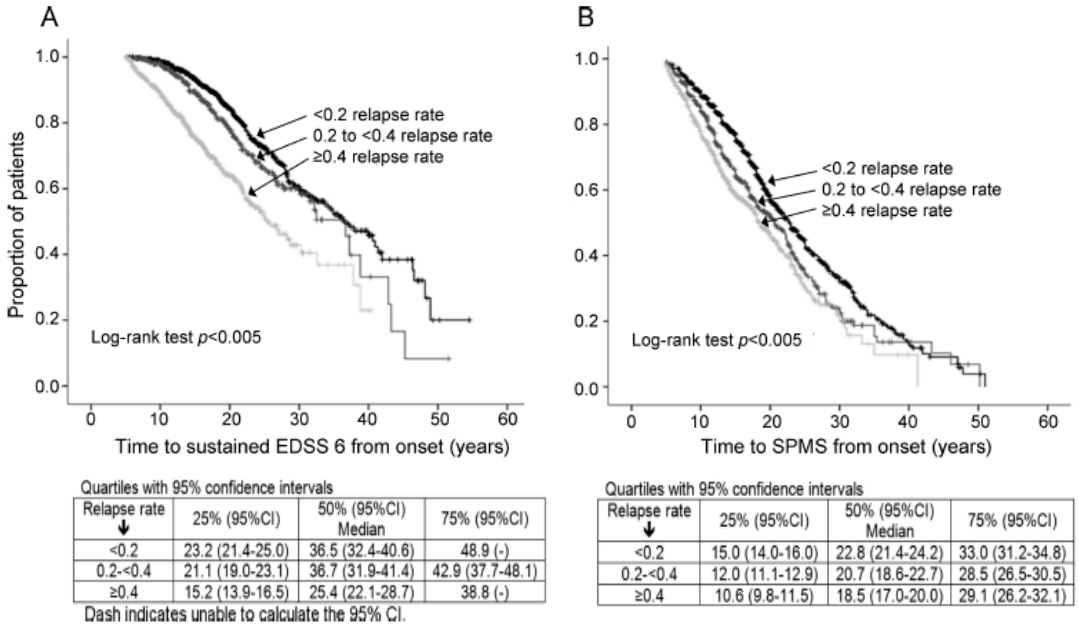
Figure 4.3 Analysis from the Goteborg database: time to SP in patients grouped by number of relapses in the first 5 years (Eriksson et al., 2003).



In the British Columbia database, patients were grouped according to the relapse rate during the first 5 years. A higher attacks frequency predicted significantly shorter times to EDSS 6 and to SP. The group with a high rate (≥ 0.4) was the main driver of this association, as differences between those with low (< 0.2) and intermediate (0.2-<0.4) rate were small (0.2 median years to attain EDSS 6 and 2.1 median years to attain SP) (Figure 4.4). By using the Cox Regression analysis it was estimated that one attack, within 5 years post onset, increased the risk of reaching EDSS 6 (HR = 1.48) and SP (HR = 1.29) during this time period. This effect diminished with time, becoming smaller if the endpoints were attained between 5 and 10 years

from onset (HRs = 1.25 and 1.11, respectively) or after 10 years (HRs = 1.10 and 1.02, respectively). The relatively large proportion of censored information (72% for reaching EDSS 6 and 45% for reaching SP) had a potential impact on these findings (Tremlett et al., 2009a).

Figure 4.4 Analysis from the British Columbia database: time to EDSS 6 and to SP in patients grouped by the relapses rate during the first 5 years (Tremlett et al., 2009a).



Quartiles with 95% confidence intervals

Relapse rate ↓	25% (95%CI)	50% (95%CI) Median	75% (95%CI)
<0.2	23.2 (21.4-25.0)	36.5 (32.4-40.6)	48.9 (-)
0.2-<0.4	21.1 (19.0-23.1)	36.7 (31.9-41.4)	42.9 (37.7-48.1)
≥0.4	15.2 (13.9-16.5)	25.4 (22.1-28.7)	38.8 (-)

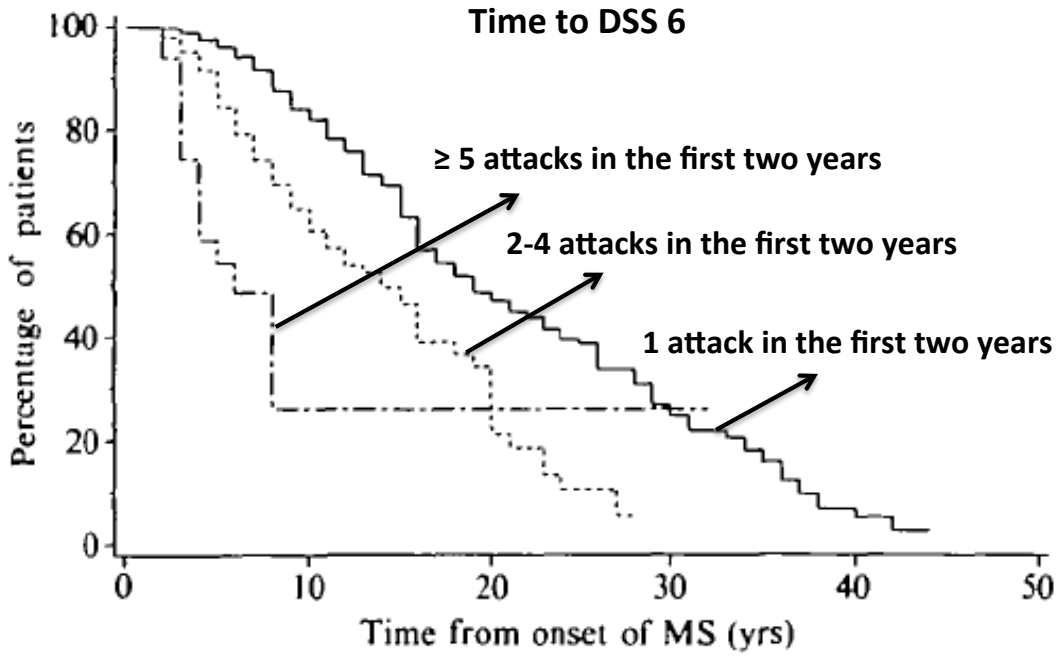
Dash indicates unable to calculate the 95% CI.

Quartiles with 95% confidence intervals

Relapse rate ↓	25% (95%CI)	50% (95%CI) Median	75% (95%CI)
<0.2	15.0 (14.0-16.0)	22.8 (21.4-24.2)	33.0 (31.2-34.8)
0.2-<0.4	12.0 (11.1-12.9)	20.7 (18.6-22.7)	28.5 (26.5-30.5)
≥0.4	10.6 (9.8-11.5)	18.5 (17.0-20.0)	29.1 (26.2-32.1)

Previous analysis of the LO database demonstrated an association between a larger number of attacks during the first two years and a shorter times to DSS 6 (Weinshenker et al., 1989c) (Figure 4.5). This was also confirmed more recently among patients from the Rennes database: the group with ≥ 2 attacks during the first two years attained DSS 3 and DSS 6, 4.5 years and 2 mean years earlier than the group with 1 attack (Leray et al., 2010). However, the time for advancing from DSS 3 to DSS 6 was not affected by the number of early relapses (7 median years in both groups) (Leray et al., 2010).

Figure 4.5 Analysis from the LO database: time to DSS 6 grouped by number of relapse during the first 2 years (Weinshenker et al., 1989c).



4.1.2.2 Late relapses and the disease evolution

The predictive effect of late relapses has been poorly investigated. This is probably due to the difficulties of collecting information on inflammatory attacks, occurring later in the disease course. In the British Columbia database, similarly to early relapses, the effect of late relapses on the outcome diminished with time (Tremlett et al., 2009a). One attack, occurring between 5 to 10 years after onset, increased significantly the hazard of reaching EDSS 6 (HR = 1.31) and SP (HR = 1.23), within this period of time, however it impacted less significantly on the risk of attaining the two endpoints after 10 years from onset (HR = 1.07 and 1.06, respectively). In addition, relapses occurring more than 10 years post onset, only marginally increased the probability of reaching DSS 6 (HR = 1.12) and SP (HR = 1.08).

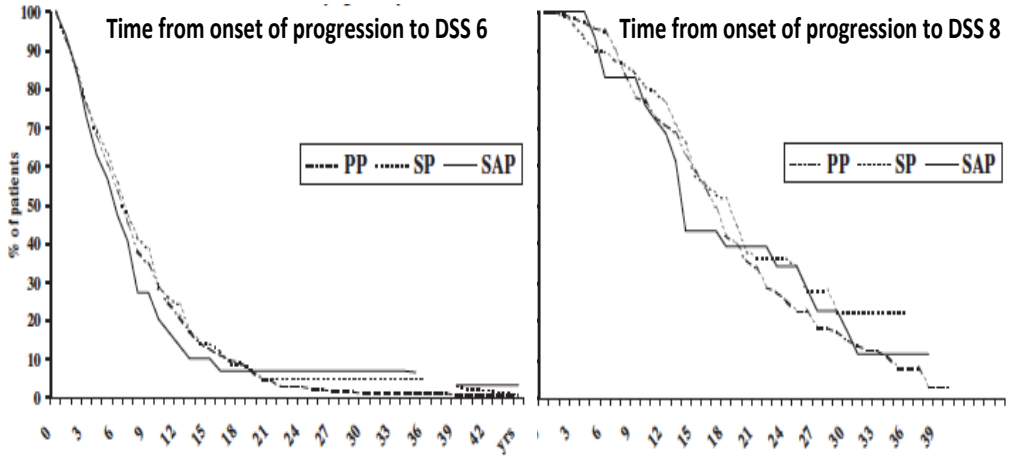
The predictive effect of relapses occurring after the attainment of DSS 3, among patients still experiencing the RR phase, was investigated in the Rennes database. The group with no attacks took longer time for attaining DSS 6 from moderate

disability, compared to group with relapses, however the differences were not statistically significant (12 versus 9 median years; p = 0.67) (Leray et al., 2010).

4.1.2.3 Total relapses and the disease evolution

The effect of total relapses during the RR phase on late outcome has never been directly investigated. Previous analyses of the LO database compared the evolution of the progressive phase, among progressive subtypes. Patients with no (PP MS), one (SAP MS) and many (SP MS) attacks before progression took equal times for reaching DSS 6 and DSS 8, from the onset of progression (Figure 4.6). These results demonstrated that the disability accumulation during the progressive phase was largely unaffected by inflammatory attacks preceding its onset (Kremenchutzky et al., 2006a).

Figure 4.6 Analyses from the LO database: times to DSS 6 and to DSS 8 from onset of progression among PP, SAP and SP MS patients (Kremenchutzky et al., 2006a).



4.1.2.4 Superimposed relapses and the disease evolution

The effect of relapses occurring during the progressive phase on late outcomes has been extensively investigated. Among PP patients from the LO database, 28% experienced superimposed relapses. These occurred in 50% within 10 years from onset, in 40% between 10 and 20 years and in 10% even after 20 years. These attacks were generally very discrete, followed by good recovery and usually affecting

extra-spinal locations (Kremenchutzky et al., 1999a). The analysis showed almost identical survival curves between PP and PR patients, demonstrating no significant effect of superimposed relapses on the disease evolution (Figure 4.7). Same results were obtained in a small dataset from California (US); 83 PP and 12 PR MS patients took equal times to reach EDSS 6 (Andersson et al., 1999).

In the Lyon database, the predictive effect of relapses occurring during the progressive phase was assessed in both primary and secondary progressive MS (Confavreux et al., 2000). The analysis confirmed the same rate of disability accumulation, between PP and PR patients (Figure 4.8). In addition, SP patients, with or without superimposed attacks, took equal times from DSS 4 (Confavreux et al., 2000) (Figure 4.8) and from the onset of progression (Vukusic and Confavreux, 2003) to DSS 6. Similarly, among patients in the British Columbia database, relapses occurring during the SP phase did not affect the attainment of late outcomes (Tremlett et al., 2009a).

Overall, this data consistently demonstrated that the rate of disability accumulation during the progressive phase, preceded or not by the RR phase, is not influenced by superimposed relapses.

Figure 4.7 Analysis from the LO database: comparison of times to DSS 6 and to DSS 8 from the disease onset, between PP and PR patients (Kremenchutzky et al., 1999a).

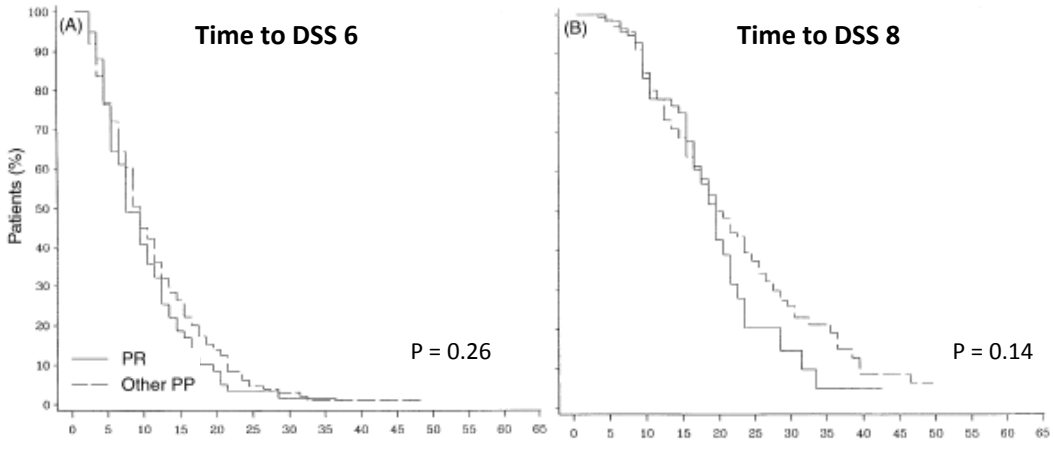
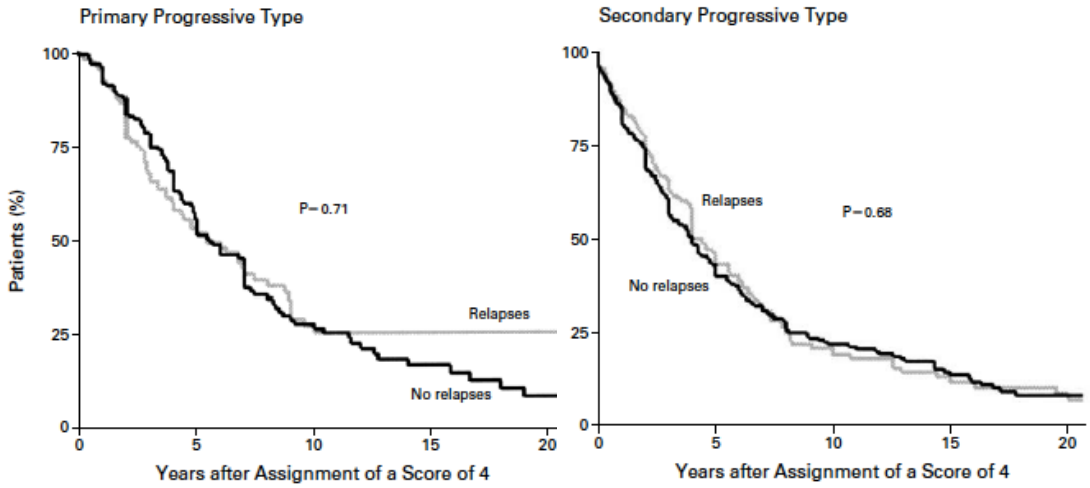


Figure 4.8 Analysis from the Lyon database: comparison of times from DSS 4 to DSS 6, among primary and secondary progressive patients with and without superimposed relapses (Confavreux et al., 2000).



4.1.3 The predictive effect of the first inter-attack interval

It is largely agreed that a shorter first inter-attack interval associates with a more rapid disease progression. Previous analysis of the LO database demonstrated a faster attainment of DSS 6, among patients with short (0-2 years), compared to those with intermediate (3-5 years) or long (≥ 6 years) interval (Weinshenker et al., 1989c). In the Lyon and Lorraine databases, the outcome was worse among patients experiencing the second attack in less than 2 years (Confavreux et al., 2003; Debouverie et al., 2008). However, the analysis from the Goteborg database could not confirm the association between a short first inter-attack interval and a worse prognosis (Runmarker and Andersen, 1993).

Only few studies have evaluated factors predicting time to the second attack. First attack severity and location were shown to strongly influence the severity and location of the subsequent relapse (Mowry et al., 2009a; Mowry et al., 2009b). In addition, the probability of experiencing a second attack within one year from the clinical onset was significantly higher among younger patients, presenting with poly-regional symptoms (Mowry et al., 2009c).

4.1.4 Aim of the study presented in this chapter

This study aimed at assessing the relationship between relapses, occurring during the RR phase, and the attainment of SP and of hard disability outcomes (DSS 6 and DSS 8). Inflammatory attacks during the early and late stages of the disease, before entering the progressive phase, were examined separately. This allowed to assess the validity of attacks number as clinical indicator of the disease activity, to test the reliability of the relapse frequency as surrogate marker for late outcomes, and to further elucidate potential mechanisms driving disability accumulation.

4.2 Methods

Among 806 relapsing onset patients, the relationship between disability outcomes and the following variables was investigated: A) the number and the type of neurological systems involved at disease onset; B) the number of relapses during the first 2 years of the disease (**early relapses**); C) the time between the first and the second attack; D) the time from the disease onset to the attainment of moderate disability (DSS 3); E) the number of relapses from year 3 up to the onset of secondary progression (**late relapses**); F) the total number of relapses during the RR phase (**total relapses**).

Kaplan Meier analysis was used to estimate the time to SP and the times to reach DSS 6 and to DSS 8 from the disease onset, from DSS 3 and from the onset of SP, among patients grouped according to the variables listed above. Groups were computed in order to maintain, to the possible extent, similar numbers in each category (Table 4.3). However, the analyses were also carried out among patients with different stratifications, for confirming results. The Log rank test, with p values level of significance < 0.05, was used to assess differences among groups. To eliminate bias from the immortal time (Suissa, 2008), the following criteria were applied for excluding patients from the analyses; A) relapsing onset patients attaining the endpoints in < 2 years (SP = 24, DSS 6 = 10, DSS 8 = 1) when assessing the effect of early attacks; B) SP patients attaining the endpoints before entering the progressive phase (DSS 6 = 16, DSS 8 = 0) when assessing the effect of late and total relapses.

The Cox proportional hazard analysis was used to investigate relapses, first inter-attack interval and time to DSS 3 as continuous variables. The analysis allowed to calculate the risk of attaining the endpoints according to increasing number of attacks, the increasing time between the first two attacks and the increasing time from the disease onset to accumulation of moderate disability (DSS 3). Hazard ratios (HR), expressing the size of the risk of reaching the endpoints, were obtained through comparison versus 0 relapses and versus 0 years interval between the first

two attacks and between the disease onset and DSS 3. Proportional hazards assumption was checked by visual inspections of Schoenfeld residual plots and corresponding statistical tests. The predictive effect of the variables was also analysed in multivariate models of disability, allowing to simulate clinical scenarios according to specific number of relapses and first inter-attack interval. Models were also compared to assess the difference in the hazard of reaching the endpoints between different scenarios.

Information on the time to endpoints (Chapter 3) and on variables (Table 4.3) was not always available, resulting in slightly different numbers of patients contributing to each analysis. Patients not reaching given endpoints, but followed for known period, were right censored.

Table 4.3 Grouping, according to variables, used when estimating the times to disability endpoints with Kaplan Meier analysis.

No of relapses in the first 2 years		**Number of patients**
Low	1	389
Intermediate	2	183
High	≥ 3	158
First inter-attack interval (yrs)		
Short	0 - 2	388
Intermediate	3 – 5	141
Long	≥ 6	155
Time to reach DSS 3 (yrs)		
Short	0 - 2	123
Intermediate	3 - 7	192
Long	≥ 8	463
No of relapses from the 3rd year to onset of progression		
Low	0	107
Intermediate	1 – 2	164
High	≥ 3	165
Total no of relapses before the onset of progression		
Low	1 – 2	158
Intermediate	3 – 4	138
High	≥ 5	163

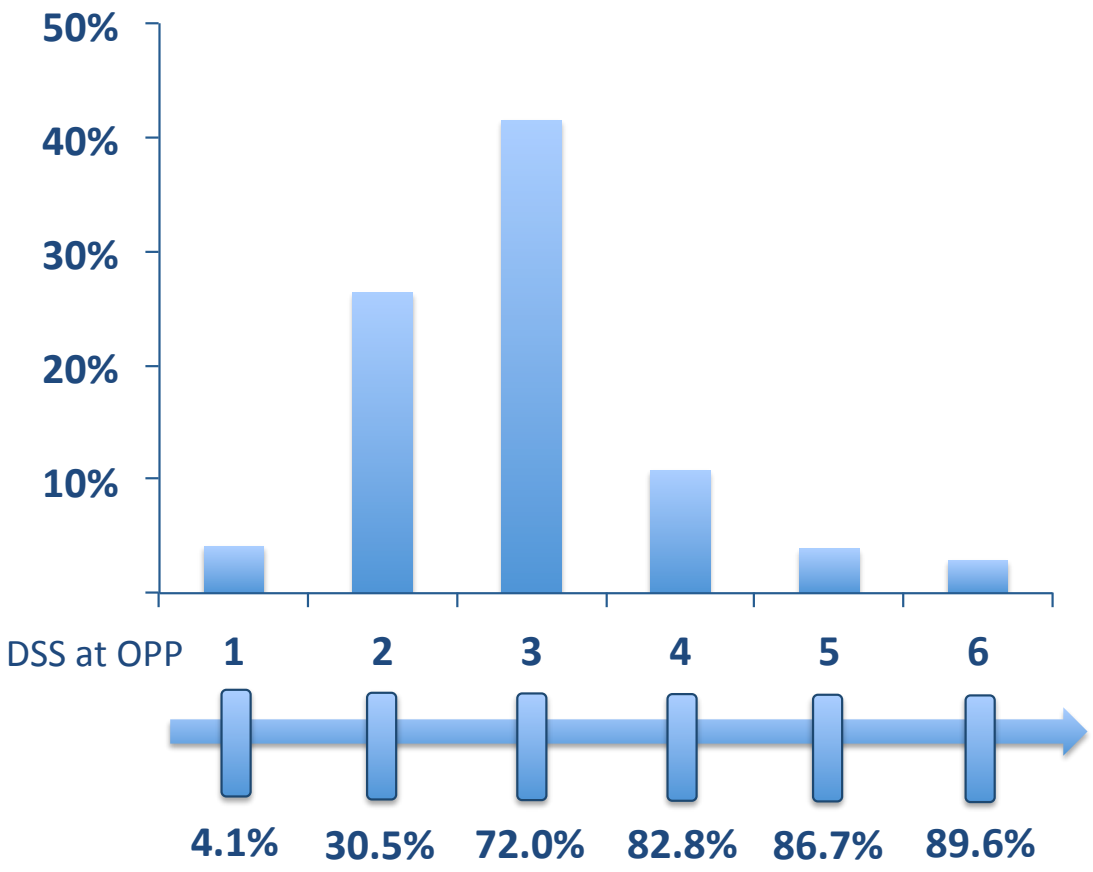
4.3 Results

Table 4.4 Clinical and demographic features of 806 relapsing onset patients. * SP patients only.

Relapsing-remitting onset MS	
No of patients	806
No of males	252 (31.2%)
No of females	554 (68.8%)
Sex ratio (F/M)	2.19
Disease course (at the end of theobservation period: 1972-2000)	
Relapsing remitting: n (%)	272 (33.8%)
Secondary progressive: n (%)	534 (66.2%)
Disease duration: years	
Mean [95% CI]	24.4 [23.7-25.1]
Median	23
Age at onset: years	
Mean [95% CI]	28.5 [27.8-29.1]
Median	27
Age at onset of progression: years	
Mean [95% CI]	40.2 [39.3-41.0]
Median	39
First inter-attack interval: years	
Mean [95% CI]	3.8 [3.5-4.2]
Median	2
Relapses rate: mean attacks/year [95% C.I.] (median)	
Year 1 + Year 2 (early relapses)	0.93 [0.88-0.97] (0.50)
Year 3 to SP (late relapses)*	0.41 [0.35-0.48] (0.21)
Year 1 to SP (total relapses)*	0.65 [0.58-0.73] (0.40)
DSS at onset of SP*	
Mean (95% CI)	2.9 (2.8-3.0)
Median	3
Systems involved at onset: number of patients (%)	
Motor	145 (17.9%)
Sensory	438 (54.3%)
Cerebellar	51(6.3%)
Brainstem	167 (20.7%)
Optic	174 (21.5%)
Bowel/bladder	25 (3.1%)
Number of symptoms at disease onset: number of patients (%)	
Monosymptomatic	535 (67.1%)
Polysymptomatic	262 (32.9%)
Kaplan Meier analysis: mean [95% C.I.] (median) years from disease onset to	
DSS 3	12.6 [11.8-13.5] (10)
DSS 6	21.3 [19.8-22.6] (18)
DSS 8	30.9 [29.1-32.8] (28)
DSS 10	46.9 [44.3-49.5] (54)
Onset of SP	21.3 [19.5-23.1] (15)
Kaplan Meier analysis: mean [95% C.I.] (median) years from DSS 3 to	
DSS 6	7.9 [7.2-8.5] (5)
DSS 8	17.6 [16.3-19.0] (15)
DSS 10	31.4 [29.4-33.4] (33)

The analysis focused on 806 patients with relapsing remitting onset. Their clinical and demographic features (Table 4.4) were extensively described in chapter 3, and here are summarized. Most of the patients were females (68.8%), the mean age at onset was 28.5 years and the mean disease duration was 24.4 years, reflecting the long observation period and the low rate of lost to follow up. A mono-symptomatic onset, characterized by sensory disturbances, was the most commonly observed clinical presentation. The mean age at onset of progression was 40.2 years, and most of the patients entered the progressive phase when they were scored between DSS 2 and DSS 4; more than 70% within DSS 3 (Figure 4.9). By the end of the observation period, the majority of patients had converted to SP MS (n = 534; 66.2%) and in large percentages had attained DSS 3 (n = 657; 81.5%), DSS 6 (n = 543; 67.4%) and DSS 8 (n = 390; 48.4%), in 15, 10, 18 and 28 median years respectively (Table 4.4).

Figure 4.9 DSS scores (x-axis) recorded when patients converted to SP MS. On the y-axis is indicated the percentage of patients, at each DSS score category, when the SP phase occurred. Below the x-axis it is indicated the cumulative percentage of patients entering the SP phase within each DSS score: this was obtained by adding the percentages of patients in each DSS score category up to the specific DSS level. OPP = onset of progressive phase.



4.3.1 Early relapses

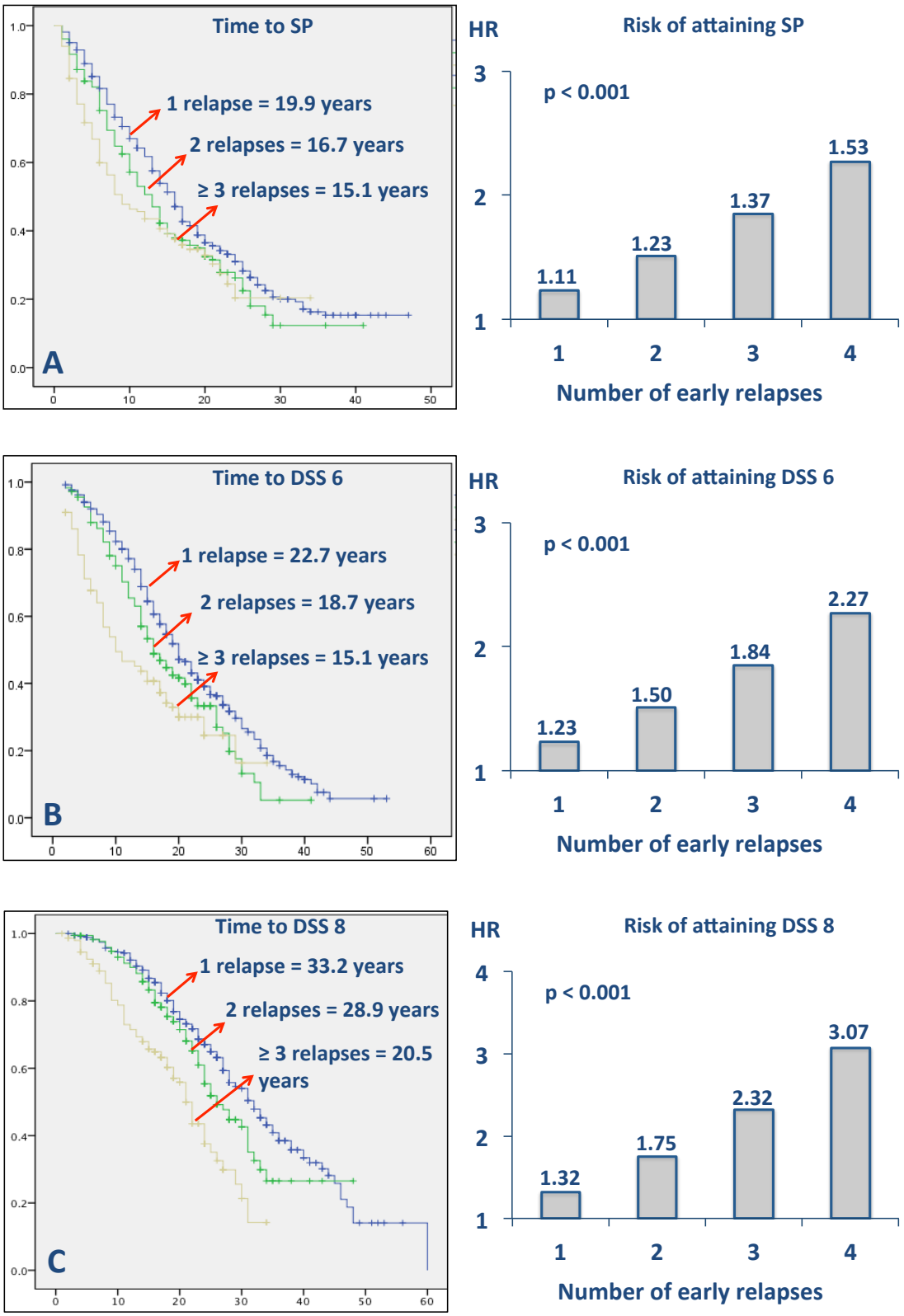
Information on the frequency of relapses during the first 2 years was available for 730 patients, who experienced 1363 relapses. The number of attacks ranged from 1 to 8, and the mean relapse rate was 0.93 attack/year. At 2 years from the clinical presentation, more than half of patients (n = 389) experienced the onset attack only, 25% (n = 183) had 2 relapses and 20% (n = 158) had 3 or more relapses (Table 4.3).

The analysis confirmed (Weinshenker et al., 1989c) that the number of attacks during the first 2 years predicted the long-term disease evolution. The groups with higher early relapses frequency attained the disability endpoints from the disease onset in significantly shorter times (Table 4.5 and Figure 4.10). Between patients with 1 and ≥ 3 attacks, there was a mean difference of 7.6 years and of 4.3 years for reaching DSS 6 and DSS 8, respectively. The Cox regression analysis demonstrated a strong association between the attacks during the first 2 years and late outcomes (HR = 1.23 for DSS 6; HR = 1.32 for DSS 8). The risk of developing severe disability increased proportionally with the number of attacks (Figure 4.10 B-C). Patients with 3 relapses, compared to those with 1 attack, had approximately 50% and 90% higher hazard of reaching DSS 6 and DSS 8, respectively (Figure 4.10).

Table 4.5 Kaplan Meier estimated times to SP, to DSS 6 and to DSS 8 from the disease onset in patients grouped by the number of early relapses. P values were obtained using the Log Rank test by comparison with the reference category (*).

No of relapses y1-y2	Time to onset of SP		Time from disease onset to DSS 6		Time from disease onset to DSS 8	
	mean [95% CI] years (median)	p value	mean [95% CI] years (median)	p value	mean [95% CI] years (median)	p value
1 relapse	19.9 [18.3-21.5] (16)	0.014	22.7 [21.1-24.1] (20)	< 0.001	33.2 [31.0-35.5] (32)	< 0.001
2 relapses	16.7 [14.6-18.9] (13)	0.38	18.7 [16.9-20.4] (16)	0.010	28.9 [26.2-31.6] (26)	0.001
≥ 3 relapses*	15.1 [12.8-17.4] (9)		15.1 [12.5-16.7] (10)		20.4 [18.6-22.3] (21)	

Figure 4.10 Kaplan Meier analysis (left): estimated times to SP (A), to DSS 6 (B) and to DSS 8 (C), among groups with low (1 attack), intermediate (2 attacks) and high (≥ 3 attacks) number of relapses during the first two years. Cox regression analysis (right): variation of the risk (HRs on y-axis) of attaining the endpoints according to the increasing number of early relapses (x-axis). The exact HRs are indicated on top of each column.



The effect of early relapses was primarily exerted by increasing the probability of entering the SP phase and by shortening the latency to SP (Table 4.5 and Figure 4.10 A). A higher number of attacks during the first 2 years predicted a higher risk (HR = 1.11) of converting to SP MS and a shorter time to the onset of progression. This association was mainly driven by the group with ≥ 3 attacks, which attained SP 4.8 mean years earlier than the group with 1 attack (p = 0.014). The comparison between the patients with low and intermediate early relapses frequency did not demonstrate any statistically significant (p = 0.38) difference (Table 4.5).

Additional analyses further demonstrated that the conversion to SP MS was the key determinant of the early relapses' predictive effect. The risk of reaching DSS 3 and DSS 6 from the disease onset, according to the number of attacks during the first two years, became much larger when it was assessed in the SP group only: 3 relapses yielded HRs 1.59 and 2.87 for attaining moderate disability, and HRs 1.84 and 2.45 for requiring a walking aid, in RR and SP patients, respectively (Table 4.6). Moreover, among those who converted to SP MS, the predictive effect of early relapses decreased proportionally with the duration of the RR phase (Table 4.7). Three attacks yielded HRs 3.03, 2.27 and 2.02 for reaching DSS 6 in the group with short (1-5 years), intermediate (6-12 years) and long (≥ 13 years) latency to progression, respectively (Table 4.7).

The number of early relapses also significantly influenced the times to the endpoints and the probability of developing severe disability from DSS 3 (Table 4.8), however the impact was much smaller than from the disease onset (Table 4.9). Patients with high early relapse frequency, compared to those with low frequency, reached DSS 6, from onset, 10 median years earlier (Table 4.5), but the two groups differed by only 2 median years when attaining the endpoint from DSS 3 (Table 4.8). Accordingly, the effect of early relapses on the risk of requiring a walking aid was larger from the disease onset than from DSS 3 (3 attacks HR = 1.84 and 1.39, respectively) (Table 4.9).

Finally, the number of attacks during the first two years also affected the evolution of the SP phase. Among SP patients, those with higher early relapse frequency had significantly shorter times to DSS levels and a higher probability of attaining the endpoints from the onset of SP (Tables 4.8 and 4.9). The predictive effect of early attacks was much larger from the onset of progression than from the disease onset and from DSS 3 (Table 4.9), further emphasizing the importance of the conversion to SP MS on the way to severe disability development.

Table 4.6 Cox regression analysis: risk (HR) of attaining DSS 3 and DSS 6 according to the number of early relapses in RR and SP patients.

	Risk of attaining DSS 3		Risk of attaining DSS 6	
No of relapses y1-y2	RRMS	SPMS	RR/SPMS	SPMS
	HR (p = 0.03)	HR (p < 0.001)	HR (p < 0.001)	HR (p < 0.001)
1	1.16	1.42	1.23	1.34
2	1.36	2.02	1.50	1.81
3	1.59	2.87	1.84	2.45
4	1.86	4.08	2.27	3.30

Table 4.7 Cox regression analysis in SP patients: risk (HR) of attaining DSS 6 according to the number of early relapses in patients stratified by the duration of the RR phase (short, intermediate, long).

	Risk of attaining DSS 6		
Duration of RR phase	**1-5 years**	**6-12 years**	**≥ 13 years**
Relapses Y1-2 (n)	**HR [95% CI] (p < 0.001)**	**HR [95% CI] (p < 0.001)**	**HR [95% CI] (p = 0.03)**
1	1.45 [1.28-1.63]	1.31 [1.14-1.51]	1.26 [1.02-1.57]
2	2.09 [1.64-2.67]	1.73 [1.31-2.28]	1.60 [1.04-2.46]
3	3.03 [2.10-4.37]	2.27 [1.49-3.44]	2.02 [1.06-3.85]

Table 4.8 Kaplan Meier analysis: times to DSS 6 and to DSS 8 from DSS 3 and from the onset of secondary progression, in patients grouped by the number of early relapses. P values were obtained with Log Rank test by comparison with the reference category (*).

No of relapses y1-y2	Time to DSS 6			
	From DSS 3		From onset of progression	
	mean [95% CI] years (median)	p value	mean [95% CI] years (median)	p value
1 relapse	8.1 [7.2-8.9] (6)	< 0.001	6.1 [5.2-6.8] (4)	< 0.001
2 relapses	7.7 [6.7-8.8] (6)	< 0.001	5.3 [4.3-6.2] (4)	< 0.001
≥ 3 relapses*	5.6 [4.4-6.4] (4)		2.5 [1.7-3.1] (1)	
	Time to DSS 8			
	From DSS 3		From onset of progression	
	mean [95% CI] years (median)	p value	mean [95% CI] years (median)	p value
1 relapse	18.2 [16.4-19.9] (16)	0.001	16.4 [14.5-18.2] (14)	< 0.001
2 relapses	17.3 [15.5-19.1] (16)	0.010	14.2 [12.5-15.9] (13)	< 0.001
≥ 3 relapses*	13.7 [11.9-15.5] (12)		9.6 [8.2-11.1] (9)	

Table 4.9 Cox regression analysis: risk (HR) of attaining DSS 6 from the disease onset, from DSS 3 and from the onset of progression according to the number of early relapses.

No of relapses y1-y2	Risk of attaining DSS 6		
	From disease onset	From DSS 3	From onset of SP
	HR (p < 0.001)	HR (p < 0.001)	HR (p < 0.001)
1	1.23	1.12	1.39
2	1.50	1.25	1.92
3	1.84	1.39	2.67

4.3.1.1 Frequent early relapses

In table 4.10 are compared the clinical and demographic features, between patients with low (1-2 attacks; n = 572) and high (≥ 3 attacks; n = 158) early relapse frequency. In both subgroups women predominated, and the mean age at the disease onset differed very little (28.8 versus 27.4 mean years; p = 0.11). However, patients with frequent early relapses (≥ 3 attacks) were significantly younger at the onset of the SP phase (35.3 versus 41.5 mean years; p < 0.001), secondary to the shorter duration of the RR phase (6.9 versus 11.7 mean years; p < 0.001). By the end of the observation period, among patients with low attacks frequency (1-2 attacks), 84.2% had attained DSS 3, 70.6% had attained DSS 6 and 46.3% had attained DSS 8 in 11, 19 and 31 estimated median years, respectively. This was significantly longer than patients with frequent early relapses (Table 4.10).

The group with ≥ 3 attacks during the first two years ("*frequent early relapsers*") represented 20% (n = 158) of the total relapsing onset population and drove the association between early relapses and late outcomes. Among *frequent early relapsers*, the Kaplan Meier estimated time to the onset of SP was 15.1 mean years (95% C.I. 12.8-17.4): 50% (n = 79) of patients entered the SP phase after 9 years from the disease onset (median time), increasing to 65.2% (n = 103) after 24 years (Figure 4.12). The remaining 34.8% (n = 55), despite the high early relapses frequency, stayed in the RR phase (Figure 4.11).

Among patients who had converted to SP MS by the end of the observation period (n = 103), 50% attained the progressive phase in 5 years and 75% in 9 years (Figure 4.11). In this subgroup, patients experienced a rapid disease evolution; all of them reached DSS 3, 94% (n = 97) reached DSS 6 and 75% (n = 77) reached DSS 8 in 4.5, 9.1 and 17.2 estimated mean years, respectively (Table 4.11). On the contrary, within the RR subgroup, less than half (n = 24; 43%) attained moderate disability (DSS 3) in estimated 16.2 mean years and very few (n = 8; 14%) attained DSS 6 through relapses (Table 4.11). There was a difference of 11.7 mean years for reaching DSS 3 between the RR and the SP patients (Figure 4.12).

Table 4.10 Comparison of clinical and demographic features between patients with low (1-2) and high (≥ 3) early relapses frequency. * Chi-square test; ** Student T-test; ¶ Log-Rank test.

	Early relapses		
	1-2 attacks	**≥ 3 attacks**	**p**
No of patients	572	158	
No (%) of males	180 (31.5)	48 (30.4)	0.79 *
No (%) of females	392 (68.5)	110 (69.6)	
Sex ratio (F/M)	2.1	2.3	
Type of disease course at the end of follow up			
RR MS n (%)	166 (29.0)	55 (34.8)	0.16 *
SP MS n (%)	406 (71.0)	103 (65.2)	
Disease duration			
Mean years (95%C.I.)	26.2 (25.3-27.0)	19.3 (18.1-20.3)	<0.001 **
Median	25	19	
Age at disease onset			
Mean years (95% C.I.)	28.8 (28.0-29.)	27.4 (26.0-28.6)	0.105 **
Median	27	27	
Duration of the RR phase			
Mean years (95% C.I.)	11.7 (11.0-12.5)	6.9 (5.8-8.0)	<0.001 **
Median	10.5	5.5	
DSS at onset of SP			
Mean (95% C.I.)	2.8 (2.7-2.9)	3.4 (3.1-3.6)	<0.001 **
Median	3	3	
Age at onset of progression			
Mean years (95% C.I.)	41.5 (40.5-42.4)	35.3 (33.4-37.2)	<0.001 **
Median	41	35	
Kaplan Meier estimated mean [95% C.I.] (median) years to			
DSS 3	13.5 [12.5-14.4] (11)	8.3 [7.0-9.6] (4)	<0.001 ¶
DSS 6	21.6 [20.4-22.9] (19)	14.6 [12.5-16.7] (10)	<0.001 ¶
DSS 8	32.4 [30.5-34.4] (31)	20.4 [18.6-22.3] (21)	<0.001 ¶

Figure 4.11 Kaplan Meier analysis of time to SP, among 158 patients with ≥ 3 attacks during the first two years: cumulative percentage of patients converting to SP MS. The median time to SP (dotted line) refers to all patients (RR + SP). The percentiles of the time to onset of progression refer to SP patients only.

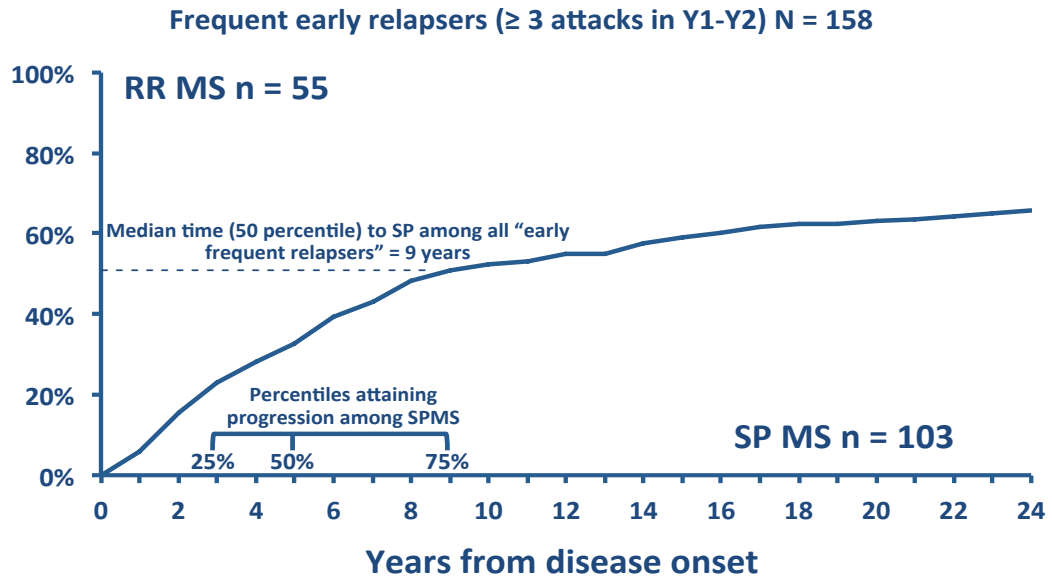


Figure 4.12 Kaplan Meier analysis of time to DSS 3 from the disease onset, among patients with frequent early relapses (≥ 3 attacks): comparison between RR (n = 55) and SP (n = 103) patients.

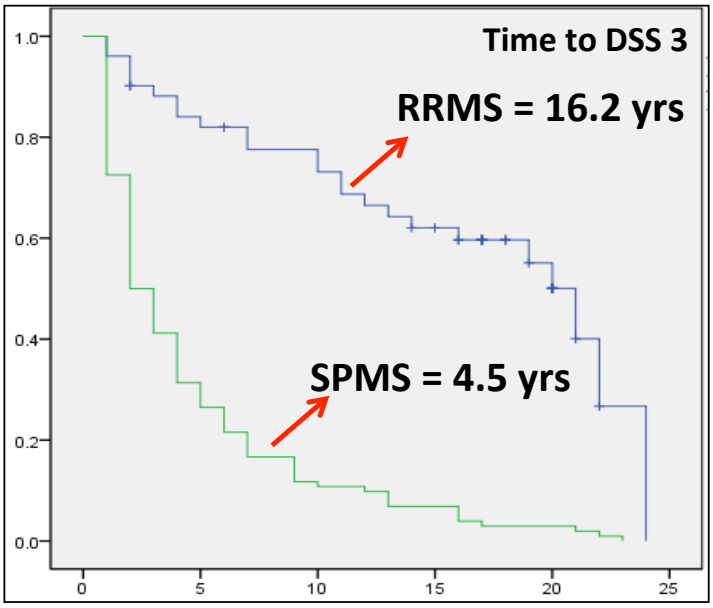
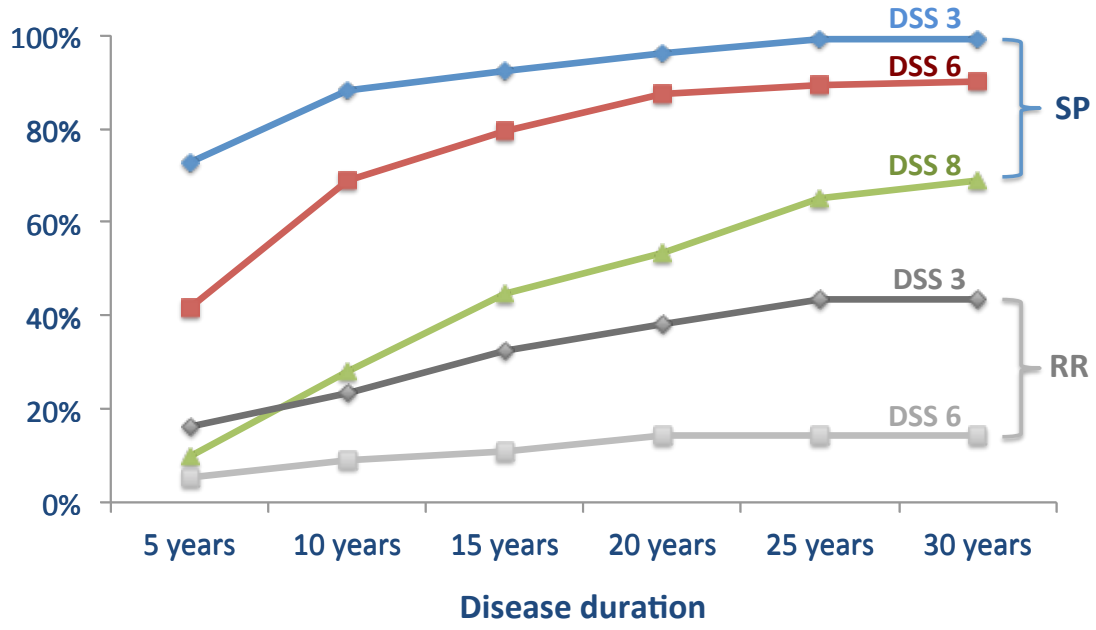


Figure 4.13 Cumulative percentage (y-axis) of patients with frequent early relapses (≥ 3 attacks), stratified by type of disease course (RR = 55; SP= 103), reaching each DSS level at specific time points (x-axis) from the disease onset. The analysis of each disability level is independent from the others. Values were calculated from the Kaplan Meier analysis of times to the endpoints.



The rate of disability accumulation was widely different in the two subgroups, sharing the same high early relapse frequency (Figure 4.13). At 5 years from the disease onset 72% and 41% of the SP subgroup already reached moderate disability (DSS 3) and required a walking aid (DSS 6), respectively. By 10 years, 23% of RR patients had attained DSS 3 and 28% of SP patients had already become bedbound (DSS 8). At 20 years, these percentages increased to 38.1% and 53.3%, respectively.

Clinical and demographic features were compared between RR (n = 55) and SP (n = 103) patients with frequent early relapses. In both subgroups, the number of early attacks was similar (Table 4.12), and most of the patients presented with sensory symptoms and had a mono-symptomatic onset attack (Table 4.11). Among those who remained in the RR phase there was a larger percentage of women (81.8% versus 63.1%; p = 0.02) and the age at the disease onset was slightly younger (25.5 versus 28.4 mean years; p = 0.01) (Table 4.11). The mean disease duration was slightly (p = 0.003) shorter in the RR subgroup (17.2 mean years [95% C.I. 15.4-18.8]) compared to the SP subgroup (20.3 mean years [95% C.I. 18.8-21.7]). However, more

than 80% of RR patients were observed for longer than 10 years and more than 70% for longer than 15 years (Figure 4.14).

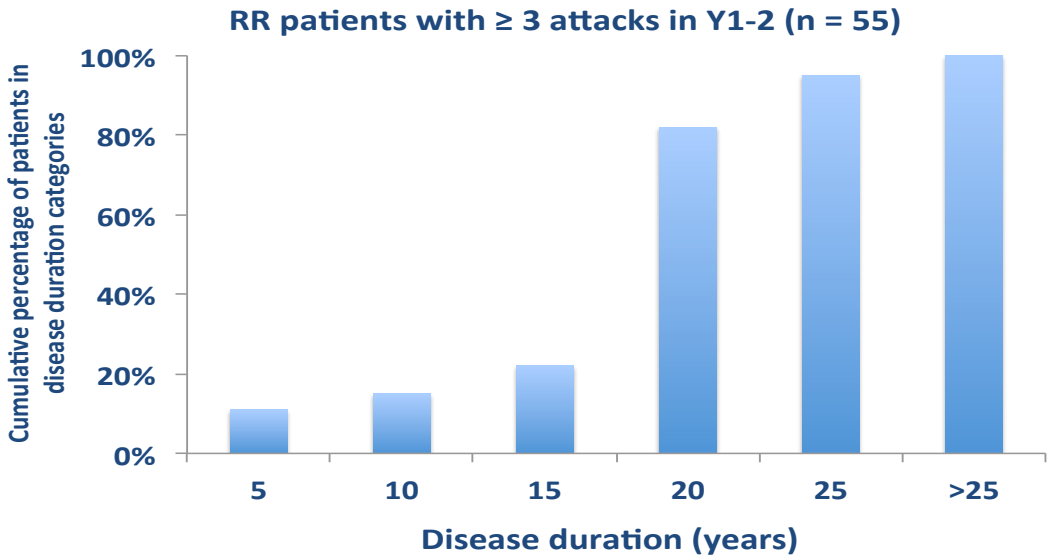
Table 4.11 Clinical and demographic features of patients with high (≥ 3) early relapse frequency, stratified by the type of the disease course (RR and SP) at the end of the observation period. * Chi-square test; ** Student T-test; ¶ Log-Rank test.

	158 patients with ≥ 3 relapses in Y1-2		
	RRMS	**SPMS**	**p**
N	55	103	
Females; n (%)	45 (82%)	65 (63%)	0.02*
Males; n (%)	10 (18%)	38 (37%)	
F/M ratio	4.5	1.7	
Mean age at onset (median)	25.5 (25) years	28.4 (27) years	0.01**
Mean disease duration (median)	17.2 (17) years	20.3 (21) years	0.003**
Monosymptomatic onset	62%	70%	0.30*
Polysymptomatic onset	38%	30%	
Type of initital presentation; n (%)			
Motor	6 (10.9%)	15 (14.6%)	0.52*
Sensory	35 (63.6%)	56 (54.4%)	0.26*
Cerebellar	3 (5.5%)	9 (8.7%)	0.46*
Brainstem	14 (25.5%)	20 (19.4%)	0.38*
Optic	8 (14.5%)	28 (27.2%)	0.07*
Bowel/Bladder	2 (3.6%)	2 (1.9%)	0.52*
Mean time to onset of SP (median)	n/a	6.9 (5) years	
Mean DSS score at onset of SP (median)	n/a	3.4 (3)	
Kaplan Meier estimated mean (median) times from disease onset to DSS levels; [% reaching the endpoint]			
DSS 3	16.2 (21) years; [43%]	4.5 (2) years; [100%]	< 0.001¶
DSS 6	27.3 (NA) years; [14%]	9.1 (7) year; [94%]	< 0.001 ¶
DSS 8	n/a	17.2 (17) years; [75%]	

Table 4.12 Number of attacks experienced during the first two years among *frequent early relapsers*, stratified by the type of disease course (RR = 55; SP = 103).

	Frequent early relapsers	
Attacks in y1-y2	RR MS: n (%)	SP MS: n (%)
3	28 (50.9%)	52 (50.4%)
4	14 (25.4%)	29 (28.1%)
5	9 (16.3%)	14 (13.5%)
6	1 (1.8%)	4 (3.8%)
7	3 (5.4%)	2 (1.9%)
8	0	2 (1.9%)

Figure 4.14 Distribution of the disease duration, expressed as cumulative percentages, among patients with frequent early relapses (≥ 3 attacks), in whom conversion to SP MS did not occur.



4.3.2 First inter-attack interval

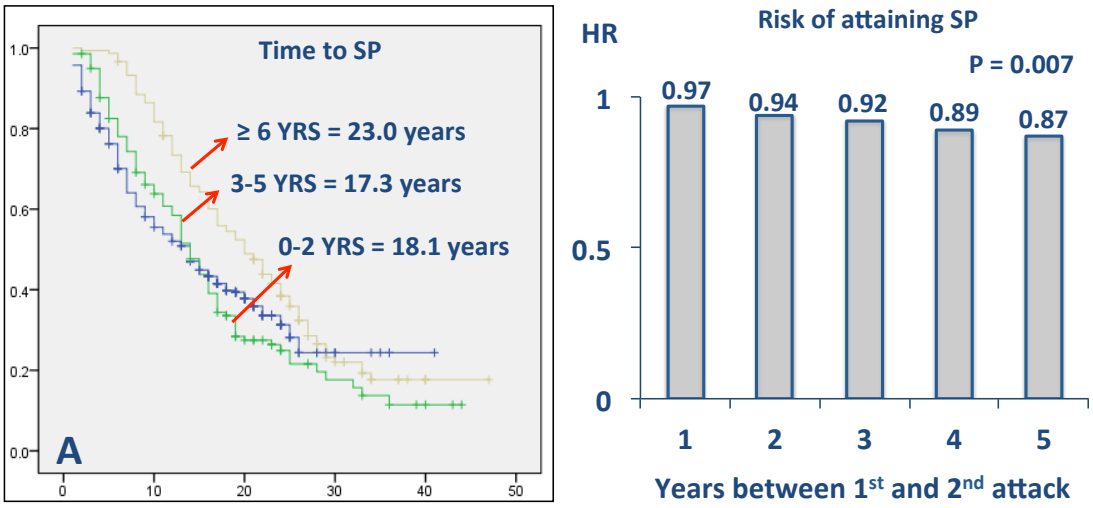
The First inter-attack interval was known for 684 patients and it spanned from 1 to 34 years (median time = 2 years). More than half (n = 388) of patients had second attack within 2 years, 20% (n = 141) after 3-5 years and 22% (n = 155) after 6 years or more. The analysis confirmed (Weinshenker et al., 1989c) that a shorter time between the first two attacks associated with significantly shorter times to disability endpoints and with a greater risk of developing severe disability (Figure 4.15 and Table 4.13).

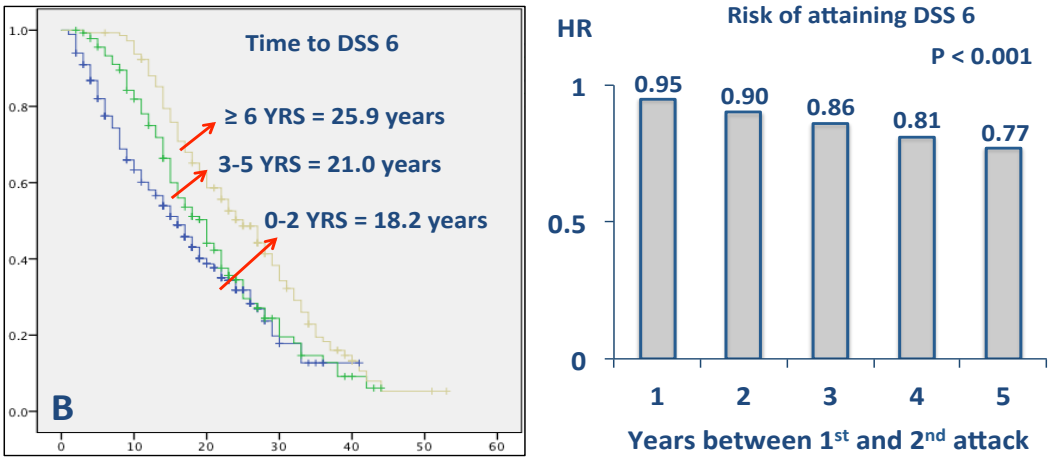
There was a difference of 4.9, 7.7 and 10.4 mean years between the group with short (0-2 years) and long (≥ 6 years) first inter-attack interval when attaining SP, DSS 6 and DSS 8, respectively (Table 4.13). Accordingly, a longer time to the second attack associated with a proportionally lower hazard of SP (HR = 0.97) and of becoming disabled (HR for DSS 6 = 0.95; HR for DSS 8 = 0.93). Although significant, the size of this effect was relatively small; the probability of reaching SP, DSS 6 and DSS 8 decreased by approximately 13% (HR = 0.87), 23% (HR = 0.77) and 28% (HR = 0.72), respectively, when the second attack occurred after 5 years (Figure 4.15). In addition, the first inter-attack interval did not significantly affect the disease evolution from DSS 3, and only marginally impacted on the evolution of the SP phase (times to endpoints from onset of SP) (Table 4.14 and 4.15).

Table 4.13 Kaplan Meier estimated times to SP, to DSS 6 and to DSS 8 from the disease onset in patients grouped by the interval between the first two attacks. P values were obtained using the Log Rank test by comparison with the reference category (*).

1st inter-attack interval	Time to onset of SP		Time from disease onset to DSS 6		Time from disease onset to DSS 8	
	mean [95% CI] years (median)	p value	mean [95% CI] years (median)	p value	mean [95% CI] years (median)	p value
0-2 years	18.1 [16.2-19.9] (14)	0.002	18.2 [16.2-19.8] (16)	< 0.001	25.3 [23.6-26.8] (25)	< 0.001
3-5 years	17.3 [14.9-19.6] (14)	0.002	21.0 [18.9-23.0] (20)	0.005	32.5 [29.5-35.4] (31)	0.18
≥ 6 years*	23.0 [20.7-25.2] (20)		25.9 [23.7-27.9] (25)		35.7 [32.6-38.6] (33)	

Figure 4.15 Kaplan Meier analysis (left): estimated times to SP (A), to DSS 6 (B) and to DSS 8 (C) among groups with short (0-2 years), intermediate (3-5 years) and long (≥ 6 years) interval between the first two attacks. Cox regression analysis (right): variation of the risk (HRs on y-axis) of attaining the endpoints according to increasing years between the first two attacks (x-axis). The exact HRs are indicated on top of each column.





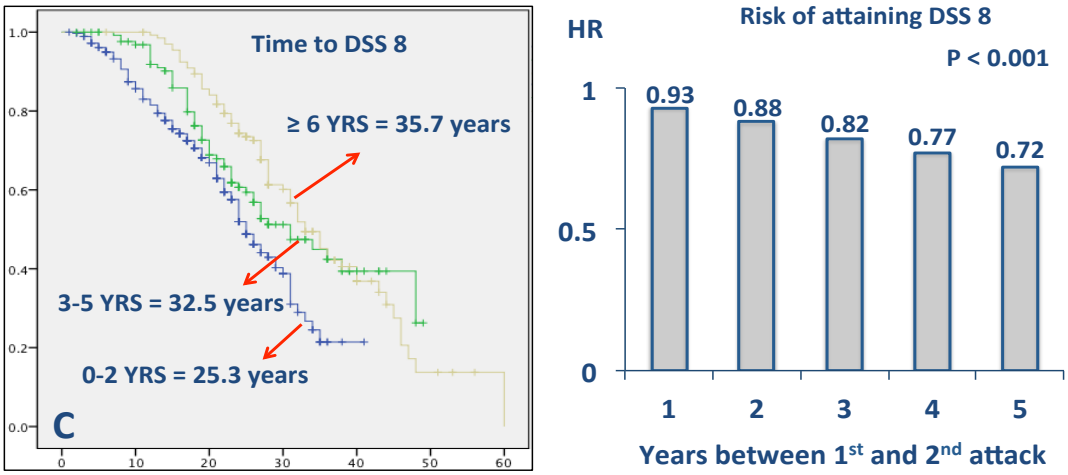


Table 4.14 Kaplan Meier analysis: times to DSS 6 and to DSS 8 from DSS 3 and from onset of progression (SP) in patients grouped by the interval between the first two attacks. P values were obtained with Log Rank test by comparison with the reference category (*).

1st inter-attack interval	Time to DSS 6			
	From DSS 3		From onset of progression	
	mean [95% CI] years (median)	p value	mean [95% CI] years (median)	p value
0-2 years	6.9 [6.1-7.6] (5)	0.05	4.0 [3.3-4.7] (3)	< 0.001
3-5 years	7.9 [6.6-9.2] (5)	0.48	5.6 [4.4-6.7] (3)	0.24
≥ 6 years*	8.4 [7.1-9.8] (6)		6.6 [5.3-8.0] (5)	
	Time to DSS 8			
	From DSS 3		From onset of progression	
	mean [95% CI] years (median)	p value	mean [95% CI] years (median)	p value
0-2 years	16.0 [14.5-17.3] (15)	0.10	12.3 [11.0-13.4] (11)	0.01
3-5 years	22.3 [18.5-26.0] (16)	0.65	17.7 [13.9-21.3] (13)	< 0.001
≥ 6 years*	17.4 [15.5-19.2] (16)		16.2 [13.5-18.8] (14)	

Table 4.15 Cox regression analysis: risk of attaining DSS 6 from disease onset, from DSS 3 and from onset of progression according to interval (years) between the first two attacks.

1st inter-attack interval	Risk of attaining DSS 6		
	From disease onset	From DSS 3	From onset of SP
	HR (p < 0.001)	HR (p < 0.20)	HR (p < 0.001)
1 year	0.95	0.99	0.96
2 years	0.90	0.97	0.92
3 years	0.86	0.96	0.89
4 years	0.81	0.95	0.85
5 years	0.77	0.94	0.82

4.3.3 Late relapses

Within the SP population (n = 534), the number of relapses experienced from the third year up to onset of progression was known for 436 patients and it ranged from 0 to 17 attacks. A total of 1038 attacks was recorded, with a mean relapse rate of 0.41 attacks/year; 107 patients had no attack after year 2, before entering the SP phase.

The number of late relapses did not affect negatively the attainment of late outcomes. Indeed, the analysis highlighted a proportional relationship with the disease evolution. A higher late relapses frequency associated with significantly longer times to disability endpoints and with a significantly lower risk of developing severe disability (Figure 4.16 and Table 4.16). Patients with no attacks, compared to patients with ≥ 3, took significantly shorter times to SP (5.4 mean years difference; $p < 0.001$) and to DSS 6 (4.3 mean years difference; $p = 0.003$). The same trend was observed for the attainment of DSS 8, although differences did not reach statistical significance ($p = 0.07$) (Table 4.16). Late relapses yielded a negative regression coefficient for reaching SP (- 0.0956; HR = 0.90; $p < 0.001$) and DSS 6 (- 0.0531; HR = 0.94; $p = 0.015$), indicating that the hazard of accumulating severe disability decreased proportionally to the number of attacks. The size of this effect was larger on the probability of entering the SP phase (5 attacks reduced the risk by approximately 38%; HR = 0.62) than on the probability of attaining DSS 6 (5 attacks reduced the risk by approximately 24%; HR = 0.76). Similar impact, although not statistically significant, was exerted on the risk of DSS 8 (- 0.0489; HR = 0.95; $p = 0.063$). The predictive effect of late relapses was also tested within the subgroup (n = 165) with frequent late attacks (≥ 3). The times to SP, to DSS 6 and to DSS 8 were not significantly different among those with 3-4 (n = 93), 5-6 (n = 44) or ≥ 7 attacks (n = 28) (Figure 4.17). Finally, the number of late relapses did not significantly affect the times to disability endpoints from DSS 3 and from the onset of SP (Table 4.17).

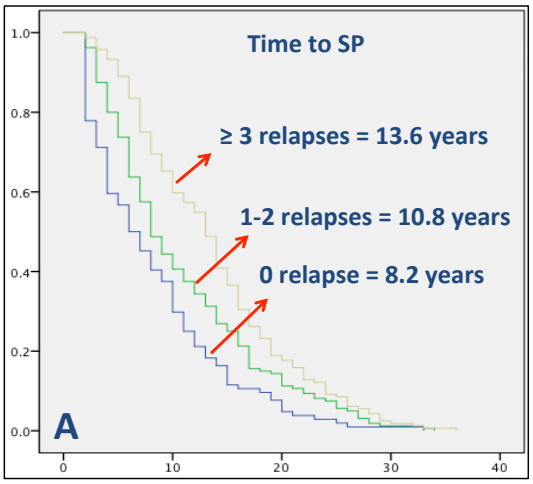
Table 4.16 Kaplan Meier estimated times to SP, to DSS 6 and to DSS 8 from the disease onset in patients grouped by the number of relapses from years 3 to the onset of SP. P values were obtained with Log Rank test by comparison with the reference category (*).

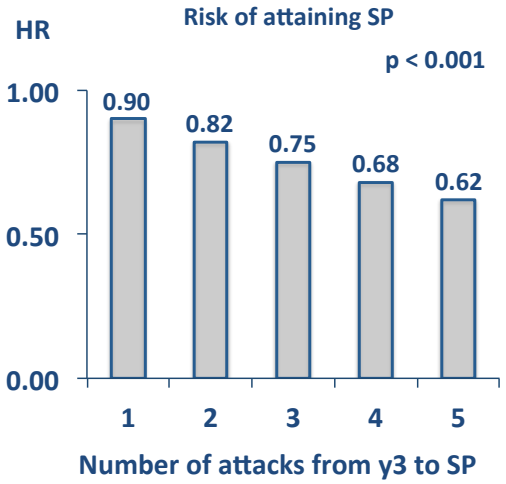
No of relapses y3 to SP	Time to onset of SP		Time from disease onset to DSS 6		Time from disease onset to DSS 8	
	mean [95% CI] years (median)	p value	mean [95% CI] years (median)	p value	mean [95% CI] years (median)	p value
0 relapse	8.2 [7.0-9.4] (6)	< 0.001	13.1 [11.2-14.9] (12)	0.003	24.6 [21.6-27.0] (23)	0.07
1-2 relapses	10.8 [9.6-11.9] (8)	0.003	16.3 [14.7-17.8] (15)	0.180	26.1[24.0-29.3] (24)	0.17
≥ 3 relapses*	13.6 [12.5-14.7] (13)		17.4 [15.9-18.9] (15)		28.1 [25.5-29.9] (26)	

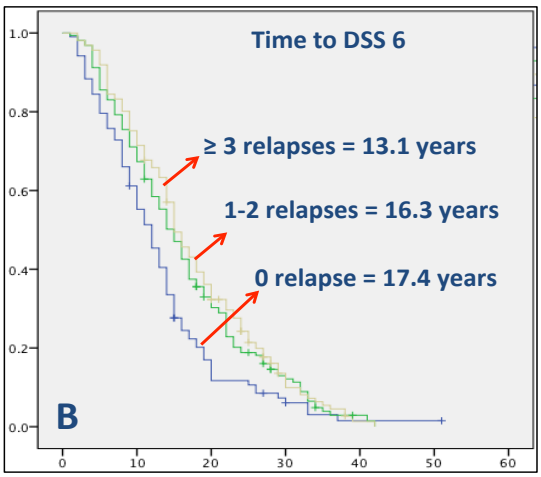
Table 4.17 Kaplan Meier analysis: times to DSS 6 and to DSS 8 from DSS 3 and from onset of progression (SP) in patients grouped by number of attacks from year 3 to SP onset. P values were obtained with Log Rank test by comparison with the reference category (*).

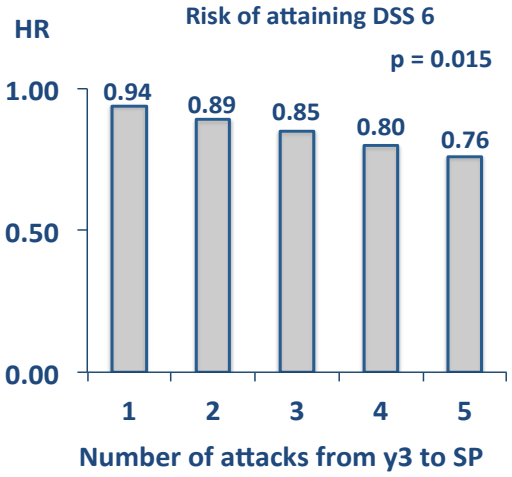
No of relapses y3 to SP	Time to DSS 6			
	From DSS 3		From onset of progression	
	mean [95% CI] years (median)	p value	mean [95% CI] years (median)	p value
0 relapse	5.5 [4.5-6.6] (4)	0.20	5.4 [4.2-6.6] (4)	0.09
1-2 relapses	6.8 [5.9-7.8] (5)	0.59	5.8 [4.8-6.7] (4)	0.01
≥ 3 relapses*	6.4 [5.5-7.3] (5)		4.2 [3.4-5.0] (3)	
	Time to DSS 8			
	From DSS 3		From onset of progression	
	mean [95% CI] years (median)	p value	mean [95% CI] years (median)	p value
1 relapse	16.2 [14.1-18.2] (14)	0.87	15.5 [13.4-17.5] (13)	0.10
2 relapses	16.5 [14.0-19.0] (14)	0.75	15.4 [12.7-17.9] (12)	0.27
≥ 3 relapses*	17.0 [15.0-18.8] (15)		14.1 [12.0-16.2] (11)	

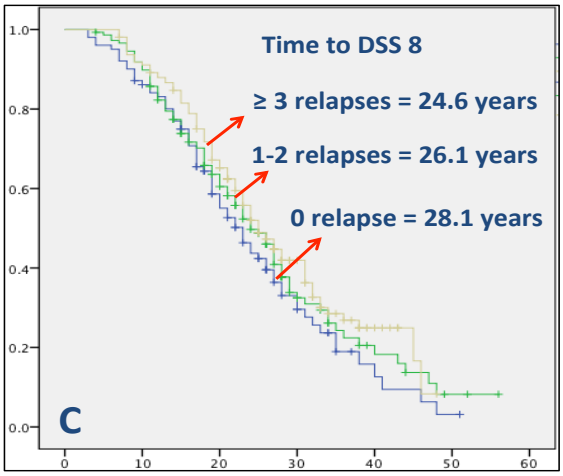
Figure 4.16 Kaplan Meier analysis (left): estimated times to SP (A), to DSS 6 (B) and to DSS 8 (C) among groups with low (0 attacks), intermediate (1-2 attacks) and high (≥ 3 attacks) number of relapses from year 3 to SP onset (late relapses). Cox regression analysis (right): variation of the risk (HRs on y-axis) of attaining the endpoints according to the increasing number of relapses from year 3 up to SP onset (x-axis). The exact HRs are indicated on top of each column.











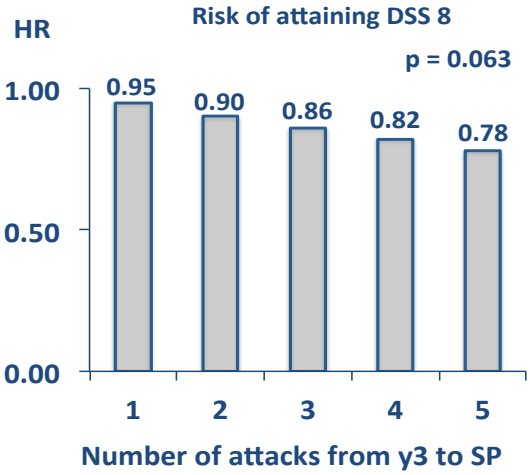
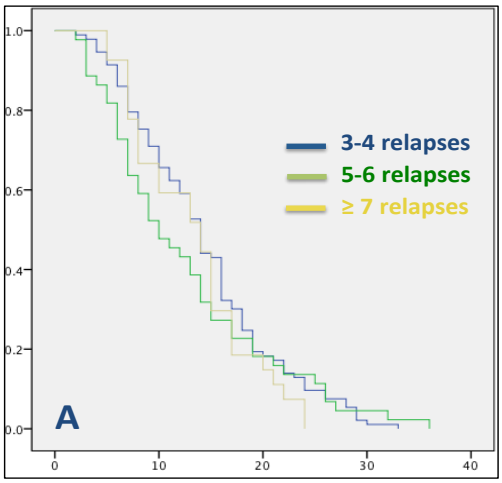
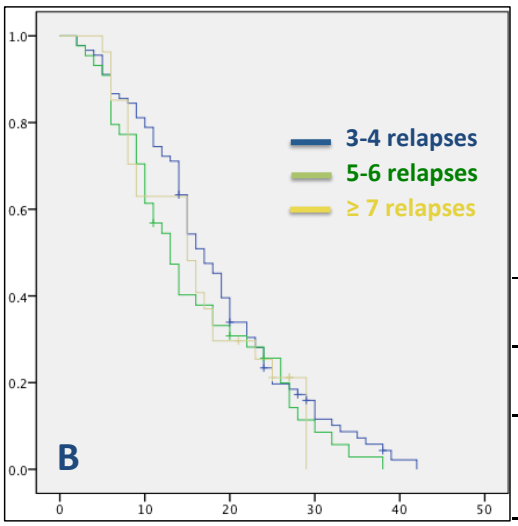


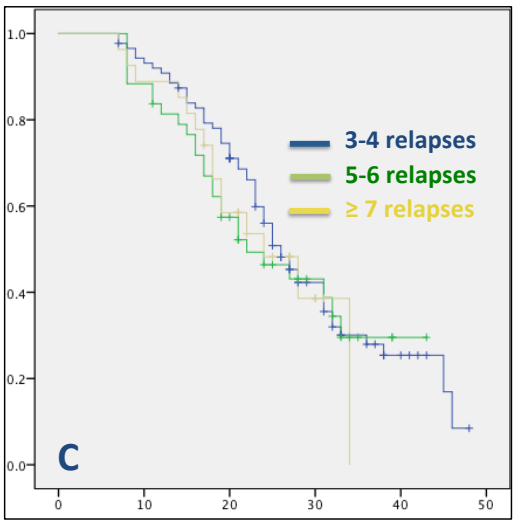
Figure 4.17 Kaplan Meier analysis and Cox regression analysis: estimated times to SP (A), to DSS 6 (B) and to DSS 8 (C) among groups with frequent late relapses (n = 165; ≥ 3 attacks). Comparison of survival curves among those with low (3-4 attacks), intermediate (5-6 attacks) and high (≥ 7 attacks) frequency. HRs (risk of attaining the endpoints) and p values were obtained with Cox Regression analysis by comparison with reference category (*).



No of relapses y3 to SP	Time to onset of SP		
	mean years (median)	HR	p value
3-4 relapses	14.3 (14)	0.82	0.38
5-6 relapses	12.5 (10)	0.94	0.83
≥ 7 relapses*	13.2 (14)		



No of relapses y3 to SP	Time from disease onset to DSS 6		
	mean years (median)	HR	p value
3-4 relapses	18.3 (17)	0.84	0.46
5-6 relapses	15.9 (13)	1.07	0.770
≥ 7 relapses*	16.2 (15)		



No of relapses y3 to SP	Time from disease onset to DSS 8		
	mean years (median)	HR	p value
3-4 relapses	28.5 (26)	0.85	0.59
5-6 relapses	26.1 (22)	0.98	0.95
≥ 7 relapses*	24.2 (24)		

Late relapses were recorded during a necessarily variable time, from year 3 up to SP onset. This makes problematic the comparison with early relapses, which were counted in the fixed time of 2 years from the disease onset. The predictive effect of late relapses was therefore tested among patients matched by the duration of the year 3-SP period. In order to make a more appropriate comparison with early relapses, the association between late relapses and the disease evolution was analysed in small subgroups, divided by serial 2-year intervals of the time from year 3 to SP. For each 2-years interval, past year 2 no significant impact of late relapses on the times to DSS 6 and to DSS 8 was found (Figure 4.18). In addition, the predictive effect of relapses after year 2 was assessed in two subgroups of patients matched by the duration of the RR phase (short = 1-13 years, n = 270; long ≥ 14 years, n = 145). This allowed ruling out the independent impact of the latency to progression on the outcomes. Again, the time to disability endpoints remained largely unaffected by the late relapses frequency (Figure 4.19).

Figure 4.18 Kaplan Meier analysis: time to DSS 6 and to DSS 8 in subgroups of patients stratified by the number of late relapses and by the duration of the period from year 3 to SP onset. P values were obtained with Log Rank Test.

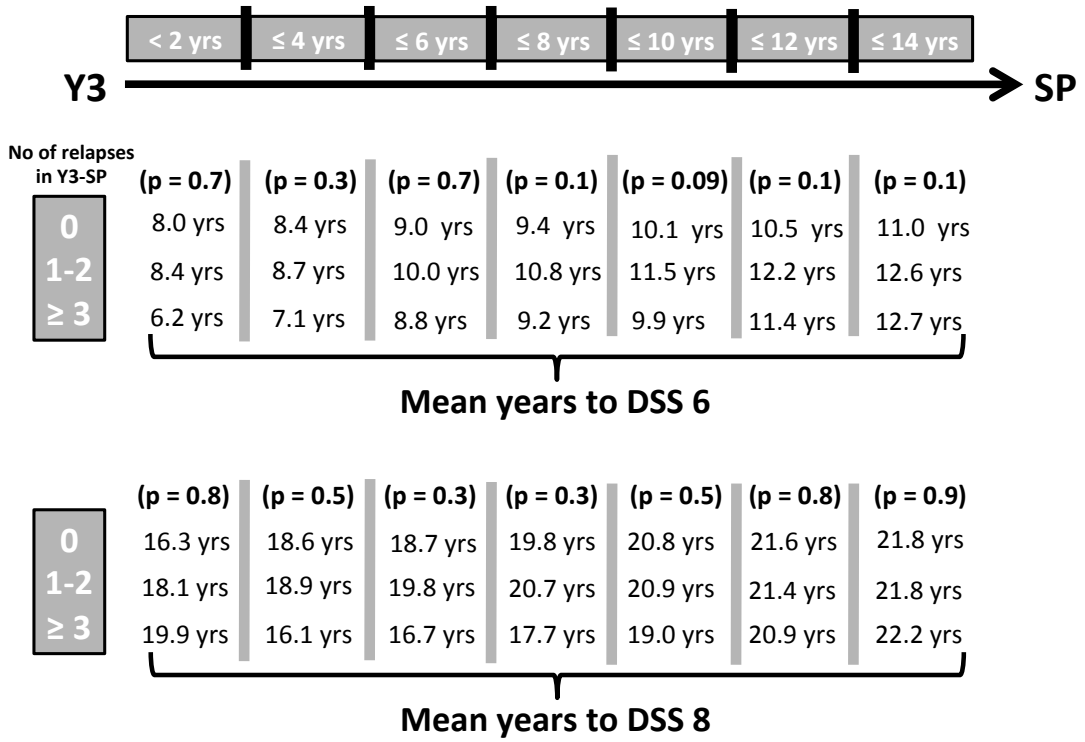
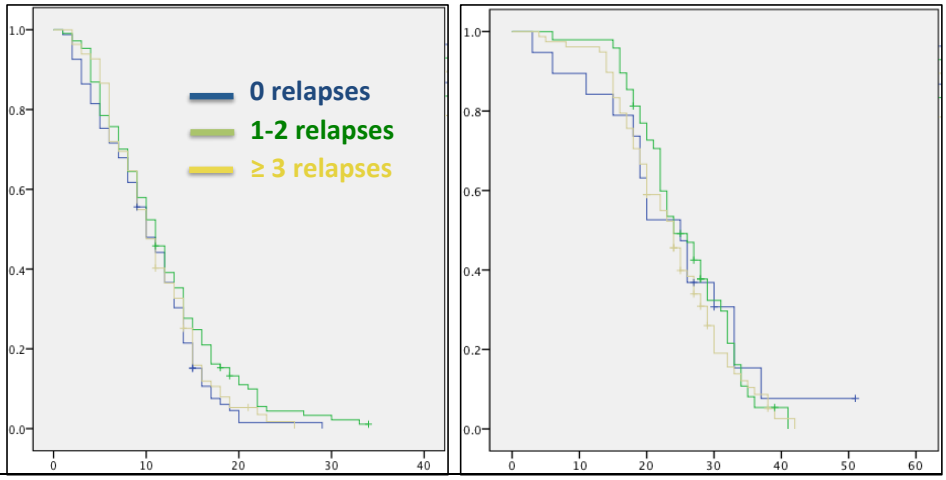


Figure 4.19 Kaplan Meier and Cox regression analyses: time to DSS 6 and risk (HRs) of reaching DSS 6 in two subgroups of patients stratified by the duration of the RR phase (short 1-13 years, long ≥ 14 years). Comparison of survival curves among patients grouped by the number of late relapses (0; 1-2; ≥ 3). P values were obtained from Cox regression analysis by comparison with reference category (*).



RR phase = 1-13 years | RR >/- 14 years

Relapses from y3 to SP	Mean (median) years to DSS 6	HR	p	Mean (median) years to DSS 6	HR	p
0 relapse	10.2 (10)	1.07	0.64	24.6 (25)	0.82	0.48
1-2 relapses	11.7 (11)	0.83	0.24	25.7 (24)	0.85	0.43
≥ 3 relapses*	10.8 (10)			23.9 (24)		

4.3.4 Total relapses

Within the SP group (n = 534), information on the total number of attacks during the RR phase was available for 459 patients, who experienced a total of 1882 relapses. The frequency spanned from 1 to 18 attacks, with a mean relapse rate before SP of 0.65 relapses/year.

Total relapses exerted no significant impact on the disease evolution (Figure 4.21 and Table 4.18). More attacks during the RR phase were associated with a longer time to SP onset (3.4 mean years difference between those with 1-2 attacks versus those with ≥ 5 attacks, $p < 0.001$) and with a lower risk (regression coefficient = -0.0417; HR = 0.96, $p = 0.02$) of entering the SP phase. Although small in size, this effect was statistically significant, and it was unlikely to be an artefact of how the onset of progression was defined. Indeed, a larger number of total relapses correlated with a significantly higher DSS score at conversion to SP MS ($r = 0.46$; $p <$

0.001), secondary to the longer latency to progression. The mean DSS score was 2.4 (median = 2), 2.8 (median = 3) and 3.4 (median = 3) among patients with 1-2, 3-4 and ≥ 5 attacks, respectively (Figure 4.20). In addition, the analysis in subgroups starting to progress at DSS ≤ 2 and at DSS ≤ 3, where the onset of the progressive phase was not clouded by concomitant relapses and therefore more easily pinpointed (Kremenchutzky et al., 2006a), yielded the same results, demonstrating a shorter time to SP among patients with fewer relapses (Table 4.19).

The times to disability endpoints from the disease onset were remarkably similar among the groups with low (1-2), intermediate (3-4) or large (≥ 5) number of attacks during the RR phase. The 3 groups required a walking aid (DSS 6) and become bedbound (DSS 8) in 15 and 25-26 mean years, respectively (Table 4.18). Among patients seen from onset only (SO subgroup) the analysis confirmed no effect of total relapses on times to disability endpoints (mean years to DSS 6: 1-2 relapses = 9.5, 3-4 relapses = 10.8, ≥ 5 relapses = 8.8; p = 0.65), demonstrating that recollection bias did not affect the results. In addition, total relapses did not affect the disease evolution from DSS 3, and only modestly impacted on the evolution of the SP phase (Table 4.19). Finally, the comparison of the two subgroups at the extremes of the total relapse frequency (1 attack n = 68 versus ≥ 7 attacks n = 70) further demonstrated no significant impact of the attacks during the RR phase on the long-term outcome. The latency to progression was shorter among those with 1 attack, and the times to DSS 6 and to DSS 8 were almost the same between two subgroups (Figure 4.22).

Figure 4.20 DSS score (y-axis) at conversion to SP MS, among patients grouped by the number of total relapses during the RR phase (x-axis).

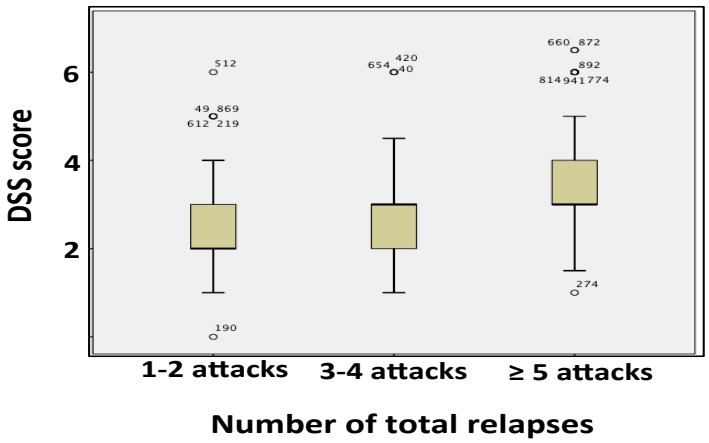


Table 4.18 Kaplan Meier estimated times to SP, to DSS 6 and to DSS 8 from the disease onset in patients grouped by the number of total relapses during the RR phase. P values were obtained with Log Rank test by comparison with the reference category (*).

Total attacks during the RR phase	Time to onset of SP		Time from disease onset to DSS 6		Time from disease onset to DSS 8	
	mean [95% CI] years (median)	p value	mean [95% CI] years (median)	p value	mean [95% CI] years (median)	p value
1-2 relapses	8.8 [7.7-9.8] (7)	< 0.001	15.6 [13.9-17.3] (14)	0.66	26.2 [23.7-28.4] (26)	0.74
3-4 relapses	11.3 [10.0-12.6] (10)	0.34	15.7 [14.0-17.4] (14)	0.98	25.8 [23.0-28.6] (24)	0.64
≥ 5 relapses*	12.2 [11.1-13.4] (11)		15.9 [14.3-17.5] (14)		26.6 [24.0-29.1] (24)	

Table 4.19 Kaplan Meier estimated times to SP in patients grouped by the number of total relapses during the RR phase, and differentiated by the DSS score at conversion to SP MS. P values were obtained with Log Rank test by comparison with the reference category (*).

Total attacks during the RR phase	Time to onset of SP (onset of progression ≤ DSS 2)		Time to onset of SP (onset of progression ≤ DSS 3)	
	mean years (median)	p value	mean years (median)	p value
1-2 relapses	9.1 (8)	0.004	8.7 (7)	< 0.001
3-4 relapses	11.4 (12)	0.33	10.7 (9)	0.028
≥ 5 relapses*	13.7 (12)		13.1 (13)	

Table 4.20 Kaplan Meier analysis: times to DSS 6 and to DSS 8 from DSS 3 and from the onset of progression (SP) in patients grouped by the total number of relapses during the RR phase. P values were obtained with Log Rank test by comparison with the reference category (*).

Total attacks during the RR phase	Time to DSS 6			
	From DSS 3		From onset of progression	
	mean [95% CI] years (median)	p value	mean [95% CI] years (median)	p value
1-2 relapses	6.4 [5.4-7.3] (5)	0.79	6.9 [5.7-8.0] (5)	< 0.001
3-4 relapses	6.4 [5.3-7.4] (5)	0.91	4.6 [3.9-5.4] (4)	0.045
≥ 5 relapses*	6.2 [5.3-7.1] (5)		3.7 [2.9-4.5] (2)	
	Time to DSS 8			
	From DSS 3		From onset of progression	
	mean [95% CI] years (median)	p value	mean [95% CI] years (median)	p value
1-2 relapses	16.6 [14.7-18.3] (15)	0.87	16.8 [14.8-18.7] (14)	0.004
3-4 relapses	15.6 [13.7-17.3] (13)	0.68	13.0 [11.4-14.6] (12)	0.48
≥ 5 relapses*	16.6 [14.6-18.6] (14)		13.7 [11.4-15.8] (11)	

Figure 4.21 Kaplan Meier analysis (left): estimated times to SP (A), to DSS 6 (B) and to DSS 8 (C) among groups with low (1-2 attacks), intermediate (3-4 attacks) and high (≥ 5 attacks) number of relapses during the RR phase (total relapses). Cox regression analysis (right): variation of the risk (HRs on y-axis) of attaining the endpoints according to the increasing number of relapses during the RR phase (x-axis). The exact HRs are indicated on top of each column.

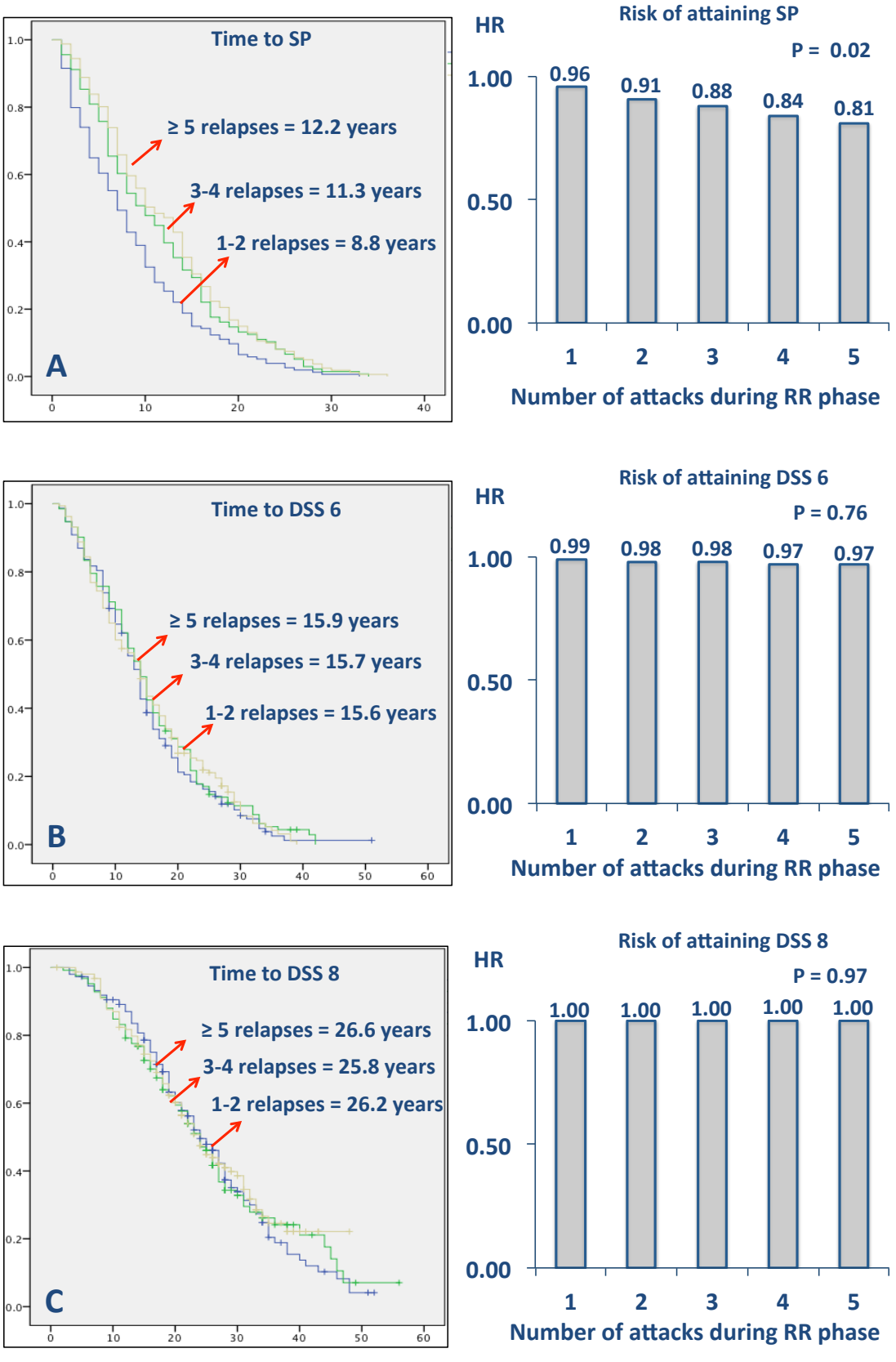
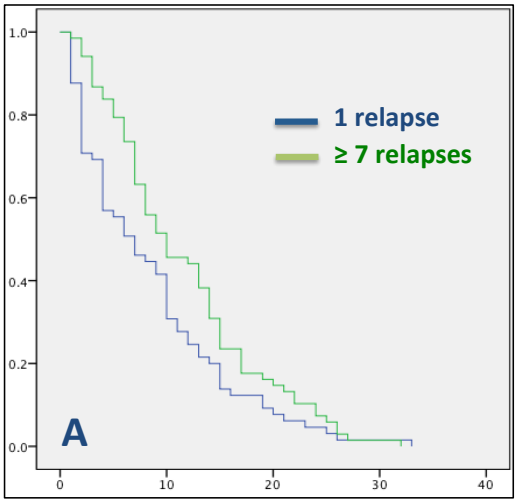
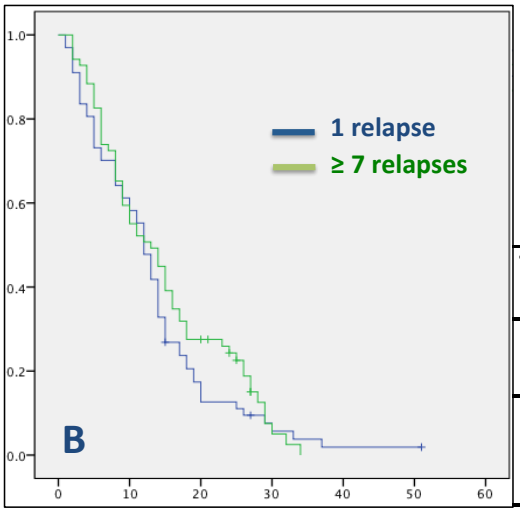


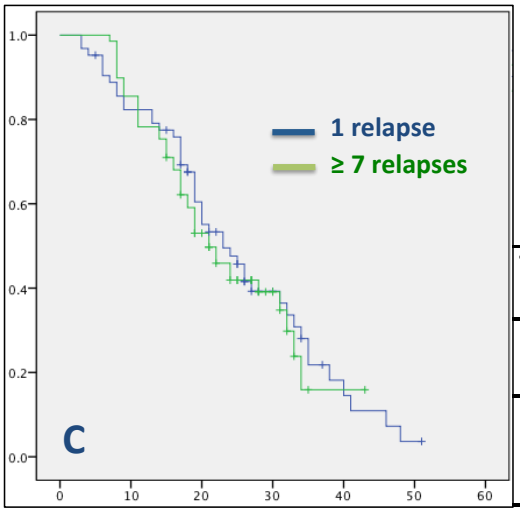
Figure 4.22 Kaplan Meier analysis and Cox regression analysis: estimated times to SP (A), to DSS 6 (B) and to DSS 8 (C) among groups with very low (n = 68, 1 attack) and very high (n = 70, ≥ 7 attacks) number of total relapses. HRs (risk of attaining the endpoint) and p values were obtained with Cox Regression analysis by comparison with reference category (*).



Total attacks during the RR phase	Time to onset of SP		
	mean years (median)	HR	p value
1 relapse	8.5 (7)	1.39	0.06
≥ 7 relapses*	11.6 (10)		



Total attacks during the RR phase	Time from to DSS 6		
	mean years (median)	HR	p value
1 relapse	13.1 (12)	1.16	0.39
≥ 7 relapses*	14.6 (13)		



Total attacks during the RR phase	Time from to DSS 8		
	mean years (median)	HR	p value
1 relapse	25.1 (23)	0.92	0.70
≥ 7 relapses*	23.9 (21)		

4.3.5 Multivariate models of disability

The concomitant effect of the variables on the outcomes was tested in multivariate models of disability.

4.3.5.1 Early relapses and the type of clinical onset

The type and the number of neurological systems involved at clinical onset did not exert any significant impact on the long-term outcome. Polysymptomatic onset did not affect times to SP (HR = 1.02, p = 0.76), to DSS 6 (HR = 1.03, p = 0.58) and to DSS 8 (HR = 1.12, p = 0.12). In addition, among the types of symptoms characterizing the first attack, only brainstem presentation associated with a higher risk of reaching DSS 6 (HR = 1.31, p = 0.02) and DSS 8 (HR = 1.59, p = 0.001). The predictive effect of early relapses remained unchanged when tested in the multivariate model along with type and number of symptoms at onset (Table 4.21).

Table 4.21 Cox Regression multiple analysis: risk of attaining SP, DSS 6 and DSS 8 according to the concomitant effect of the number of early relapses, the type and the number of neurological systems involved at the clinical onset.

	Risk of SP		Risk of DSS 6		Risk of DSS 8	
	HR	**p**	**HR**	**p**	**HR**	**p**
Relapses y1-y2	1.11	0.03	1.22	< 0.001	1.33	< 0.001
Motor presentation	1.20	0.18	1.13	0.35	1.25	0.16
Sensory presentation	0.91	0.52	0.98	0.91	0.98	0.91
Cerebellar presentation	1.05	0.78	1.21	0.30	1.43	0.09
Brainstem presentation	1.11	0.48	1.33	0.05	1.65	0.003
Optic presentation	0.95	0.73	0.94	0.65	0.89	0.48
Polysymptomatic onset	0.89	0.41	0.96	0.74	0.94	0.70

4.3.5.2 Early relapses and the first inter-attack interval

When assessed simultaneously, early relapses and the first inter-attack interval exerted the same impact on the risk of entering the SP phase (HR = 1.10, p = 0.02 and HR = 0.98, p = 0.05, respectively) and of attaining DSS 6 (HR = 1.18, p < 0.001 and HR = 0.96, p < 0.001, respectively) and DSS 8 (HR = 1.28, p < 0.001 and HR = 0.96, p < 0.001, respectively), compared to when assessed separately (univariate analysis).

4.3.5.3 Early relapses and late relapses

In the multivariate model, the size of the predictive effect exerted by early relapses increased, while the impact of late relapses on the outcomes remained unchanged. The analysis confirmed that, among SP patients, the hazard of entering the progressive phase, and of reaching DSS 6 and DSS 8 increased proportionally with the number of attacks during the first 2 years (HRs 1.34, $p < 0.001$; 1.34, $p < 0.001$; 1.34. $p < 0.001$, respectively) and decreased proportionally to the number of attacks from year 3 up to SP onset (HRs 0.90, $p < 0.001$; 0.93, $p = 0.003$; 0.94, $p = 0.020$, respectively).

The Multiple Cox regression analysis allowed calculating the probability of attaining DSS 6, according to the concomitant effect of early and late relapses. Among SP patients, grouped by the number of early relapses, the hazard of reaching DSS 6 (1 attack HR = 1.34, 2 attacks HR = 1.80, 3 attacks HR = 2.43) in each group reduced with the increasing number of attacks after year 2, and become about 30-40% smaller if 3 relapses occurred during the year 3-SP period (HRs 1.10, 1.48, 2.00, respectively) (Figure 4.23). The Kaplan Meier analysis was used to estimate the times to the endpoint, in subgroups of patients selected according to the early and late relapses frequencies, and confirmed the results from the Cox regression multiple analysis. Among patients with 1, 2 or 3 attacks during the first two years, the times to DSS 6 increased proportionally to the number of late relapses (Table 4.22). In addition, the multivariate model further highlighted year 2 as the watershed, after which the predictive effect of relapses reversed. Two hypothetical patients, experiencing 3 relapses at different timing before entering the progressive phase, were compared. The time to DSS 6 was much shorter and the risk of reaching the endpoint was much higher when the onset attacks was followed by two additional relapses occurring during the first two years (3 attacks in y1-y2 + 0 attacks in y3-SP: time to DSS 6 = 7.4 years, HR = 2.43), compared to when two additional relapses occurred during the year 3-SP period (1 attack in y1-y2 + 2 attacks in y3-SP: time to DSS 6 = 22.7 years, HR = 1.18) (Figure 4.23 and Table 4.22). The comparison of these

two clinical scenarios demonstrated a difference of almost 50% less probability of reaching the endpoint (HR = 0.48), among those with 2 relapses after year 2.

Figure 4.23 Multiple Cox regression analysis in SP patients: calculated risk (HR) of attaining DSS 6 from the disease onset according to the concomitant effect of early and late relapses. On the y-axis is shown the variation of the HRs (on top of each column) among patients with 1, 2, 3 attacks during the first two years (x-axis), experiencing increasing number of attacks from year 3 to SP onset.

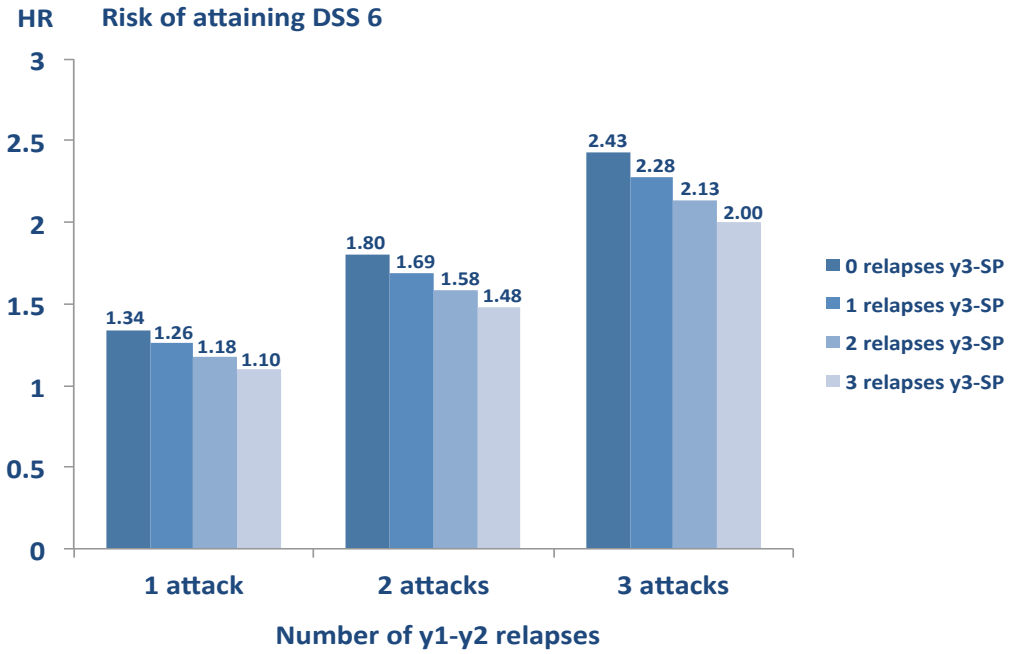


Table 4.22 Kaplan Meier analysis in SP patients. Estimated times to DSS 6 based on the number of early and late relapses.

Relapses		patients	Mean (median)
y1-y2 (n)	y3-SP (n)	(n)	years to DSS 6
1	0	59	14.3 (13)
1	1	54	18.3 (17)
1	2	35	22.7 (22)
1	3	34	17.5 (15)
2	0	23	12.8 (10)
2	1	23	13.2 (12)
2	2	9	15.7 (13)
2	3	14	15.5 (14)
3	0	9	7.4 (5)
3	1	12	9.2 (6)
3	2	9	11.7 (8)
3	3	5	10.6 (6)

4.3.5.4 Early relapses, first inter-attack interval and late relapses

When the first inter-attack interval (HR = 0.92) was included in the multivariate analysis, calculating the probability of reaching DSS 6 from the disease onset, the impact of early relapses slightly decreased (HR = 1.23), while the effect of late relapses remained unchanged (HR = 0.91) (Table 4.23). The multivariate model allowed assessing more precisely the influence of the timing of the second attack on the outcome, by simulating different hypothetical clinical scenarios (Table 4.24). Model A refers to a patient experiencing 2 relapses before converting to SP MS. Two attacks within 2 years from the disease onset (first inter-attack interval = 1 years) yielded HR 1.42 for reaching DSS 6. However, when the second attack occurred at year 3 (first inter-attack interval = 2 years) the hazard reversed (HR = 0.97) (Table 4.24 model A) and became about 32% lower (comparison of the two scenarios yielded HR = 0.68). These results indicated that the predictive effect of the first inter-attack interval largely depended on whether or not the second attack occurred within the first two years. In addition, the risk of attaining the endpoint decreased modestly and proportionally to the duration of the interval between the first two attacks (Table 4.24 model A). Finally, model B confirmed that the number of late relapses associated with a proportionally lower probability of developing severe disability even when it was adjusted to the first inter-attack interval (Table 4.24).

Table 4.23 Cox regression multivariate analysis. Risk (HR) of attaining DSS 6 from the disease onset, from the onset of progression (SP) and from DSS 3, according to the concomitant effect of early relapses, first inter-attack interval and late relapses. RC = regression coefficient.

	Risk of reaching DSS 6								
	From disease onset			**From onset of progression**			**From DSS 3**		
	RC	HR (95% CI)	p value	RC	HR (95% CI)	p value	RC	HR (95% CI)	p value
Relapses y1-y2	0.213	1.23 (1.13-1.35)	< 0.001	0.282	1.32 (1.18-1.48)	< 0.001	0.082	1.08 (0.99-1.18)	0.06
First inter-attack interval	-0.073	0.92 (0.90-0.95)	< 0.001	-0.010	0.98 (0.96-1.01)	0.37	-0.011	0.98 (0.96-1.01)	0.33
Relapses Y3 to SP	-0.088	0.91 (0.87-0.95)	< 0.001	0.034	1.03 (0.99-1.07)	0.09	-0.032	0.96 (0.92-1.01)	0.14

Table 4.24 Cox regression multivariate analysis. Risk of attaining DSS 6 calculated by combining the effect of early relapses, first inter-attack interval and late relapses.

Model A	Risk of attaining DSS 6		
Relapses y1-y2 (n)	**INT-ATT (yrs)**	**Relapses y3-SP (n)**	**(HR)**
2	1	0	**1.42**
1	2	1	**0.97**
1	3	1	**0.90**
1	4	1	**0.84**
1	5	1	**0.78**

Model B	Risk of attaining DSS 6		
Relapses y1-y2 (n)	**INT-ATT (yrs)**	**Relapses y3-SP (n)**	**(HR)**
1	2	1	**0.97**
1	2	2	**0.89**
1	2	3	**0.81**
1	2	4	**0.74**
1	2	5	**0.68**

4.3.5.5 Early relapses and time to moderate disability

The interval between the disease onset and moderate disability (DSS 3) was known for 634 patients and ranged from 1 to 40 years. Among patients who reached the endpoint, the mean time to DSS 3 was 9.5 years (95% C.I. 8.9-10.1) and the median time was 8 years. The analysis demonstrated that the time to DSS 3 predicted the long-term disease evolution. A longer interval associated with modestly, but significantly longer times from DSS 3 to DSS 6 and to DSS 8 (Table 4.25) and with a lower risk of attaining the endpoints (DSS 6, HR = 0.97, $p < 0.001$; DSS 8 HR = 0.96, $p < 0.001$) (Table 4.26). In addition, the predictive effect of the time to DSS 3 was independent of the number of early relapses and of the first inter-attack interval, as it remained unchanged in the multivariate analysis (Table 4.26). In contrast, when adjusted for the time to reach DSS 3, the effect of total attack during the first two years on the outcomes became smaller (Table 4.26) than the univariate analysis (Tables 4.9), although it remained marginally significant.

Table 4.25 Kaplan Meier analysis: times from DSS 3 to DSS 6 and to DSS 8 in patients grouped by interval from disease onset to moderate disability (DSS 3). P values obtained with Log Rank test. * Reference category.

Interval from disease onset to DSS 3	**Time from DSS 3 to DSS 6**		**Time from DSS 3 to DSS 8**	
	mean [95% CI] years (median)	**p value**	**mean [95% CI] years (median)**	**p value**
0-2 years	5.1 [4.2-6.1] (4)	< 0.001	14.1 [12.1-16.1] (11)	< 0.001
3-7 years	7.7 [6.6-8.8] (6)	0.70	17.1 [15.1-19.2] (16)	0.05
≥ 8 years*	8.7 [7.8-9.6] (6)		18.4 [17.0-19.8] (17)	

Table 4.26 Cox regression multiple analysis. Risk of attaining DSS 6 calculated by combining the effect of early relapses, first inter-attack interval and time to moderate disability (DSS 3). RC = regression coefficient.

	Risk of reaching DSS 6 from DSS 3			**Risk of reaching DSS 8 from DSS 3**		
Univariate analysis	**RC**	**HR (95% CI)**	**p value**	**RC**	**HR (95% CI)**	**p value**
Time to DSS 3	-0.029	0.97 (0.95-0.98)	< 0.001	-0.034	0.96 (0.95-0.98)	< 0.001
Multivariate analysis						
Time to DSS 3	-0.034	0.96 (0.94-0.98)	< 0.001	-0.039	0.96 (0.93-0.98)	0.001
Relapses y1-y2	0.082	1.08 (1.00-1.17)	0.04	0.11	1.11 (1.01-1.23)	0.02
First Inter-attack	0.024	1.02 (0.99-1.05)	0.05	0.031	1.03 (0.99-1.06)	0.05

4.4 Discussion

The relationship between relapses and the accumulation of severe disability is perhaps the most controversial topic in MS. It keeps fuelling discussions and debates among clinicians and scientists around the world. The inflammatory attacks can be sufficient for the experienced physicians when making the diagnosis, yet they are the most puzzling clinical feature of the disease. Because of their random presenting pattern and because they can unpredictably determine a residual deficit, relapses contribute not only to the physical but also to the psychological burden of the disease.

Since the earliest reports (Charcot, 1880), MS has been described as an inflammatory disease, which primary targets the myelin sheaths in the CNS and causes the formation of the demyelinated plaques. As permanent disability develops over time, it is reasonable to assume that the axonal damage, which is the essential cause of disease progression (Trapp and Nave, 2008), is a consequence of successive exacerbations. The frequency of inflammatory attacks is therefore commonly used for monitoring the disease activity (Cohen et al., 2004), as indicator of the treatment response (Sormani et al., 2012) and as surrogate marker of the short term disease evolution (Sormani et al., 2011). In addition, the efficacy of MS therapies in RCTs is normally measured by assessing their impact on relapse related endpoints.

However, the contribution of inflammatory attacks to the mechanisms driving the evolution of the progressive course, and leading to the development of permanent disability, remains ambiguous. The therapeutic relapse suppression was shown to prevent the EDSS worsening within the short observation period of clinical trials (1993; 1998a; Coles et al., 1999; Jacobs et al., 1996a; Johnson et al., 1995; Kappos et al., 2006a; Polman et al., 2006), but its impact on the hard outcomes in the long term is unclear (Bermel et al., 2010; Ebers et al., 2010; Goodin et al., 2012; Kappos et al., 2006c; Shirani et al., 2012). This highlighted a possible dissociation between exacerbations and the pathological processes responsible for the disease

progression. The axonal damage is known to occur in the context of acute myelin disruption (Kuhlmann et al., 2002; Trapp et al., 1998) and, to variable extent, within chronic demyelinated lesions (Bjartmar and Trapp, 2001; Mews et al., 1998). The mechanisms causing the axonal loss have never been fully elucidated (Dutta and Trapp, 2011). The infiltrating inflammatory cells might exert a direct toxic effect on the axons, by releasing proteolytic enzymes, cytokines and oxidative products (Smith et al., 2001; Trapp and Nave, 2008; Weiner, 2009). Nevertheless, the neurodegeneration could also be a consequence of sodium and calcium mediated injuries (Craner and Fugger, 2011; Micu et al., 2006; Waxman et al., 2004), and could be unrelated to the inflammation (Chaudhuri and Behan, 2004). The controversy is further fuelled by pathological studies describing extensive axonal damage within the NAWM (Bjartmar et al., 2001; DeLuca et al., 2006; Evangelou et al., 2000; Frischer et al., 2009; Lovas et al., 2000; Trapp et al., 1998), and suggesting that the neurodegeneration might occur independently of active demyelination (Bitsch et al., 2000). More recently, the cortical demyelination, which does not correlate with the white matter demyelination (Bo et al., 2007) and it is possibly caused by compartmentalized inflammation (Frischer et al., 2009), was suggested as a potential pathological driver of the disease progression (Kutzelnigg et al., 2005). MRI studies have also contributed to the debate. The inflammatory lesions load associates weakly with the clinical activity (Bakshi et al., 2008; McDonald et al., 1994) and was shown to predict only modestly the disability accumulation in the long term (2008; Calabrese et al., 2013; Fisniku et al., 2008a). In contrast to the white matter changes, the grey matter atrophy, which is an expression of the axonal pathology, correlates strongly with the disease severity (Calabrese et al., 2013; Fisniku et al., 2008b; Rudick et al., 2009).

Within this complicated picture, natural history studies can help to solve this endless debate, although they are not free of potential limitations. The collection of information on relapses might be affected by recollection bias. Overcoming these difficulties in the long term is particularly challenging, as demonstrated by the paucity of data on the relationship between late relapses and hard outcomes. In addition, the clinical manifestations partially dissociate from the biological events,

limiting the possibility of drawing conclusions on the disease pathogenesis. Episodic relapses are just a "filtered" expression of an underlying, constantly active inflammatory process. The MRI inflammatory lesions are known to occur 5-10 times more frequently than the clinically evident attacks (Barkhof, 2002). Moreover, the axonal damage (Trapp et al., 1998) and the brain atrophy (Filippi et al., 2003) take place already during the early stage of the disease, when the patients are free of permanent disability. Notwithstanding these limitations, the natural history studies can offer important clues to further elucidate the complex interaction between inflammation, unremitting disability and the mechanisms driving the disease progression. The data from large sample of patients, observed for a long period, gives a unique picture of the variable clinical outcome over time.

Among MS registries, the LO database contains the most extensive collection of information on relapses from a well-ascertained, untreated population, reaching in large percentages high disability levels. This offered the possibility to test the validity of the relapses number as clinical indicator of the disease activity and to estimate the impact of inflammatory attacks on unambiguous, meaningful disability outcomes, representing the heart of the medical, social and economic burden of MS.

4.4.1 The frequency of relapses

The average attacks number shows marked variation within and between individuals over time (Weinshenker and Ebers, 1987). Longitudinal assessments (Confavreux et al., 1980; Fog and Linnemann, 1970; Goodkin and Hertsgaard, 1989; Patzold and Pocklington, 1982) yielded greater frequencies compared to the retrospective studies (Leibowitz et al., 1964; Mc and Compston, 1952; Myhr et al., 2001; Panelius, 1969) (Figure 4.1). This was most likely due to the recollection bias. As previously reported in other cohorts (Mc and Compston, 1952; Patzold and Pocklington, 1982; Tremlett et al., 2008b), in the LO database the relapse rate lessened with time, mostly due to the regression to the mean. The number of attacks recorded during the first two years (0.93 mean attacks/year) decreased with the disease duration (mean 0.41 attacks/year from year 3 up to SP onset) and averaged 0.65 attacks/year

during the RR phase. This figure coheres with other natural history studies (Figure 4.1) and also conforms to the rates seen in placebo arms from RCTs (Inusah et al., 2010)

4.4.2 Relapses and the evolution of the RR phase (the latency to SP)

The probability of entering the progressive phase and the time to progression remain relatively unexplored outcomes, with a profound clinical relevance (Kremenchutzky et al., 2006a). It has been previously demonstrated that early relapses are predictors of SP (Table 4.2). However, the independent role of late and total relapses before the onset of progression, on the evolution of the RR phase has never been assessed.

4.4.2.1 Early relapses

Only few studies examined the relationship between early relapses and the attainment of SP. In the Lorraine database, the time to convert to SP MS was significantly longer between those with 1 and > 1 attacks during the first 5 years (Debouverie et al., 2008). Similarly, in the Gothenburg database 5 or more relapses during the first 5 years associated with a significantly shorter time to SP, compared to 2-4 attacks (7 median years difference) (Figure 4.3) (Eriksson et al., 2003). The British Columbia's group used a different methodology and offered the most extensive analysis. A higher attack rate within 5 years from onset associated with a higher hazard of and with a shorter time to SP (Figure 4.4). One attack increased the risk of reaching SP within 5 years (HR = 1.29) and the size of this effect decreased proportionally to the disease duration (HR = 1.11 and 1.02 for SP reached in > 5 to 10 years or in > 10 years, respectively) (Tremlett et al., 2009a).

The LO database is advantaged by a large proportion (66.2%) of patients attaining SP at the end of the observation period. In line with previous studies, the analysis confirmed that early relapses are a good predictor of the evolution of the RR phase.

A higher number of attacks during the first 2 years associated with an increased risk of converting to SP MS and with a shorter latency to progression (Table 4.5, Figure 4.10). The probability of reaching SP was significantly affected by early relapses (1 attack yielded HR = 1.11) and increased proportionally with the frequency of attacks, becoming 26% higher among patients with 3 attacks (HR = 1.37). The Kaplan Meier analysis highlighted the patients with high (≥ 3) early relapse frequency as the outliers driving this association. This subgroup attained SP 4.8 mean years earlier ($p < 0.001$) than those with 1 relapse, but only 1.6 mean years earlier ($p = 0.38$) than those with 2 relapses (Table 4.5).

4.4.2.2 Late and total relapses

In contrast with early relapses, the attacks after year 2 and the total attacks during the RR phase did not predict the time to SP. However, late and total relapses were collected among SP patients only. Therefore, the Cox regression analysis could not assess the risk of converting to SP MS, but it rather estimated the probability of attaining the endpoint in short or long times. The two variables yielded HRs below 1, which indicated that fewer late and total relapses associated with a significantly higher probability of entering the SP phase more rapidly (HR = 0.90 and 0.96, respectively) (Figures 4.16 A and 4.21 A). The Kaplan Meier analyses confirmed that the latency to progression was longer among those with a higher attacks frequency (Tables 4.16 and 4.18). SP patients with ≥ 3 late relapses attained progression 2.8 mean years and 5.4 mean years later than those with 1-2 ($p = 0.003$) and with 0 attacks ($p < 0.001$), respectively (Figure 4.16). The subgroup analysis, among those with high late relapses frequency (3-4, 5-6 and ≥ 7 attacks), further demonstrated no influence exerted by late attacks on the time to progression (Figure 4.17 A).

Therefore, neither the number of relapses after year 2 nor the number of total attacks was a good indicator of the evolution of the RR phase. A larger number of total relapses associated with a longer latency to SP. This was demonstrated by comparing patients with ≥ 5 versus 1-2 attacks (3.4 mean years difference; $p < 0.001$) (Figure 4.21 A), by comparing patients with ≥ 7 versus 1 attack (3.1 mean years

difference; p = 0.06) (Figure 4.22 A) and by including in the analysis only those patients who started to progress at DSS ≤ 2 and at DSS ≤ 3 (Table 4.19), indicating that the difficulty in defining the onset of progression did not affect the results. Indeed, previous analysis of the LO database reported a shorter duration of the RR phase among SAP (1 attack before progression) compared to SP (multiple attacks before progression) patients (time to SP = 7.6 versus 10.3 mean years) (Kremenchutzky et al., 2006a), highlighting a proportional relationship between total attacks and the latency to progression. Given the association between early relapses and the onset of SP, these results came unexpected and suggested that, after an early watershed is reached, mechanisms leading to the onset of the progressive phase dissociate from the focal inflammatory pathology. This will be more extensively addressed in Chapter 7.

The relationship between late relapses and the onset of SP was previously assessed only in the British Columbia database (Tremlett et al., 2009a). One attack, occurring between 5 to 10 years from onset, increased the risk of SP (HR = 1.23), but this effect decreased over time (HR = 1.08 for 1 attack occurring after 10 years). This methodological approach aimed at exploring the impact of relapses in the long term. However, performing separate analyses in patients categorized according to the disease duration (0-5; >5 to 10; >10 years) limited the meaningfulness of conclusions. The selection bias explains, at least partially, the decreasing predictive effect of relapses, as the groups observed for longer time are necessarily more likely to reach the endpoint less rapidly. In addition, the different definition of late relapses and the inclusion in the LO analysis of only SP patients make further problematic the comparison between results from the British Columbia and from the LO cohorts.

4.4.3 Relapses and the attainment of hard disability endpoints

4.4.3.1 Early relapses and late outcomes

Natural history studies consistently demonstrated that the number of early inflammatory attacks predicts the long-term disease evolution (Table 4.2). A high early relapses frequency associates not only with a shorter latency to progression but also with a faster attainment of disability endpoints. Overall, the effect on the times to DSS levels appears larger than the effect on the time to SP. Interestingly, in the majority of the studies, the times to DSS 6, according to the number of attacks during the first 5 (Confavreux et al., 2003; Debouverie et al., 2008; Eriksson et al., 2003) and 2 (Leray et al., 2010) years differed significantly but little, among groups. The analysis from the British Columbia database again stands out as the most comprehensive, although the survival estimates were affected, at least in part, by the large proportion (72%) of censored information. The number of attacks during the first 5 years increased significantly (HR = 1.48) the probability of reaching EDSS 6, and this effect decreased over time. The group with a high attack rate (≥ 0.4), which attained the endpoint 9 years earlier than the other two groups with a lower rate (<0.2 and 0.2-<0.4), was the main driver of the association between early relapses and late outcomes (Figure 4.4).

With the extended follow up, the analysis here presented confirmed (Weinshenker et al., 1989c) that, among LO patients, a larger number of attacks during the first two years associated with a faster attainment of hard disability outcomes from the disease onset, from the onset of SP and from DSS 3. Times to DSS 6 and to DSS 8 from onset where significantly shorter among those with ≥ 3, compared to those with 2 (3.6 and 8.5 mean years differences, respectively) and with 1 (7.6 and 12.8 mean years difference, respectively) early relapse (Table 4.5). The risk of accumulating disability increased proportionally with the number of attacks and the impact on the two endpoints was slightly different (HRs yielded by 3 attacks: DSS 6 = 1.84 and DSS 8 = 2.32) (Figure 4.10). This predictive effect was primarily exerted by

increasing the probability of entering the SP phase and by shortening the latency to progression. Among patients with a large number of attacks during the first 2 years, the faster attainment of DSS levels was secondary to the more rapid conversion to SP MS (Table 4.5). The onset of the SP phase was the key determinant of the effect of early relapses on the outcomes. In support of this, when only SP patients were included in the analysis the predictive effect of relapses became larger (Table 4.6). In addition, in the SP group, the impact exerted by early relapses on the outcome decreased proportionally with the latency to progression, and become almost not significant when it was tested among patients with a long duration of the RR phase (Table 4.7).

4.4.3.2 Late relapses and late outcomes

As seen for the attainment of progression, the analysis demonstrated that after year 2 a reversal seems to take place. The number of late relapses did not affect the long-term disease evolution. Indeed, the probability of developing severe disability decreased proportionally to the number of attacks. This might indicate that different biological mechanisms underly early and late inflammatory attacks. More relapses from year 3 up to the onset of progression predicted a significantly lower risk of attaining the endpoints; 5 attacks reduced the hazard of reaching DSS 6 by 24 % (HR = 0.76) and DSS 8 by 22% (HR 0.78) (Figure 4.16). This effect was also demonstrated by the Kaplan Meier analysis. The group with ≥ 3 attacks reached the endpoints in significantly longer times compared to the group with no relapse (4.3 mean years difference for attaining DSS 6) (Table 4.16). In addition, the similar survival times, among patients with 3-4, 5-6 or ≥ 7 attacks (Figure 4.17), further confirmed the lack of impact of late relapses on the long term disease evolution. The proportional relationship between the attacks after year 2 and the latency to SP explains the longer times to disability endpoints, among groups with a higher number of late relapses (Figure 4.16). In support of this, when patients were matched by the duration of the RR phase (1-13 or ≥ 14 years), the groups with low, intermediate and high late relapses frequency had very similar survival curves and the same risk of developing disability (Figure 4.19).

4.4.3.3 Total relapses and late outcomes

Importantly, the number of attacks during the RR phase exerted no significant effect on the attainment of the disability outcomes. The times to the endpoints from disease onset were remarkably equal among patients with small (1-2 attacks), intermediate (3-4 attacks) and high (≥ 5 attacks) number of total relapses. The 3 groups reached DSS 6 in 15.6, 15.7 and 15.9 mean years, respectively, and DSS 8 in 26.2, 25.8 and 26.2 mean years, respectively (Table 4.18). Accordingly, the risk of developing severe disability remained unchanged, despite the increasing number of attacks (4.21 B and C). Even when the two subgroups of patients at the extremes of the total relapse frequency (1 versus ≥ 7 attacks) were compared, the total number of attacks before progression exerted no impact on the disease evolution (Figure 4.23 B and C). Same results were obtained when the SO subgroup was assessed separately, demonstrating that the retrospectively collected information on relapses did not bias the analysis.

4.4.4 Relapses and the evolution of the SP phase

The relationship between relapses and the evolution of SP MS remains partially unexplored. It is well established that superimposed relapses do not affect the evolution of the progressive phase in both SP (Confavreux et al., 2000; Vukusic and Confavreux, 2003) (Figure 4.8) and PP patients (Kremenchutzky et al., 1999a) (Figure 4.7). Less is known about the effect exerted by relapses during the RR phase on the slope of the SP phase. Previous analysis of the LO database reported remarkably similar times from the onset of progression to DSS levels among SAP, SP and PP patients (Figure 4.6), indicating that the disability accumulation during the progressive phase was largely independent of attacks preceding its onset. The group from Gothenburg demonstrated no predictive effect exerted by the attacks during the first 5 years on the time from SP to EDSS 7 (Eriksson et al., 2003). In the 3 French cohorts from the EDMUS network early relapses did not significantly affect the times from DSS 3 (Leray et al., 2010) and from DSS 4 (Confavreux et al., 2003; Debouverie

et al., 2008) to higher DSS levels. However, it was not reported the percentage of patients still in the RR phase at the time when moderate disability occurred.

In this study the effect of relapses on the evolution of the SP phase was explored by assessing the times to the endpoints and the risk of developing disability both from DSS 3 and from SP, however the two approaches were methodologically different. The analysis of the disease evolution from moderate disability (DSS 3) included approximately 30% of patients who had reached this level through relapses (Figure 4.9), remained stable and free of progression for long time, or never entered the SP phase. In contrast, the analysis from the onset of progression included only SP patients, who were therefore at higher risk of developing severe disability.

4.4.4.1 Early relapses and the progressive phase

In mild contrast with previous reports (Confavreux et al., 2003; Debouverie et al., 2008; Leray et al., 2010), among the LO patients, the evolution of the SP phase was affected by early relapses. A larger number of attacks during the first two years associated with significantly shorter times from the onset of SP to the endpoints. This was primarily driven by the group with frequent early relapses (≥ 3), attaining very rapidly DSS 6 (2.5 mean years) and DSS 8 (9.6 mean years) from the onset of progression (Table 4.8). The size of the predictive effect exerted by y1-y2 attacks on the probability of developing severe disability was larger from the onset of progression than from disease onset (3 attacks for reaching DSS 6 yielded HRs 2.67 and 1.84, respectively) (Table 4.9). This was explained by the selection bias, as only SP patients were included in the analysis from progression, and again emphasized the conversion to SP MS as the key determinant of the effect of early relapses on late outcomes.

The times to disability levels from DSS 3 were significantly affected by the number of early relapses, however the differences among groups were very small (Table 4.8). In addition, the probability of reaching the endpoints, according to the number of early attacks, was much lower from DSS 3 than from the onset of SP (3 attacks for

reaching DSS 6 yielded HRs 1.39 from DSS 3 and 2.67 from SP) (Tables 4.9). The inclusion in the analysis of those patients who reached moderate disability through relapses and never entered progression, explains why the disease evolution from DSS 3 was only modestly impacted by early relapses. Moreover, the predictive effect of y1-y2 attacks from DSS 3 was also smaller than from disease onset (3 attacks for reaching DSS 6 yielded HR 1.39 and 1.84, respectively) (Table 4.9). This importantly confirmed that early relapses influenced the attainment of late outcomes primarily by shortening the time to DSS 3, which for most of the patients implied the latency to progression.

It has been suggested that the disease progresses in 2 stages, independent of each other, and separated by a clinical watershed of irreversible disability. In the Rennes and the Lyon databases, the times to DSS 3 (Leray et al., 2010) and to DSS 4 (Confavreux et al., 2003) did not affect the subsequent disease evolution from these disability landmarks. This supported the notion that the disease evolution in the late stages is amnesic to the previous clinical history (Confavreux et al., 2003). In mild contrast, the analysis in this study confirmed (Weinshenker et al., 1989c) that a longer time to DSS 3 modestly, but significantly, predicted a lower risk of disability (DSS 6, HR = 0.97, $p < 0.001$; DSS 8 HR = 0.96, $p < 0.001$) (Table 4.26) and associated with longer times to the endpoints (Table 4.25). The differences with the analyses from the French cohorts might be explained by the different percentages of patients reaching the endpoints (61.8% at DSS 3 and 48.3% at DSS 4 in the Lyon and Rennes databases, respectively, and 81.5% at DSS 3 in the LO database), which affected the survival estimates. Nevertheless, this study supported the notion that the outcome is mainly determined before the attainment of moderate disability. Past this point, the disease progression seems to enter a common final pathway, which is largely independent of the previous evolution. Indeed, in the multivariate analysis, the time to DSS 3, probably by heralding the progressive course, accounted for most of the predictive effect of early relapses, which remained only modestly significant (DSS 6: HR = 1.08, $p = 0.04$) (Table 4.26). On the contrary, the impact of the time to moderate disability on the disease progression remained unchanged when it was

adjusted for the number of y1-y2 attacks (Table 4.26), suggesting that it influenced the outcome's severity independently of the number of relapses.

4.4.4.2 Late relapses and the progressive phase

The attacks from year 3 to SP onset had no impact on the evolution of the progressive phase. The times to disability endpoints from DSS 3 and from onset of progression were similar among patients grouped by the number of late relapses (Table 4.17).

4.4.5 The different predictive effect of early and late relapses

The analyses unexpectedly highlighted a dichotomy, among SP patients, between early and late relapses, which related to the outcomes in an opposite way. The attacks from year 3 to the onset of SP seem to counterbalance the negative impact of y1-y2 relapses on the long-term disease evolution. This might imply a yet undetermined interaction between the development of the progressive course and the suppression of clinically evident attacks, possibly analogous to what occurs in PP MS. A higher number of attacks during the first 2 years associated with what is destined to be a more rapid clinical course. Among patients with a poor prognosis, the faster attainment of the SP phase probably suppresses or masks attacks occurring after year 2, explaining the converse relationship between late relapses and the times to progression and to disability endpoints. This disconnection between the relapse rate and the disease evolution seems to occur after an early watershed is reached. The biological explanations of this discrepancy remain unclear, but analyses suggest that mechanisms driving the disease evolution dissociate from the focal inflammatory pathology early in the disease course. Early and late relapses are difficult to compare because of methodological differences. Early relapses were counted in the fixed first 2 years interval and were collected, among all relapsing onset patients. On the contrary, late relapses were collected during the variable interval from year 3 to the onset of progression, among SP patients only. Inevitably,

the latency to SP affected the results from the analyses of late relapses. Indeed, when patients were stratified by the duration of the RR phase, the survival curves according to the number of attacks after year 2 were similar (Figure 4.19). For these reasons, the effect of late relapses on the times to DSS 6 and to DSS 8 was assessed in small subgroups of serial 2-years intervals from year 3 to SP, allowing to make a more appropriate comparison with early relapses. In each subgroup, patients sharing the same duration of the y3-SP period attained the endpoints in similar times, despite the different number of late attacks (Figure 4.18). Notwithstanding the small samples size, these sub-analyses further confirmed no impact of relapses after year 2 on the disease evolution.

The concomitant effect of early and late relapses on the outcome was assessed in multivariate analysis (Figure 4.23). The model confirmed that the two variables affected the disease evolution in an opposite way. The probability of developing severe disability increased proportionally with the number of attacks during the first 2 years and inversely with the number of attacks after year 2 (Figure 4.23). This allowed to calculate the risk of attaining DSS 6 according to clinical scenarios, based on specific relapses numbers. For instance, the hazard of requiring a walking aid, among those with 3 attacks during the fist two years (HR = 2.43), decreased by approximately 40% (HR = 2.00) if 3 more attacks occurred during the year 3-SP period. The Kaplan Meier analysis confirmed results from the Cox regression multivariate model (Table 4.22).

In addition, the model further emphasized year 2 as the watershed after which the predictive effect of relapses reversed. Two hypothetical patients, experiencing 3 relapses before entering the SP phase, were compared. The hazard of reaching DSS 6 was much higher when the 3 attacks occurred during the first 2 years (HR = 2.43) rather than before and after year 2 (1 early relapse + 2 late relapses: HR = 1.18), highlighting the relevance of the timing of relapses on the outcome and further emphasizing the influence of early attacks on late outcomes.

4.4.6 Frequent early relapses

The groups with a larger number of early attacks attained SP and DSS levels in significantly shorter times, however a causal relationship with the severe disability, occurring 15-20 years later, cannot be assumed. The possibility that a higher early relapse frequency is simply a concomitant to a predestined, more rapid clinical course remains open. Indeed, in all databases analyses, the association between early relapses and late outcomes was mainly driven by the subgroup with very high attack frequency, experiencing an aggressive disease course. In the LO database those patients with ≥ 3 early relapses were outliers and exhibited an unusually fast disability accumulation. They attained progression in 9 median years, and they reached DSS 6 and DSS 8 from disease onset in 10 and 21 median years, respectively, and from onset of SP in 1 and 9 median years, respectively. It remains unclear if this exceptionally bad disease course can be merely explained by a causal relationship between the frequent early inflammatory attacks and the late disability or other factors are implicated in determining the outcome's severity.

This hypothesis drove the analysis of the frequent early relapses subgroup (≥ 3 attacks), which represented 20% (n = 158) of the RR onset population. Although the disease evolution is unpredictable at individual level, this subpopulation was expected to have homogeneously a poor prognosis. However, at the end of the observation period, patients sharing the same adverse features of high early relapses frequency were distributed from one extreme of the disability spectrum to the opposite. During the first 9 years from onset (median time to SP), about 5.5% of "frequent early relapsers" every year had become progressive, and the subgroup equally split according to the type of disease course (79 SP MS/79 RR MS). In the following 15 years an additional 15.2% entered the SP phase, leaving 55 patients (34.8%) progression free (Figure 4.11). Those who remained in the RR phase had a remarkable benign disease course. Less than half (43.6%) accumulated moderate disability (DSS 3) in 16.2 mean years, which was 11 years slower than those who entered the SP phase. The RR subgroup was observed for 17 median years (70% for

longer than 15 years) (Figure 4.14) and their lack of progression cannot be simply attributable to the insufficiently long observation time. This is because the mean duration of the RR phase in the total SP population was 10 years (Chapter 6) and 75% of SP "frequent early relapsers" became progressive by 9 years (Figure 4.11). The remaining 103 (65.2%) "frequent early relapsers" rapidly attained progression (5 median years) and accumulated severe disability in large percentages. Comparison of the baseline features demonstrated that RR and SP subgroups differed little (Table 4.11). The slightly younger age at onset (25.5 versus 28.4 mean years, $p = 0.001$) and the larger percentages of females (82% versus 63%, $p = 0.02$), among patients who remained in the RR phase, cannot explain the largely different final outcome between the two subgroups.

This study for the first time analysed the long-term evolution among a very specific subgroup of patients, selected for the adverse clinical features of high early relapses frequencies. The results need confirmation from other databases. The analysis further highlighted the onset of the SP phase as the key determinant of long-term prognosis. The conversion to SP MS represented the clinical watershed differentiating the severity of the disease course among "frequent early relapsers", partially independently of inflammatory attacks. A subgroup of patients with an exceptionally severe disease course was identified. This might represent the target for a very aggressive therapeutic approach early in the disease course.

In addition, such a large variability of the clinical phenotype, within a population sharing the same adverse clinical features, also suggests that individuals susceptibility to inflammation might be controlled by other independent factors, which promote a more aggressive outcome by facilitating the onset of the progressive course. The axonal vulnerability or its resistance to degeneration might be genetically influenced, however this fascinating hypothesis needs to be tested in other suitable populations.

4.4.7 The first inter-attack interval

Previous analyses of the LO database (Weinshenker et al., 1989c), in line with the reports from the Lyon and the Lorraine databases (Confavreux et al., 2003; Debouverie et al., 2008), demonstrated an association between short first inter-attack interval and worse outcome.

This study confirmed that patients with a short interval between the first and the second attack attained SP and the DSS levels in significantly shorter times (Table 4.12). The risk of entering the progressive phase and of developing severe disability decreased proportionally with the duration of the interval. The predictive effect was modest but significant: the hazard of reaching SP (HR = 0.97), DSS 6 (HR = 0.95) and DSS 8 (HR = 0.93) decreased by 13% (HR = 0.87), by 23% (HR = 0.77) and by 28% (HR = 0.72), among patients experiencing the second attack after 5 years, respectively (Figure 4.16). This effect remained unchanged in the multivariate analysis when it was adjusted to the effect of early relapses. However, the first inter-attack interval did not significantly impact on disease evolution from DSS 3 and only marginally affected the evolution of the SP phase (Table 4.13).

The concomitant impact of early relapses, interval between the first 2 attacks and late relapses on the probability of attaining DSS 6 was assessed in the multivariate analysis. In a hypothetical patient experiencing 2 relapses in the first two years the risk of requiring a walking aid (HR = 1.42) reversed if the second exacerbation occurred 3 years after onset (2 years first inter-attack interval HR = 0.97) (Table 4.24 A). In addition, the hazard further decreased with the longer interval between the first two attacks. The model highlighted the timing of the second attack as an important determinant of the probability of developing severe disability and suggested that the predictive effect of the first inter-attack interval largely depended on whether or not a second relapse occurred within the first 2 years.

4.4.8 Clinical presentation and residual deficit from relapses

Previous analysis of the LO database reported that polysymptomatic presentation was strongly associated with worse prognosis in PP patients (Cottrell et al., 1999b). In contrast, this study demonstrated that, among RR patients, the number of neurological systems involved at onset did not influence the risk of converting to SP MS (HR = 1.02, p = 0.76) and to attain disability endpoints (DSS 6 H = 1.03, p = 0.58; DSS 8 HR 1.12, p = 0.12). In addition, the type of symptoms at clinical onset was found not to affect the disease evolution, beside a modest predictive effect exerted by the brainstem presentation (Table 4.21). This data does not cohere with other studies, showing worse outcome in patients presenting with motor disturbances (Confavreux et al., 2003; Debouverie et al., 2008; Eriksson et al., 2003; Tremlett et al., 2006). However, the comparison among studies remains difficult because of the different criteria and terminology used when attributing the symptoms to specific anatomic locations (Table 3.2).

Previous analyses from the other cohorts consistently showed that an incomplete recovery from the onset attack associated with a faster attainment of disability endpoints (Table 4.1), but it did not affect the disease evolution from established disability (Confavreux et al., 2003; Debouverie et al., 2008; Leray et al., 2010). One of the shortcomings of this study is the lack of data on the residual deficit from the first relapse, which did not allow to test the predictive effect of the degree of recovery from the onset attack, among the LO patients. More importantly, the evaluation of only the number of relapses, but not of the type of symptoms characterizing the attacks, represents the biggest limitation, which is shared by all the natural history studies. The available data on the development of disability as a direct consequence of an inflammatory attack were obtained during short observation times, from patients who had already reached EDSS 2.5-3.5 (Hirst et al., 2008a; Lublin et al., 2003; Young et al., 2006) and possibly, at least in part, had already entered the SP phase. Indeed, conclusions were contradicting. A residual deficit (0.5-1 EDSS point worsening) from exacerbations was reported in 42% of 224 patients from placebo

groups (Lublin et al., 2003) and in 49% of 182 patients attending an MS clinic (Hirst et al., 2008a). These results could not be replicated by a separate analysis of 576 placebo patients, showing no consistent effect of relapses on the development of sustained EDSS score (Young et al., 2006). In addition, among 2578 attacks observed in a MS clinic over 15 years, only 7 exacerbations caused the attainment of EDSS 6 sustained for at least 6 months (Bejaoui and Rolak, 2010).

This study could not address the important question whether unremitting disability can be directly caused by inflammatory attacks. However, most of patients in the LO database converted to SP MS at or before DSS 3; more than 80% within DSS 4 (Figure 4.9). This indicates that, in most of the patients, the accumulation of severe disability (requiring a walking cane or worse) did not occur during the RR phase, but almost invariably resulted from the steady evolution of the progressive phase. It remains possible that, among some patients, a residual deficit from relapses accelerated the attainment of DSS 3 and therefore promoted a faster accumulation of severe disability. However, given the proportional relationship between late relapses and the time to DSS levels, this appears improbable. Groups with high late relapse frequency would be expected to experience more likely a partial recover from the attacks, nonetheless they had better outcome than those with no relapse.

4.4.9 Conclusions

The data from this study indicated that the relapse frequency during the first two years is a good predictor of the late disability accumulation and influenced the outcome primarily by affecting the probability of entering the SP phase. The results suggest that mechanisms responsible for the development of a progressive course are probably already active during the early stage of the disease, representing the only plausible window of therapeutic opportunity. The number of early attacks can be potentially used for selecting patients at high risk of a fast disease progression and therefore needing more aggressive treatments. This information is available early in the disease course and therefore is extremely useful for optimizing an aggressive therapeutic approach. In addition, in view of their exceptionally severe

disease course, patients with frequent early attacks (≥ 3) appear a good candidate for an induction therapy with strong immunosuppression.

However, the analyses did not provide any evidence that relapse frequency after year 2 is a reliable indicator of the disease evolution and a valid surrogate marker for late outcomes. There was no indication that LO database patients with more late and total attacks accumulated disability more rapidly, even in groups with very high number of attacks. In addition, the proportional relationship between the number of attacks from year 3 up to the onset of progression, which are normally counted in RCTs, and the disease evolution questions the validity of measuring the treatments efficacy by using relapse related endpoints. It remains possible that, among patients under treatment, inflammatory attacks have different biological meaning and relate differently to the outcome. Models predicting disability based exclusively on the relapses number cannot sufficiently explain the large variability of the disease evolution among patients. The data consistently indicated that the onset of the SP phase is the key determinant of the long-term prognosis and is a robust outcome measure for late disability.

Chapter 5

Age and the long-term disability accumulation

5.1 Introduction

Relapses and the steady accumulation of disability (progression) can present alone or in combination and define the MS clinical phenotypes (Lublin and Reingold, 1996), however their interaction remains uncertain. It has been suggested that common pathophysiological mechanisms might drive the disease evolution among progressive patients (Revesz et al., 1994), independent of the occurrence of clinical inflammatory attacks (Confavreux and Vukusic, 2006a). Indeed, a large body of evidence, from natural history studies, indicates that MS subtypes share a similar rate of disability accumulation during the progressive phase. In the LO database, SAP, SP and PP patients attained disability endpoints from the onset of progression in remarkably similar times (Kremenchutzky et al., 2006a). In addition, the disease evolution from established disability was shown to be unaffected by the type of the initial disease course. In the EDMUS French cohorts, progressive onset and relapsing onset patients took the same times to progress from DSS 3 (Leray et al., 2010) and from DSS 4 (Confavreux and Vukusic, 2006a; Debouverie et al., 2008) to higher DSS levels. This was also observed among LO patients (Chapter 3). The accumulation of disability from DSS 3 differed little between the RR/SP and the PP group, and was almost equal between the SP and the PP group (Figures 3.21 and 3.22). These results lent support to the unifying concept of the disease course and further confirmed the amnestic nature of the disease evolution, characterized by two independent stages (Confavreux and Vukusic, 2006a; Leray et al., 2010). The attainment of moderate disability (DSS 3/DSS 4) represents the clinical watershed, after which a common final pathway, not influenced by the previous clinical history, drives the disease progression.

The predictive effect of relapses was shown to be limited to the first 2 years of the disease (early relapses) (Chapter 4). This suggested that, after an early watershed is reached, a disconnection occurs between biological mechanisms underlying inflammatory attacks and those driving the disease evolution. The progressive, steady accumulation of permanent disability gradually prevails and possibly

suppresses or masks relapses. However, it remains largely unclear which factors cause the changes in this complex interaction between inflammation and degeneration, and shifts the balance in favour of the latter.

Age has often been indicated as an important prognostic factor in MS. In view of the similar outcome (Kremenchutzky et al., 2006a) and of the strikingly similar age at the onset of progression, among all progressive subtypes (Table 3.3) (Confavreux and Vukusic, 2006a; Koch et al., 2007; Tutuncu et al., 2013), it has been hypothesized that age-related neurodegenerative mechanisms might determine the emergence of the progressive phase and drive its evolution (Confavreux and Vukusic, 2006b; Kremenchutzky et al., 2006a). The disease course among MS subgroups could be mainly a function of the different ages at which pathological process become clinically evident.

5.1.1 Age at disease onset and disability outcomes

It has been overwhelmingly demonstrated that an older age at disease onset associates with a worse outcome. This was consistently shown by early (Bonduelle, 1967; Leibowitz et al., 1964; McAlpine, 1961; Panelius, 1969) and late (Confavreux et al., 1980; Confavreux et al., 2003; Debouverie et al., 2008; Eriksson et al., 2003; Kantarci et al., 1998; Koch et al., 2007; Leray et al., 2010; Myhr et al., 2001; Phadke, 1990; Poser et al., 1982b; Riise et al., 1992; Tremlett et al., 2006; Trojano et al., 1995; Visscher et al., 1984) natural history studies, using different age grouping and methodologies (Table 5.1). Being older at first symptoms unequivocally associated with shorter times from disease onset to EDSS/DSS levels.

In the three French EDMUS cohorts, the age at onset was investigated by analysing the total population, grouped in 5 age categories. An older age at first symptoms predicted shorter times from the disease onset to the endpoints (Confavreux et al., 2003; Debouverie et al., 2008; Leray et al., 2010). However, the age at onset did not affect the disease evolution from DSS 3 (Leray et al., 2010), from DSS 4 (Confavreux et al., 2003; Debouverie et al., 2008) and from SP (Vukusic and Confavreux, 2003). In

addition, patients older at onset were significantly older when attaining the DSS levels (Confavreux and Vukusic, 2006b; Leray et al., 2010), highlighting the complex interaction between age and prognosis.

Table 5.1 The association between age at onset and the attainment of disability endpoints, among MS registries. * Age reference category.

Database	Age grouping	Populations and endpoints analyzed	Association found
Lyon	0-19*; 20-29; 30-39; 40-49; ≥ 50	total (n = 1844): times from onset to DSS 4, DSS 6, DSS 7	older age ---> shorter times to endpoints
		total (n = 1844): times from DSS 4 to DSS 6, DSS 7	no effect
		total (n = 1844): times from brith (age at) DSS 4, DSS 6, DSS 7	older age at onset ---> older age at disability
Rennes	< 20; 20 - <30; 30 - < 40; 40 - < 50; ≥ 50*	total (n = 1415): times from onset to DSS3, DSS 6	older age ---> shorter times to endpoints
		total (n = 1415): times from DSS3 to DSS 6	no effect
		total (n = 1415): times from birth (age at) to DSS 3, DSS 6	older age at onset ---> older age at disability
Lorrainne	< 20; 20 - <30; 30 - < 40; 40 - < 50; ≥ 50	total (n = 2518): times from onset to EDSS 3, EDSS 4, EDSS 6, SP	older age ---> shorter times to endpoints
		total (n = 2518): times from EDSS 3 to EDSS 4, from EDSS 4 to EDSS 6	older age ---> shorter time from EDSS 3 to EDSS 4; no effect on time from EDSS 4 to EDSS 6
British Columbia	< 20*; 20 - <30; 30 - < 40; 40 - < 50; ≥ 50	total (n = 2319): time from onset to EDSS 6	older age ---> shorter times to endpoint
		total (n = 2319): time from birth (age at) to EDSS 6	older age at onset ---> older age at disability
		SP (n = 1245): time to SP from onset and from birth (age at)	older age ---> shorter time to and older age at SP
		SP (n = 1245): time from SP to EDSS 8	older age ---> longer time to endpoint
	< 30*; 30 - < 40; 40 - < 50; ≥ 50	RR (n = 5162): time to SP from onset and from birth (age at)	older age ---> shorter time to and older age at SP
Gothenburg	n/a	RR (n = 219): time from onset to SP, DSS 6	older age ---> shorter time to SP and to DSS 6
	< 30; ≥ 30	RR (n = 220): time from onset to SP, DSS 7	older age ---> shorter time to SP; no effect on time to DSS 7
Groningen	< 20; 20-29; 30-39; ≥ 40	RR (n = 228): time from onset to SP	older age ---> shorter time to SP
Salpetriere	10-19; 20-29; 30-39; 40-49; 50-59	RR (n = 884): time from onset to SP	older age ---> shorter time to SP
		total (n = 957): probability of having a PP course	older age ---> higher probability of having a PP course
Italy	≤ 25; > 25	RR (n = 249): probability of reaching SP	older age ---> higher risk of SP

Tremlett et al. reached similar conclusions by analysing the total population from the British Columbia database, stratified in 5 age categories. The groups younger at onset took longer times to EDSS 6, and the risk of developing disability increased proportionally with the age, becoming double (HR for EDSS 6 = 2.22) among patients aged > 50. However, patients younger at onset were also significantly younger when attaining the endpoint (Tremlett et al., 2006). The analyses from the Gothenburg

database yielded partially contradicting results. Among relapsing onset patients, being older was associated with significantly shorter times to DSS 6 (Runmarker and Andersen, 1993) but did not affect the risk of attaining DSS 7 (Eriksson et al., 2003).

5.1.2 Age at disease onset and the onset of the progressive phase

The effect of the age at disease onset on the risk of conversion to SP MS has been extensively investigated. Two separate analyses from the British Columbia group demonstrated that patients older at first symptoms attained secondary progression in a significantly shorter time, but were also older at the onset of SP MS (Koch et al., 2010; Tremlett et al., 2008a). The risk of entering the SP phase rose by 27.1% for every 5 years increase of the age at onset (HR for 1 year = 1.05) (Koch et al., 2010). Controversially, patients older at the onset of the RR phase had a shorter latency to progression and exhibited a slower evolution of the SP phase. There was a 8.8 mean years difference for reaching EDSS 8 from the onset of progression, between the group aged < 20 and the group aged > 40 (Tremlett et al., 2008a). The authors did not address the possible explanations of these results, which are largely in contrast with the faster disability accumulation from the disease onset observed among patients older at onset.

The association between older age at onset and a greater risk of conversion to SP MS was confirmed in the Gothenburg database (Eriksson et al., 2003), in the Lyon database (Vukusic and Confavreux, 2003) and in a small dataset (n = 249) from Italy (Trojano et al., 1995). In addition, shorter times to SP, in groups older at onset, were reported in several cohorts, from Lorraine (Debouverie et al., 2008), from Groningen (n = 228) (Koch et al., 2007) and from Salpetriere (n = 844) (Stankoff et al., 2007). These results strongly indicate that, by growing older, relapsing onset patients become more likely to experience the SP course. Similarly, the risk of having a PP course was shown to increase proportionally with the age at the disease onset (OR = 1.13 per additional year at onset) (Stankoff et al., 2007).

5.1.3 Age at onset of progression

The age at onset of progression is largely unaffected by the type of the disease course. This was first reported more than 40 years ago by Fog et al., who demonstrated exactly the same age (36.3 years) at conversion to SP MS and at onset of PP MS (Fog and Linnemann, 1970). Same results were lately yielded by the analysis of a Dutch MS cohort (n = 342), which showed very similar age at the onset of the progressive phase between SP (37.5 mean years) and PP (35.7 mean years) patients (Minderhoud et al., 1988b). More recently, natural history studies used the Kaplan Meier analysis from birth to compare the age at onset of primary and secondary progressive MS. This was reported strikingly similar between PP and SP patients from the Lyon database (mean years: SP = 39.5, PP = 39.3; p = 0.47) (Confavreux and Vukusic, 2006a) and from the Groningen database (mean years: SP = 39.5, PP = 39.1; p = 0.95) (Koch et al., 2007).

Interestingly, same conclusions were reached when analysing progressive patients stratified by the number of relapses before the onset of progression. The analyses from the LO database (Table 5.2) (Kremenchutzky et al., 2006a) and from the Mayo Clinic database (Figure 5.1) (Tutuncu et al., 2013) demonstrated a remarkably similar age at the onset of progression among those with one (SAP MS), many (SP MS) and no (PP MS) attacks before the progressive phase.

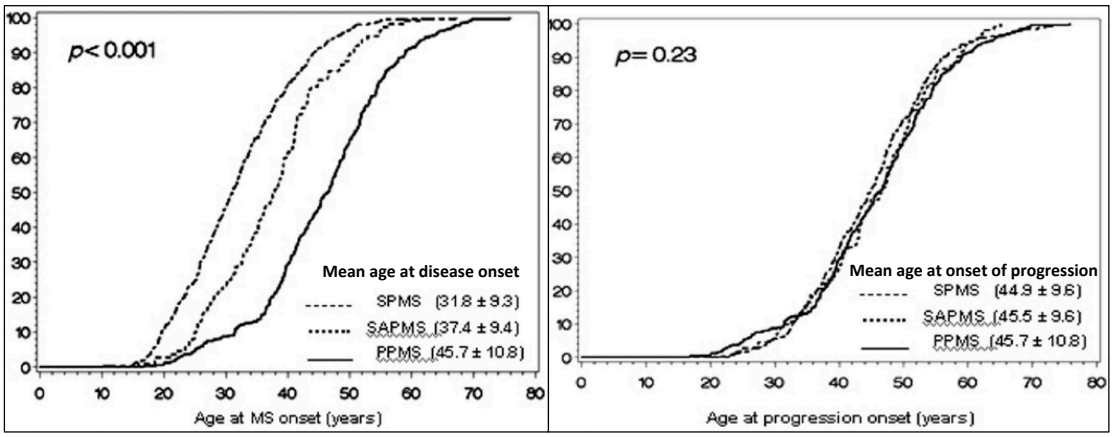
The only study that failed to replicate these results was from the British Columbia database. All relapsing onset patients at risk of SP were included in the Kaplan Meier analysis. The median age at the onset of secondary progression was significantly older than the median age at the onset of primary progression (49.0 versus 41.0 years; $p < 0.005$) (Tremlett et al., 2009b). However, when only SP patients were analysed, the age differences with the PP group became narrower, although they remained statistically significant (43.1 versus 41.0; $p < 0.005$). The authors commented that the exclusion from the analysis of patients still at risk of entering the SP phase represents a methodological caveat, which explains why in the other cohorts the age at onset of primary and secondary progression did not differ. It is

worth noticing that a separate analysis from the British Columbia database, including a larger sample, reported a different estimate of the median age at onset of SP (53.7 years) (Koch et al., 2010).

Table 5.2 Mean age at the disease onset and at the onset of progression, among progressive subtypes in the LO database: SP (excluding SAP), SP (all), SP starting to progress at DSS ≤ 2-3, SAP and PP patients (Kremenchutzky et al., 2006a).

Progressive MS subtypes	Age at disease onset (mean years)	Age at onset of progression (mean years)
SP (excluding SAP)		39.2
SP at DSS ≤ 2	30.4	40.2
SP at DSS ≤ 3	29.7	40.1
SP (all)		39.4
SAP	33.3	40.9
PP	38.6	38.6

Figure 5.1 Kaplan Meier analysis from birth to the disease onset and to the onset of progression: comparison of SAP, SP and PP patients from the Mayo Clinic database (Tutuncu et al., 2013).



5.1.3.1 Age at onset of progression and prognosis

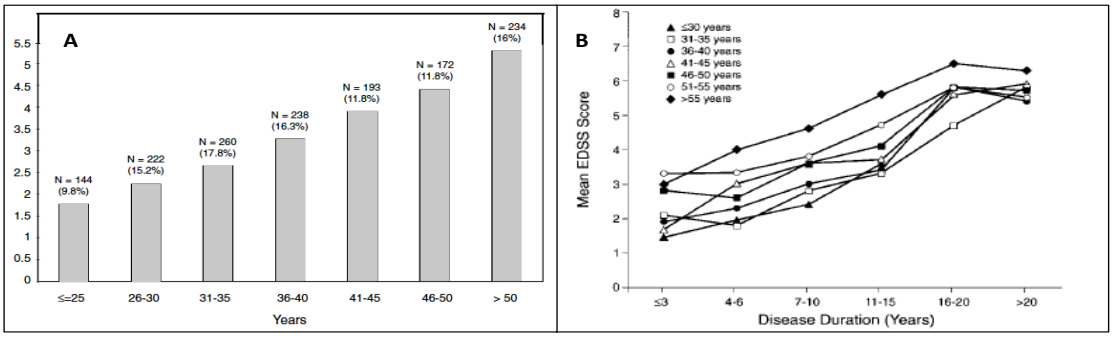
Only few studies have investigated the relationship between the age at the onset of progression and prognosis. Among patients from the LO database (Cottrell et al., 1999b) and from the Lorraine database (Leray et al., 2010), the evolution of the PP phase was shown to be unaffected by the age at its onset.

Two separate analyses from the British Columbia database, including different samples (n = 352 and 424), reported significantly longer times to EDSS 6 among PP patients aged < 30, but very similar outcome in the others age groups (Koch et al., 2009; Tremlett et al., 2005). In addition, the age at the onset of progression was found to affect the evolution of the SP phase. Times from the onset of SP to EDSS 8 were significantly longer among patients older at conversion to SP MS (Tremlett et al., 2008a).

5.1.4 Current age

The effect of current age on the disability accumulation has been extensively investigated by Trojano et al. (Liguori et al., 2000; Trojano et al., 2002). Among 1463 patients from two separate datasets, the EDSS score increased significantly by growing older (Liguori et al., 2000) (Figure 5.2 A), even when patients were stratified by the duration of the disease. (Figure 5.2 B) (Trojano et al., 2002).

Figure 5.2 Current age and disability accumulation among 1463 patients from two Italian databases. A) Mean EDSS score (y-axis) among current age groups (x-axis) (Liguori et al., 2000); B) Mean EDSS score (y-axis) among patients stratified by current age (dotted lines) and disease duration (x-axis) (Trojano et al., 2002).



5.1.5 Age and relapses

Only few studies have examined the relationship between age and the number of relapses. Overall, results indicate that the relapse rate reduces by growing older. Tremlett et al. reported a gradual decrease of the attacks frequency over time, proportional to the patients' age (Figure 4.2 B, Chapter 4). Similarly, in the database from Salpetriere, the median number of relapses before the onset of SP diminished with the increasing age at onset (Stankoff et al., 2007). In addition, in both the LO and in the Mayo Clinic databases, patients with many attacks (SP) were younger at disease onset, compared to patients with only one attack (SAP) before progression (Kremenchutzky et al., 2006a; Tutuncu et al., 2013) (Table 5.2 and Figure 5.5). This further demonstrated that the relapses frequency during the RR phase reduces proportionally with an older age. Interestingly, among patients from the Mayo Clinic database, the age at the last relapse, over the entire disease course, was shown to be unaffected by the type of the disease course (mean years: SP = 48.1, SAP = 49.6, PP = 47.8; $p > 0.05$) (Tutuncu et al., 2013).

5.1.6 Age at disability milestones

Confavreux et al. offered the most extensive analysis of factors affecting the age at the attainment of disability milestones (DSS 4, 6 and 7) (Confavreux and Vukusic, 2006b). For the total population of 1844 patients, median age at DSS 6 was 54.7 years (95% CI 53.5-55.8). Factors that associated with a younger age at disability levels were male sex and young age at the disease onset. Interestingly, relapsing onset and progressive onset patients differed little in the age at the attainment of DSS 4 (44.8 versus 42.2 median years; $p < 0.001$) and of DSS 6 (55.3 versus 53.0 median years; $p = 0.002$). In addition, the two subgroups reached DSS 7 (62.8 versus 63.1 median years; $p = 0.24$) at the same age.

The analysis from the Rennes' database confirmed that the age at disability milestones was not affected by the type of the disease course. The median ages at DSS 3 were 41.9 and 42.4 years ($p = 0.23$), and at DSS 6 were 52.1 and 51.1 years ($p =$

0.91) between progressive onset and relapsing onset patients, respectively (Leray et al., 2010). In addition, men attained DSS levels at significant younger age than women, in both disease phenotype subgroups (Leray et al., 2010).

Factors affecting the age at EDSS 6 were also assessed among patients from the British Columbia database. The analysis confirmed a slightly younger age among men, compared to women, when reaching severe disability. In addition, relapsing onset patients were significantly ($p < 0.005$) older (61.4 mean years) at EDSS 6, compared to progressive onset patients (57.3 mean years) (Tremlett et al., 2006).

5.1.7 Aim of the study presented in this chapter

The overall aim of this study was to assess the effect of age on the disease evolution before and after the onset of the progressive phase. This allowed to investigate the contribution of age related factors to the accumulation of severe disability and to clarify how age affects the expression of the clinical phenotypes.

5.2 Methods

The analyses were carried out in the total population, in the relapsing onset and in the progressive onset groups, and in all progressive patients pooled together. The relationships between the disability accumulation and 1) **the age at disease onset**, 2) **the age at onset of progression** and 3) **current age** were investigated. Variables were assessed both as categorical (Table 5.3) and as continuous. Groups were computed in order to maintain, to the possible extent, similar numbers in each category. However, the analyses were also carried out among patients with different stratifications, for confirming results.

The binary logistic regression analysis was used to assess 1) the risk of experiencing a PP course according to the increasing age at onset; 2) the risk of entering the SP phase and of attaining DSS levels, according to the increasing age at onset, to the increasing current age ("growing older") and to the increasing disease duration; 3) the risk of attaining DSS levels, according to the increasing age at onset of progression. The Kaplan Meier analysis was used for estimating, among age groups, the time from birth to the onset of the disease, the times to conversion to SP MS from the disease onset and from birth (age at), and the times to DSS 6-8 from birth (age at), from the disease onset, from DSS 3 and from the onset of progression. The Log Rank test investigated the differences among groups. When testing the effect of age at onset of SP on the attainment of disability outcomes from the disease onset, to eliminate bias from immortal time (Suissa, 2008), SP patients attaining the endpoints before entering the progressive phase (DSS 6 = 16, DSS 8 = 0) were excluded from the analysis. The Cox regression multiple analysis was used to assess the risk of attaining the endpoints among age categories, to investigate the concomitant effect of the age and of the number of relapses on the outcomes, and for validating results from binary logistic regression analysis. Spearman rank correlation test assessed the correlation between age and the number of relapses.

Table 5.3 Stratification by the age at the disease onset and by the age at the onset of progression in the total population (n = 1023), the RR/SP group (n = 806), the PP group (n = 217) and the PP/SP group (n = 751). * SP only.

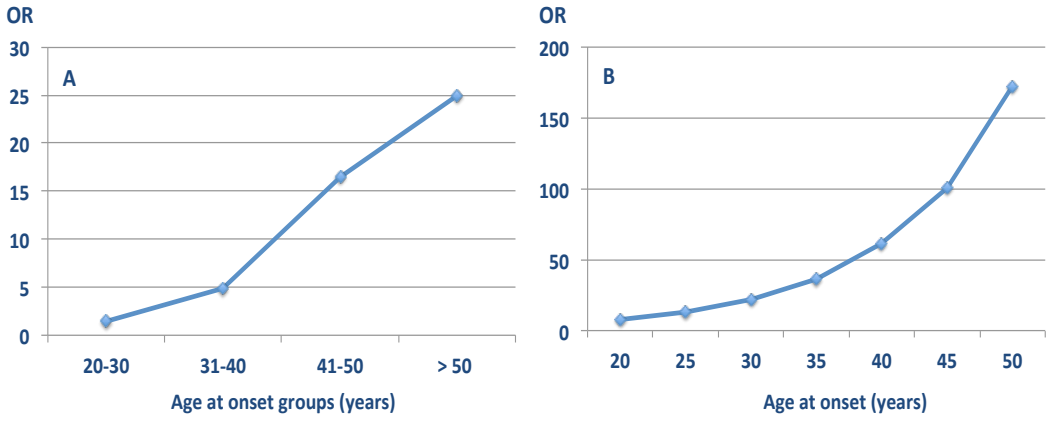
	Age at disease onset	n (%)	Age at onset of progression	n (%)
Total population				
	< 20	112 (10.9%)		
	20-30	452 (52.9%)		
	31-40	266 (26.0%)		
	41-50	157 (15.3%)		
	< 50	40 (3.9)		
missing		6 (0.5%)		
Tot		1023		
RR/SP group	**≤ 20**	145 (17.9%)	**≤ 30***	90 (16.8%)
	21-30	371 (46.0%)	**31-45***	264 (49.4%)
	> 30	285 (35.3%)	**> 45***	155 (29.0%)
missing		5 (0.6%)		25 (4.6%)
Tot		806		534
PP group	**< 35**	76 (35.0%)		
	35-45	83 (38.2%)		
	> 45	57 (26.2%)		
missing		1 (0.4%)		
Tot		217		
PP/SP group			**≤ 35**	243 (32.3%)
			36-45	270 (35.9%)
			> 45	212 (28.2%)
missing				26 (3.4%)
Tot				751

5.3 Results

5.3.1 The age at the disease onset

Information on the age at disease onset was available for all but 6 patients (5 SP and 1 PP MS) (Table 5.3). In the total population, the binary logistic regression analysis was used to calculate the probability of experiencing the PP course, among age at onset categories. The relative risk (OR) increased proportionally with the age and, compared to patients aged < 20, was 1.4 ($p = 0.34$) for the group aged 20-30, 4.9 ($p < 0.001$) for the group aged 31-40, 16.5 for the group aged 41-50 ($p < 0.001$) and 25.0 ($p < 0.001$) for the group aged > 50 (Figure 5.3 A). By assessing the effect of the age at onset as continuous variable, the risk of experiencing the PP course at specific ages was also calculated (regression coefficient 0.103, OR = 1.10 per additional year at onset; $p < 0.001$) (Figure 5.3 B). In both analyses, the probability of having PP MS increased dramatically past the age of 40 (Figure 5.3 A and B). This reflected the large percentage (65%) of PP cases between the ages 30 and 50 at onset (Figure 3.11, Chapter 3).

Figure 5.3 Binary logistic regression analysis in the total population: relative risk of experiencing the PP course according to the age at disease onset. **A**) Risk (OR) of PP (y-axis) in different age at onset groups (x-axis), compared to the group aged < 20; **B**) Risk (OR) of PP (y-axis) according to the increasing age at onset (x-axis).



The total population was stratified in 5 age categories (Table 5.3), in order to assess the relationship between the age at the disease onset and late outcomes. Patients older attained DSS levels from onset in significantly shorter times, and the risk of accumulating disability increased proportionally with the age (Table 5.4 and Figure 5.4). The disease progressed at remarkably different rates, among age groups; the youngest (< 20) patients attained DSS 6, 14 years later than the oldest (> 50) (Table 5.4). By 15 and 18 years from onset, 50% of patients aged < 20 had reached moderate disability and 50% of patients aged > 50 had become bedbound, respectively (Figure 5.4).

Among the age at onset categories, the distribution of the clinical phenotypes varied; most of RR/SP patients had the clinical onset between the age < 20 and 40 and most of PP patients between the age 30 and > 50 (Figure 3.11, Chapter 3). Therefore, in the total population, the different outcomes, among age groups, mainly reflected the different rate of the disability accumulation between the relapsing onset and the progressive onset group. In addition, the disease evolution from DSS 3 was not significantly influenced by the age at onset. Times from moderate disability to DSS 6 and to DSS 8 were remarkably similar, among patients stratified by the age at clinical onset (Table 5.5).

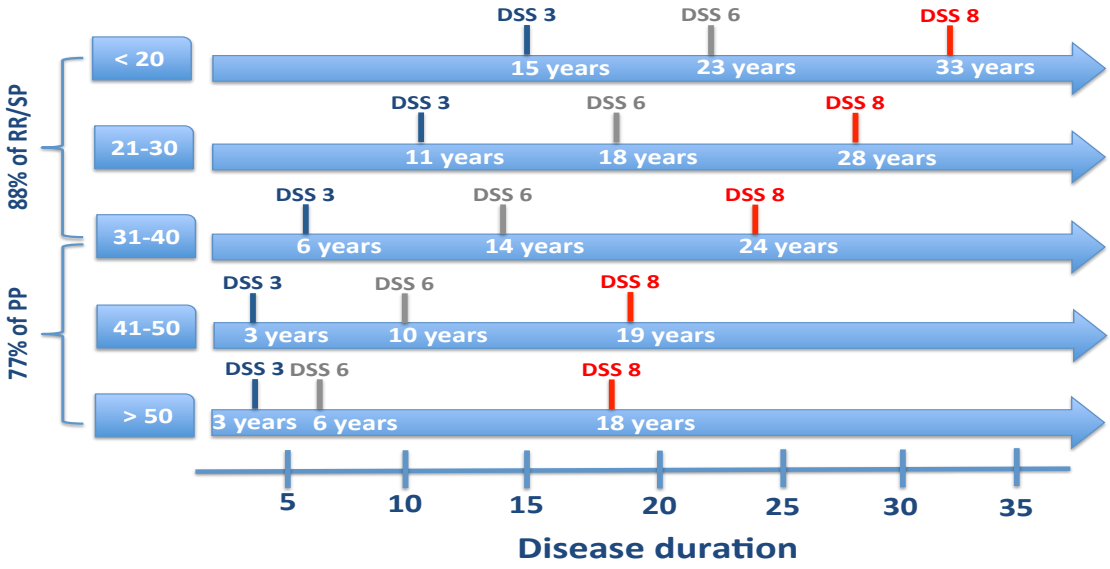
Table 5.4 Kaplan Meier analysis in the total population: times from onset to DSS 6 and DSS 8 among patients stratified by the age at the disease onset. Cox regression analysis: risk (HR) of attaining the endpoints from onset, against the reference category (*).

Age at disease onset	Time from disease onset to DSS 6			Time from disease onset to DSS 8		
	mean [95% CI] years (median)	HR	p value	mean [95% CI] years (median)		p value
< 20	24.4 [20.6-28.1] (23)	0.31	< 0.001	34.2 [29.8-38.6] (33)	0.31	< 0.001
20-30	20.7 [19.2-22-2] (18)	0.39	< 0.001	30.2 [28.2-32.2] (28)	0.38	< 0.001
31-40	15.5 [14.1-16.9] (14)	0.60	0.006	25.8 [23.8-27.8] (24)	0.51	0.003
41-50	11.8 [10.2-13.3] (10)	0.91	0.61	20.1 [18.2-22.0] (19)	0.83	0.43
> 50 *	10.3 [7.6-13.0] (6)			17.5 [14.9-20.0] (18)		

Table 5.5 Kaplan Meier analysis in the total population: times from DSS 3 to DSS 6 and to DSS 8, among patients stratified by the age at the disease onset. Cox regression analysis: risk (HR) of attaining the endpoints from DSS 3, against the reference category (*).

Age at disease onset	Time from DSS 3 to DSS 6			Time from DSS 3 to DSS 8		
	mean [95% CI] years (median)	HR	p value	mean [95% CI] years (median)	HR	p value
< 20	8.3 [6.4-10.2] (5)	0.72	0.13	16.5 [14.2-18.9] (16)	0.99	0.96
20-30	7.5 [6.7-8.3] (5)	0.78	0.17	17.6 [15.8-19.4] (16)	0.94	0.75
31-40	6.9 [5.8-7.9] (5)	0.87	0.48	17.5 [15.8-19.3] (15)	1.28	0.18
41-50	5.9 [5.0-6.8] (4)	0.97	0.91	14.3 [12.9-15.8] (13)	1.38	0.20
> 50 *	5.6 [3.8-7.3] (3)			13.2 [10.9-15.5] (13)		

Figure 5.4 Kaplan Meier analysis in the total population: median times from the disease onset to DSS levels, among age at onset groups.



5.3.1.1 The age at the onset of the RR phase

The association between an older age at onset and worse outcome was confirmed among relapsing onset patients. Groups older at the onset of the RR phase had a significantly higher risk of attaining DSS 6 (OR per additional year at onset = 1.04, $p < 0.001$) and DSS 8 (OR per additional year at onset = 1.02, $p = 0.02$), and a significantly higher risk of converting to SP MS (OR per additional year at onset = 1.04, $p < 0.001$). Onset at age 40 (OR = 4.22) and at age 50 (OR = 6.04) respectively doubled and tripled the probability of entering the SP phase, compared to onset at age 20 (OR = 2.05) (Figure 5.5).

When grouped, RR patients older at the first relapse took significantly shorter times to accumulate severe disability from the disease onset, secondary to the shorter latency to SP MS (Tables 5.6 and 5.6). Patients aged > 30, compared to patients aged ≤ 20, entered the SP phase 12 median years earlier (Table 5.6), and attained DSS 6 and DSS 8 more rapidly (11 and 9 median years difference, respectively) (Table 5.7). This effect was limited to the evolution of the RR phase and disappeared once the progressive phase supervened. Times to disability endpoints from the onset of progression and from DSS 3 were remarkably similar, among age groups (Table 5.8). This demonstrated that the disability accumulation during the SP phase was unaffected by the age at the onset of the RR phase. An older age at onset influenced adversely the outcome by increasing the probability of entering the SP phase (Figure 5.5) and by shortening the latency to progression (Figure 5.6). Indeed, when patients were stratified by the duration of the RR phase (short = 1-5 years; intermediate = 6-12 years; long ≥ 13 years), in each group, times to DSS 6 were similar among age categories and were not significantly affected by the age at the first attack (Figure 5.7).

Figure 5.5 Binary logistic regression analysis in relapsing onset patients: risk (OR) of converting to SP MS (y-axis) according to the age at the onset of the RR phase (x-axis).

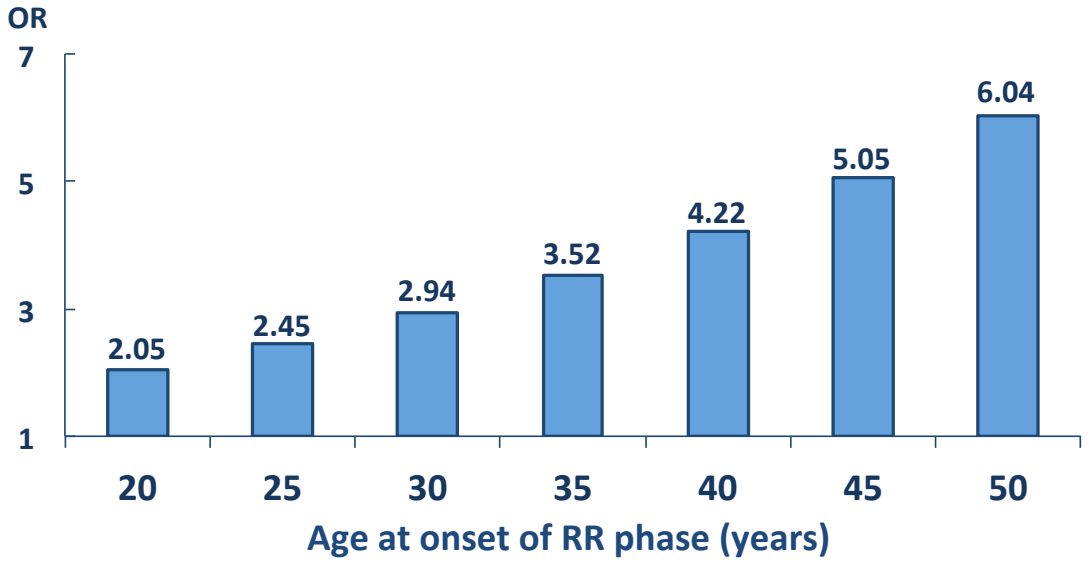


Table 5.6 Kaplan Meier analysis in relapsing onset patients: times to SP from the disease onset, among patients stratified by the age at onset of the RR phase. Cox regression analysis: risk (HR) of attaining the SP phase against the reference category (*).

Age at onset of RR phase	Time from disease onset to SP		
	mean [95% CI] years (median)	HR	p value
≤ 20	25.8 [21.7-29.9] (22)	0.51	< 0.001
21-30	20.2 [18.2-21.7] (16)	0.62	< 0.001
> 30 *	15.3 [13.3-17.3] (10)		

Table 5.7 Kaplan Meier analysis in relapsing onset patients: times from the disease onset to DSS 6 and to DSS 8, among age at onset groups. Cox regression analysis: risk (HR) of attaining the endpoints from the disease onset against the reference category (*).

Age at disease onset	Time from onset to DSS 6			Time from onset to DSS 8		
	mean [95% CI] years (median)	HR	p value	mean [95% CI] years (median)	HR	p
≤ 20	25.6 [22.1-29.1] (25)	0.52	< 0.001	34.8 [30.6-39.0] (34)	0.59	0.002
21-30	21.5 [19.9-23.2] (19)	0.67	< 0.001	31.1 [28.9-33.3] (29)	0.72	0.008
> 30 *	16.8 [15.3-18.3] (14)			26.1 [24.1-28.2] (25)		

Table 5.8 Kaplan Meier analysis in relapsing onset patients: times from DSS 3 and from the onset of SP to DSS 6 and to DSS 8, among age at onset groups. Cox regression analysis: risk (HR) of attaining the endpoints from DSS 3 and from onset of SP against the reference category (*).

Age at disease onset	Time from SP to DSS 6			Time from SP to DSS 8		
	mean [95% CI] years (median)	HR	p	mean [95% CI] years (median)	HR	p
≤ 20	5.0 [3.3-6.7] (2)	1.14	0.27	13.9 [10.2-17.7] (11)	1.28	0.11
21-30	5.0 [4.2-5.8] (3)	1.08	0.39	15.3 [13.3-17.4] (12)	1.10	0.44
> 30 *	5.5 [4.6-6.4] (4)			15.0 [13.4-16.6] (13)		
	Time from DSS 3 to DSS 6			Time from DSS 3 to DSS 8		
	mean [95% CI] years (median)	HR	p	mean [95% CI] years (median)	HR	p
≤ 20	9.1 [7.4-10.9] (6)	0.78	0.07	16.7 [14.6-18.8] (16)	1.00	0.98
21-30	7.7 [6.8-8.7] (6)	0.91	0.33	18.1 [16.0-20.3] (16)	0.99	0.93
> 30	7.4 [6.3-8.5] (5)			16.7 [15.2-18.3] (15)		

Figure 5.6 Kaplan Meier analysis in RR patients stratified by the age at disease onset: comparison of times from the disease onset to the onset of SP, and of times from the onset of SP to DSS 6 and to DSS 8. P values were obtained with Log Rank test. * Reference category.

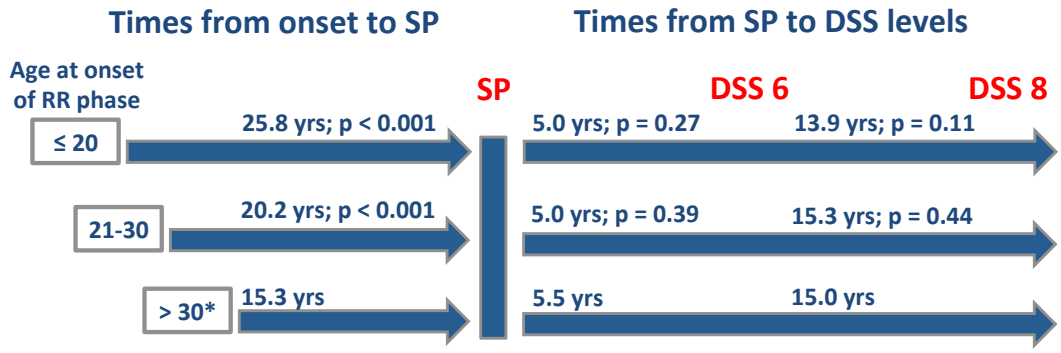
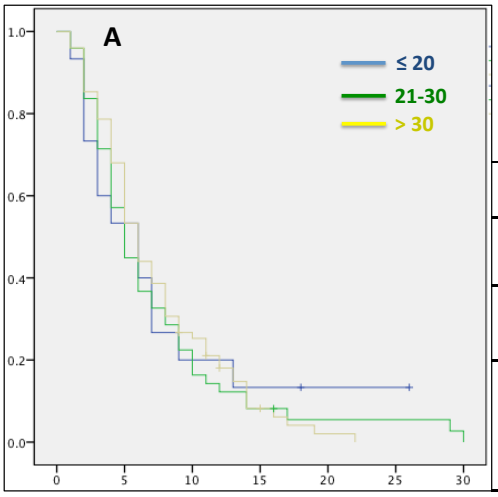
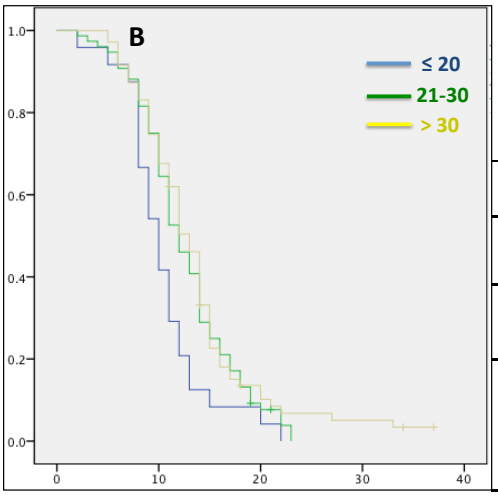


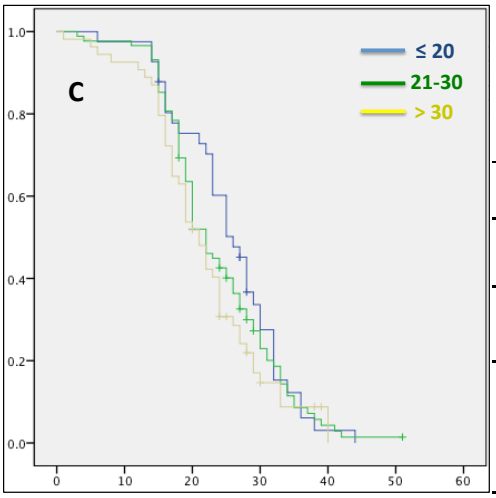
Figure 5.7 Kaplan Meier analysis in relapsing onset patients stratified by the duration of the RR phase (A: 1-5 years; **B**: 6-12 years; **C**: ≥ 13 years): comparison of times from the disease onset to DSS 6, among patients grouped by the age at disease onset. Cox regression analysis: risk (HR) of attaining DSS 6 compared to the reference category (*).



RR phase = 1-5 years			
Age at disease onset	**Time from onset to DSS 6**		
	mean [95% CI] years (median)	**HR**	**p value**
≤ 20	7.8 [3.8-11.7] (6)	0.96	0.91
21-30	7.2 [5.3-9.0] (5)	1.07	0.68
> 30 *	7.3 [6.2-8.5] (6)		



RR phase = 6-12 years			
Age at disease onset	**Time from onset to DSS 6**		
	mean [95% CI] years (median)	**HR**	**p value**
≤ 20	10.4 [8.7-12.1] (10)	1.82	0.013
21-30	12.6 [11.5-13.6] (12)	1.13	0.46
> 30 *	13.7 [12.1-15.3] (13)		



RR phase ≥ 13 years			
Age at disease onset	**Time from onset to DSS 6**		
	mean [95% CI] years (median)	**HR**	**p value**
≤ 20	25.6 [23.0-28.1] (26)	0.71	0.13
21-30	23.8 [22.0-25.7] (22)	0.32	0.83
> 30 *	21.8 [19.4-24.3] (21)		

5.3.1.2 Multiple analysis

The rate of conversion to SP MS is known to increase proportionally with the disease duration (Weinshenker et al., 1989b). The binary logistic regression analysis confirmed that the probability of entering the SP phase became higher with a longer time from the disease onset (OR = 1.07 per additional year of disease duration, $p < 0.001$). Importantly, in the multivariate analysis, the effect of the age at onset of the RR phase on the risk of conversion to SP MS remained unchanged (OR = 1.04) when adjusted to the duration of the disease (OR = 1.07). This indicated that the two variables impacted on the probability of SP independently. In addition, the Cox regression multivariate analysis was used to assess the concomitant effect of the number of early relapses and the age at onset on the risk of attaining SP, DSS 6 and DSS 8. The impact of the two variables on the outcomes did not substantially change between the univariate and the multivariate model, demonstrating that their predictive effect was independent (Table 5.9).

Table 5.9 Cox Regression univariate and multivariate analyses in relapsing onset patients: risk (HR) of attaining SP, DSS 6 and DSS 8 according to the age at the disease onset and to the number of relapses during the first two years (early relapses).

	Risk (HR [95%CI]) of attaining		
	SP	DSS 6	DSS 8
	Univariate analysis		
Age at onset of RR phase	1.02 [1.02-1.03]	1.03 [1.02-1.03]	1.02 [1.01-1.03]
Number of relapses in year 1 and year 2	1.10 [1.03-1.19]	1.22 [1.14-1.31]	1.31 [1.21-1.43]
	Multivariate analysis		
Age at onset of RR phase	1.03 [1.02-1.04]	1.03 [1.02-1.04]	1.02 [1.01-1.04]
Number of relapses in year 1 and year 2	1.13 [1.05-1.22]	1.25 [1.17-1.35]	1.35 [1.24-1.47]

5.3.1.3 The age at onset and the relapse frequency

The Spearman's test was used to assess the correlation between the number of relapses and the age at onset. The analysis demonstrated that the frequency of early relapses was not affected by the age at the first symptom ($r = -0.05$; $p = 0.13$). However, being older at the disease onset correlated with a significantly lower number of late ($r = -0.26$; $p < 0.001$) and total relapses ($r = -0.24$; $p < 0.001$) (Figure 5.8). The longitudinal analysis confirmed that, among age at onset groups, the frequency of total attacks during the RR phase peaked at 20-30 years and gradually decreased with older age (Figure 5.9). Similarly, the mean age at onset of the RR phase increased conversely with the number of total relapses. Patients with a low attack frequency had the clinical onset at older age, compared to patients with a high attack frequency (Figure 5.10).

The Kaplan Meier analysis from birth was used to estimate the influence of the total number of relapses during the RR phase on the age at the disease onset and at the onset of progression. The survival curves among patients with 1 (SAP) 2-3 and 4 or more attacks were compared. The SAP group had disease onset at significantly older age (33 median years), compared to SP groups with 2-3 (30 median years) and ≥ 4 (27 median years) total attacks. However, the age at the onset of progression varied by only 2 mean years among the three groups and was not significantly affected by the total relapses frequency (Figure 5.11).

Figure 5.8 Distribution of the number of late and total relapses according to the age at the onset of the RR phase. Spearman's correlation test was used to calculate the correlation coefficient (r).

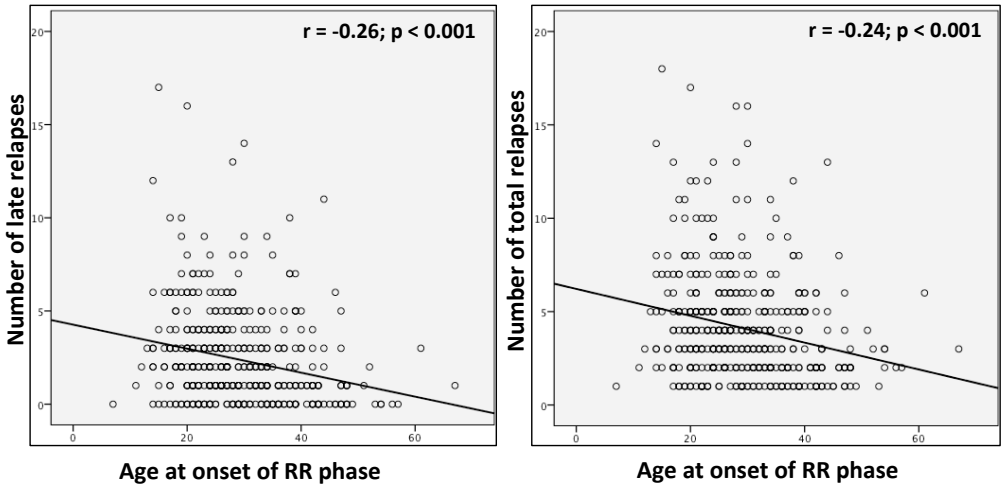


Figure 5.9 Number of total relapses during the RR phase (y-axis), among age at onset groups (x-axis); the exact number of relapses is indicated on top of each column.

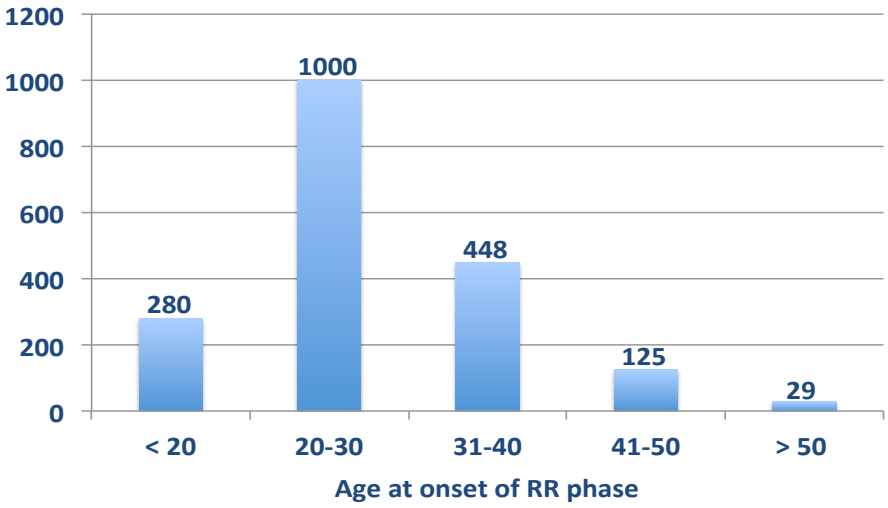


Figure 5.10 Mean age (95% C.I.) at the onset of the RR phase (y-axis), among patients grouped by the number of total relapses (x-axis).

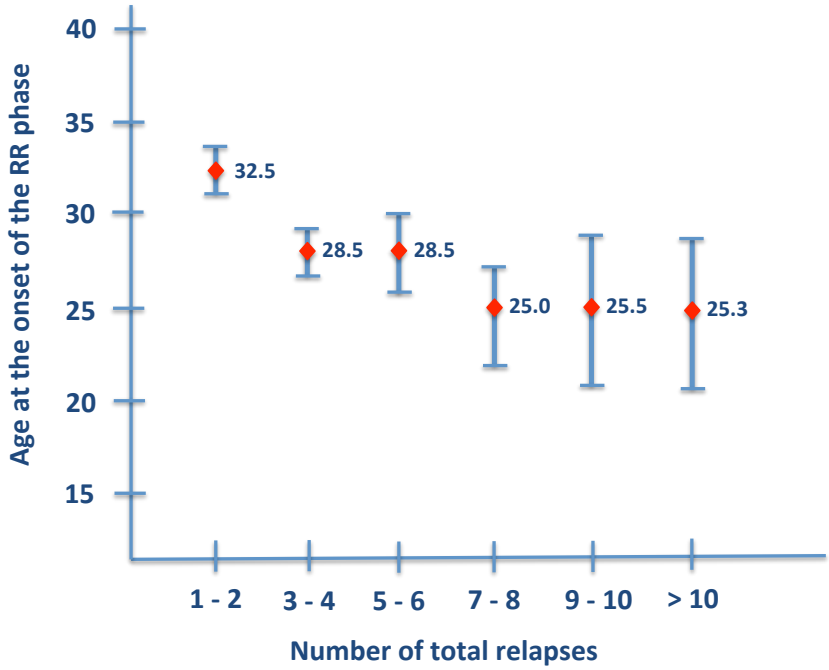
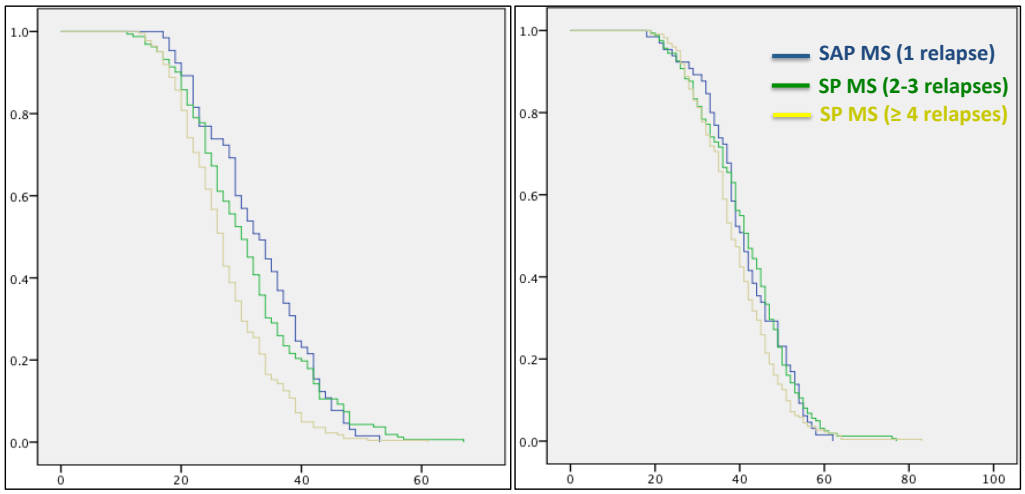


Figure 5.11 Kaplan Meier analysis from birth to the disease onset and to the onset of progression: comparison of the age at the disease onset and at the onset of progression, among SP patients with 1, 2-3, ≥ 4 total attacks during the RR phase. HR and p values were obtained with Cox regression analysis, by comparison against the reference category (*).



Total relapses	Time from birth to onset (age at onset)			Time from birth to SP (age at SP)		
	mean [95% CI] years (median)	HR	p value	mean [95% CI] years (median)	HR	p value
1	32.8 [30.6-35.0] (33)	0.58	< 0.001	41.3 [38.9-43.7] (41)	0.82	0.16
2 -- 3	31.0 [29.5-32.6] (30)	0.64	< 0.001	41.4 [39.7-43.0] (42)	0.79	0.02
≥ 4 *	27.4 [26.4-28.4] (27)			39.2 [38.0-40.5] (38)		

5.3.2 The age at the onset of progression

5.3.2.1 The age at the onset of progression and the clinical phenotype

Among 751 progressive patients, information on the age at onset of progression was not available for 26 cases (25 SP and 1 PP MS) (Table 5.3). The age at which progression started largely varied, from 14 to 83 years (median age = 39): the youngest 25% became progressive by 33 years and the oldest 25% after 47 year (Figure 5.12). The Kaplan Meier analysis was used to compare the time from birth to the disease onset and to the onset of progression between patients experiencing a progressive course, preceded (SP MS = 534) or not (PP MS = 217) by the relapsing remitting phase. The SP group had disease onset 12 median years earlier than the PP group, however the progressive phase started at almost the same age in both subpopulations (40 versus 39 median years, respectively; p = 0.09) (Figure 5.13).

The time from birth to the onset of progression was also compared among PP patients and SP patients stratified by the total number of relapses during the RR phase (1; 2-3; ≥ 4) and by the number of early relapses (1; 2; ≥ 3). Groups with a high total attacks frequency were significantly younger at the disease onset, however the SP subtypes and the PP group started to progress at similar median ages (SAP = 41 years, SP 2-3 relapses = 42 years, SP ≥ 4 = 38 years, PP = 40 years) (Figure 5.14). In contrast, SP patients with different number of early relapses had similar age at the disease onset and, compared to PP patients, had almost the same age at the onset of the progressive phase (Figure 5.15). Again, the group with frequent early attacks (≥ 3) diverged from the other SP patients and attained the progressive phase at significantly younger age (35 median years) (Figure 5.15), secondary to the faster evolution of the RR phase (Chapter 4, Table 4.11).

Figure 5.12 Percentages (y-axis) of progressive patients (PP + SP) entering the progressive phase at different ages (x-axis): percentages are calculated within each age group, independently (10-20; 21-30; 31-40; 41-50; 51-60; 61-70; 71-80). Percentiles of the time from birth to the onset of progression are also indicated.

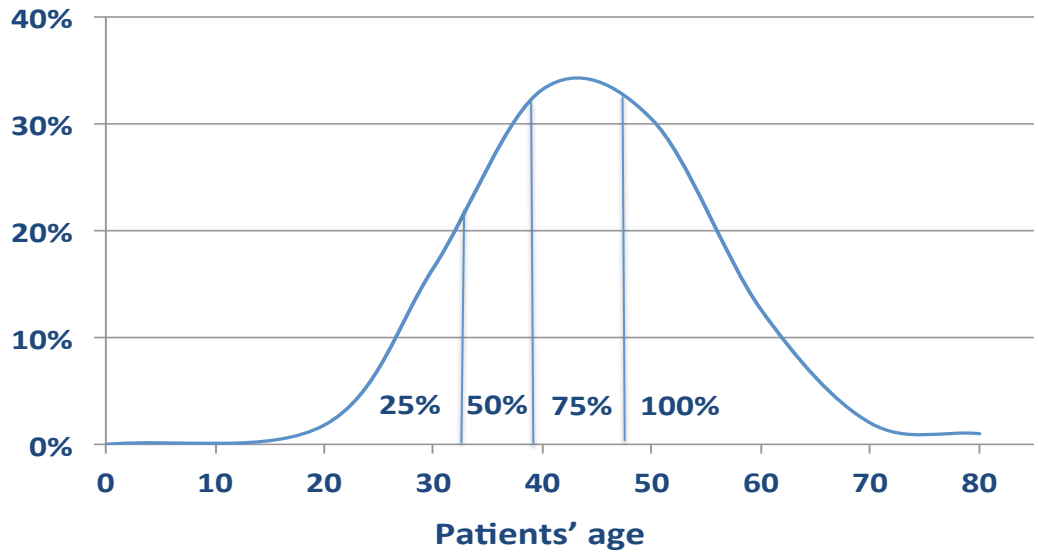
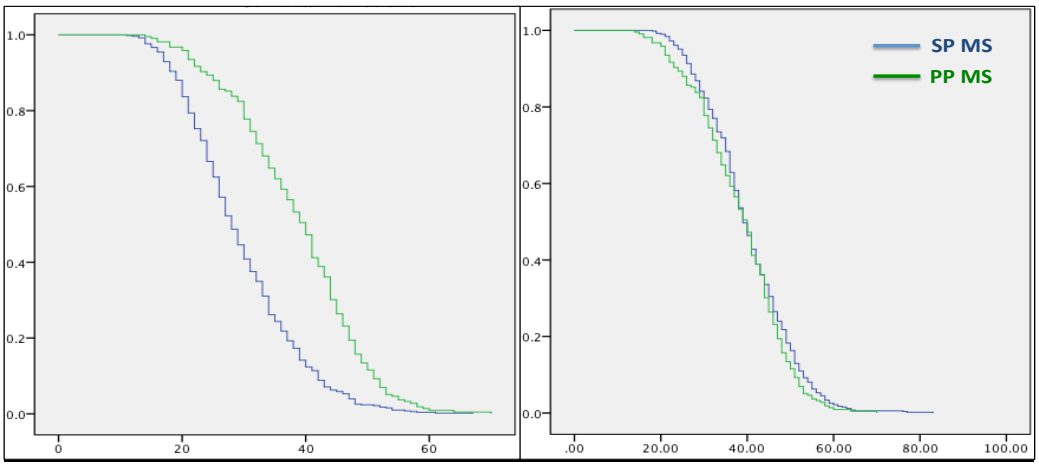
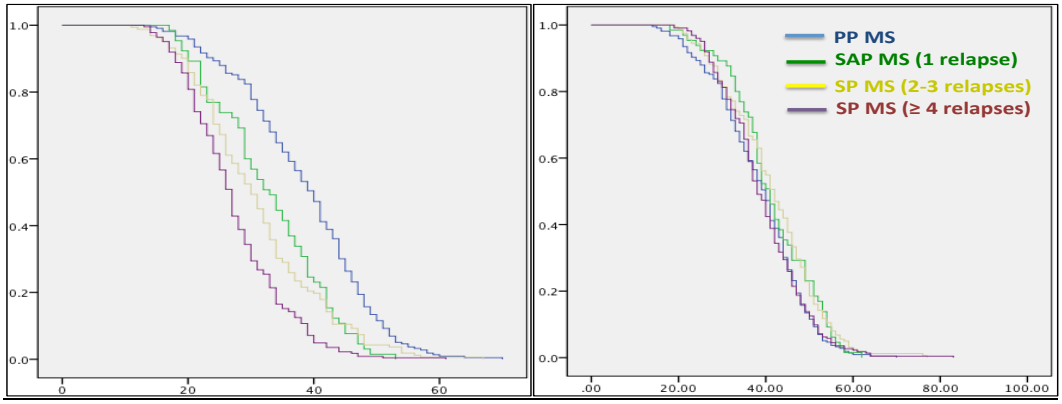


Figure 5.13 Kaplan Meier analysis in progressive patients (PP + SP): comparison of times from birth to the disease onset (age at disease onset) and to the onset of progression (age at onset of progression), between PP and SP MS. P value was obtained with Log Rank test.



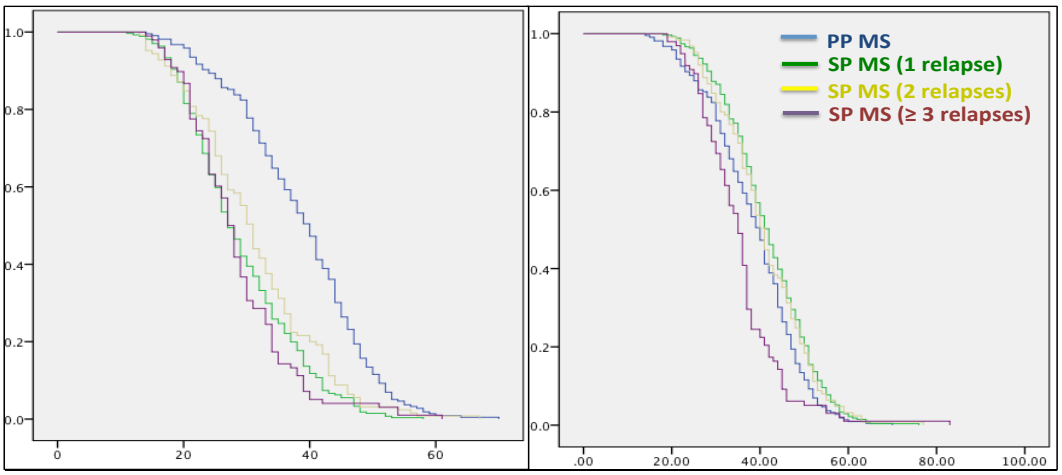
	Time from birth to onset of disease (age at disease onset)		Time from birth to onset of progression (age at onset of progression)	
	mean [95% CI] years (median)	**p value**	**mean [95% CI] years (median)**	**p value**
SP MS	29.4 [28.6-30.2] (28)	0.096	40.2 [39.3-41.0] (39)	0.096
PP MS	38.6 [37.2-39.9] (40)		38.6 [37.2-39.9] (40)	

Figure 5.14 Kaplan Meier analysis in progressive patients (PP + SP): comparison of times from birth to the disease onset (age at disease onset) and to the onset of progression (age at onset of progression) between PP and SP MS (1; 2-3; ≥ 4 total attacks). HR obtained with Cox regression analysis by comparison against the reference category (*).



Total relapses	Time from birth to onset of disease (age at onset)			Time from birth toprogression (age at progression)		
	mean [95% CI] years (median)	HR	p value	mean [95% CI] years (median)	HR	p value
0 (PP MS)	38.6 [37.2-39.9] (40)	0.33	< 0.001	38.6 [37.2-39.9] (40)	1.02	0.78
1 (SAP MS)	32.8 [30.6-35.0] (33)	0.56	< 0.001	41.3 [38.9-43.7] (41)	0.82	0.16
2 -- 3 (SP MS)	31.0 [29.5-32.6] (30)	0.60	< 0.001	41.4 [39.7-43.0] (42)	0.79	0.02
≥ 4 (SP MS)*	27.4 [26.4-28.4] (27)			39.2 [38.0-40.5] (38)		

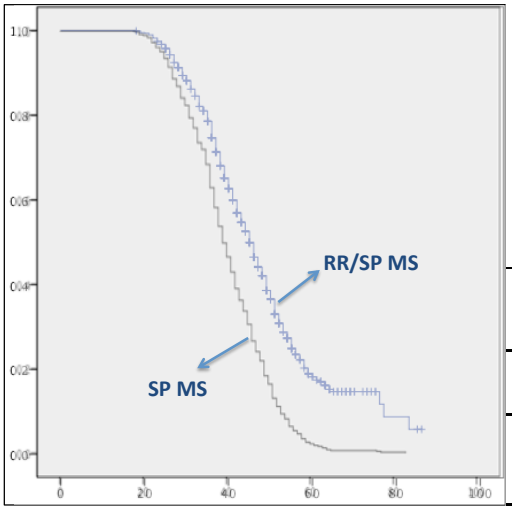
Figure 5.15 Kaplan Meier analysis in progressive patients (PP + SP): comparison of times from birth to the disease onset (age at disease onset) and to the onset of progression (age at onset of progression) between PP and SP MS (1; 2; ≥ 3 early attacks). HR obtained with Cox regression analysis by comparison against the reference category (*).



Relapses Y1-2	Time from birth to onset of disease (age at onset)			Time from birth to progression (age at progression)		
	mean [95% CI] years (median)	HR	p value	mean [95% CI] years (median)	HR	p value
(PP MS)*	38.6 [37.2-39.9] (40)			38.6 [37.2-39.9] (40)		
1 (SP MS)	29.0 [27.9-30.1] (27)	2.3	< 0.001	41.7 [40.5-42.8] (42)	0.78	0.007
2 (SP MS)	31.1 [29.2-32.9] (31)	1.79	< 0.001	41.0 [39.2-42.8] (41)	0.81	0.074
≥ 3 (SP MS)	28.3 [26.7-30.0] (27)	2.47	< 0.001	35.3 [33.4-37.2] (35)	1.44	0.003

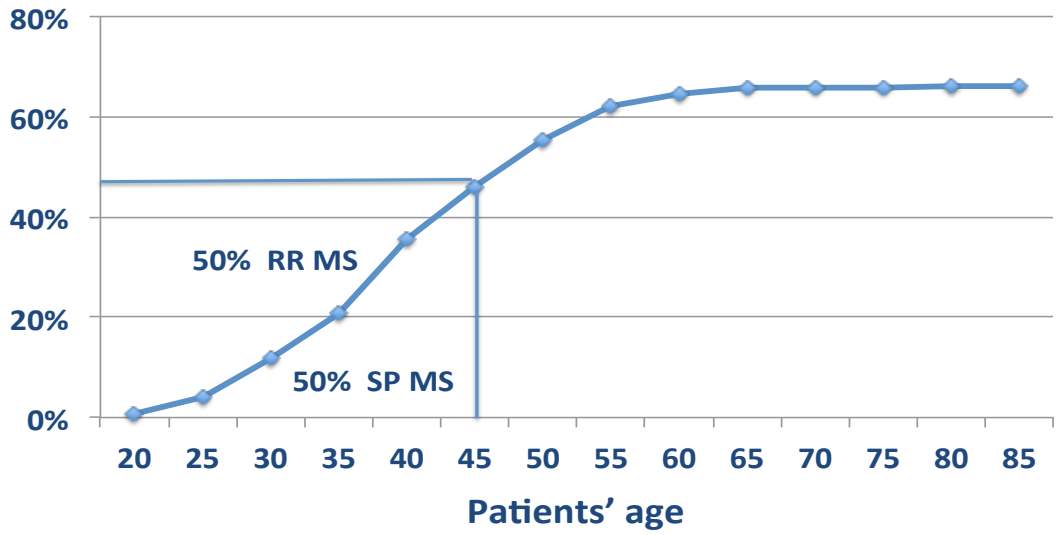
In order to estimate the probability of experiencing the SP course according to age, the time from birth to the onset of the SP phase was also calculated among all relapsing onset patients (n = 806); 37 patients (4.5% or relapsing onset population) were excluded from the analysis because of missing information on the age at SP onset or on the duration of the disease. Among the remaining 769 patients, by the end of the observation period, 509 (66.2%) converted to SP MS and 260 (33.8%) remained free of progression. The inclusion of censored information (patients not attaining the endpoint) in the analysis extended by 8 mean years the Kaplan Meier estimated age at conversion to SP MS (mean age = 48.3 years [95% CI 46.8-49.8]; median age = 45 years) (Figure 5.16). By the age of 35, 20% (n = 161) of patients had attained the SP phase; this percentage increased to 46% (n = 354), to 62% (n = 477) and to 65% (n = 506) by the age of 45, 55 and 75 years, respectively (Figure 5.17). Among those who did not enter the progressive phase (n = 260), the mean disease duration was 20.2 (95% CI 19.1-21.3) years and the mean age at last assessment was 47.0 (95% CI 45.6-48.5) years.

Figure 5.16 Kaplan Meier analysis from birth to the onset of progression among relapsing onset patients (blue line) and among SP patients only (black line).



	Time from birth to onset of progression (age at onset of progression)
	mean [95% CI] years (median)
RR/SP MS	48.3 [46.8-49.8] (45)
SP MS	40.2 [39.3-41.0] (39)

Figure 5.17 Cumulative percentage (y-axis) of patients converting to SP MS according to age (x-axis): data were taken from the Kaplan Meier analysis from birth to the onset of progression, among relapsing onset patients.



5.3.2.2 The age at the onset of progression and late outcomes

Information on the age at the onset of SP was known for 509 patients (95.3% of the SP population) (Table 5.3). The age at conversion to SP MS largely varied from 18 to 83 years (median age = 39); the youngest 25% became progressive by 33 years (25 percentiles) and the oldest after 47 year (75 percentiles).

The age at the onset of secondary progression correlated inversely with the probability of developing severe disability. The negative regression coefficient yielded by the logistic regression analysis indicates that being older at conversion to SP MS associated with a modestly, but significantly lower risk of attaining DSS 6 (OR = 0.96, $p = 0.03$) and DSS 8 (OR = 0.96, $p < 0.001$). This was secondary to the shorter latency to progression, among patients with poor prognosis (Chapter 4), becoming progressive at younger age. Accordingly, groups younger at the onset of the SP phase attained disability endpoints from the disease onset in significantly shorter times (Table 5.10). However, the age at conversion to SP MS did not affect the rate of disability accumulation during the progressive phase. Times from the onset of

progression to disability levels were remarkably similar, among patients grouped by the age at SP onset; only those aged ≤ 30 attained DSS 8 in significantly shorter time (Table 5.11).

Table 5.10 Kaplan Meier analysis in SP patients: times from onset to DSS 6 and to DSS 8, among patients grouped by the age at the onset of SP. Cox regression analysis: risk (HR) of attaining the endpoints against the reference category (*).

Age at onset of SP phase	Time from disease onset to DSS 6			Time from disease onset to DSS 8		
	mean [95% CI] years (median)	HR	p value	mean [95% CI] years (median)	HR	p value
≤ 30	9.8 [7.7-11.2] (8)	3.05	< 0.001	17.1 [14.0-19.6] (13)	3.48	< 0.001
31-45	14.8 [13.7-15.9] (14)	1.57	< 0.001	25.1 [23.7-27.0] (23)	1.47	< 0.001
> 45 *	20.3 [18.0-21.5] (18)			31.3 [28.5-34.1] (29)		

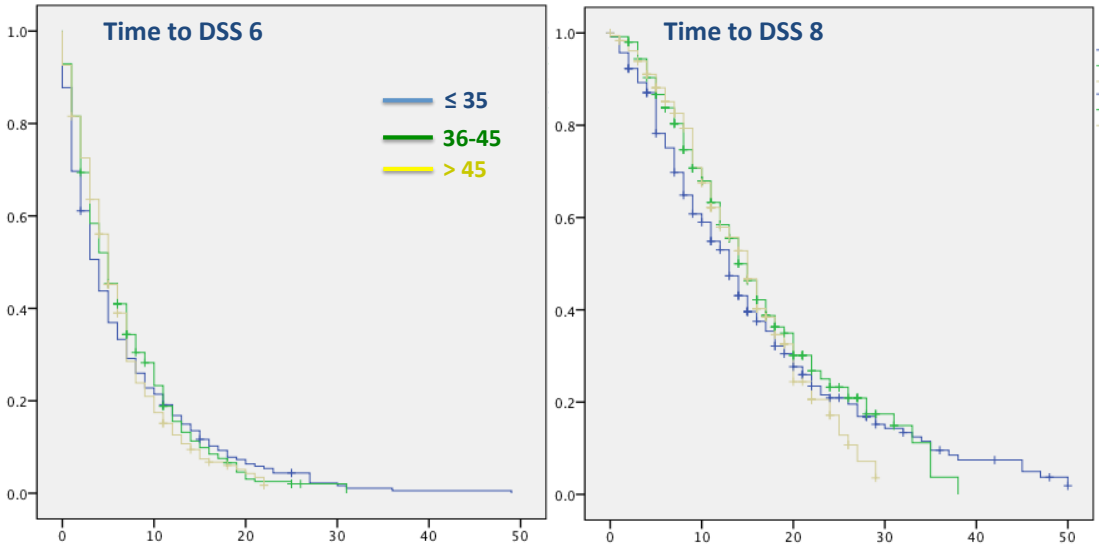
Table 5.11 Kaplan Meier analysis in SP and in PP patients: times from the onset of SP to DSS 6 and to DSS 8, among patients grouped by the age at the onset of SP, and times from the onset of PP to DSS 6 and to DSS 8, among patients grouped by the age at the onset of PP. Cox regression analysis: risk (HR) of attaining the endpoints against the reference category (*).

Age at onset of SP	Time from SP to DSS 6			Time from SP to DSS 8		
	mean [95% CI] years (median)	HR	p value	mean [95% CI] years (median)	HR	p value
≤ 30	4.6 [3.1-6.0] (2)	1.25	0.06	11.4 [8.8-14.0] (7)	1.71	0.001
31-45	5.2 [4.4-5.9] (3)	1.08	0.44	15.3 [13.8-16.8] (13)	1.04	0.76
> 45 *	5.5 [4.6-6.4] (4)			14.7 [13.1-16.4] (15)		
Age at onset of PP	**Time from PP to DSS 6**			**Time from PP to DSS 8**		
	mean [95% CI] years (median)	HR	p value	mean [95% CI] years (median)	HR	p value
< 35	10.2 [8.1-12.2] (7)	0.76	0.15	21.7 [18.6-24.8] (19)	0.64	0.045
35-45	9.6 [8.1-11.1] (9)	0.82	0.23	20.2 [17.8-22.7] (19)	0.71	0.06
> 45	8.0 [6.5-9.6] (7)			16.4 [14.2-18.5) (16)		

Similarly, among PP MS patients, the logistic regression analysis showed no significant impact of the age at the onset of the primary progressive phase on the probability of attaining DSS 6 (OR = 0.93, p = 0.13) and DSS 8 (OR = 0.99, p 0.48). In addition, the Kaplan Meier analysis demonstrated similar times to the endpoints among the 3 age groups (Table 5.11).

All progressive patients (PP + SP) were pooled together and stratified according to the age at start of progression, to further assess its effect on the disability accumulation during the progressive phase. The closely matching survival curves in the three age groups, attaining DSS levels from progression in very similar times, demonstrated no significant impact of the age at the onset of progression on the evolution of the progressive phase (Figure 5.18).

Figure 5.18 Kaplan Meier analysis in progressive patients (PP + SP): times from the onset of progression to DSS 6 and to DSS 8 in patients grouped by the age at onset of progression. Cox regression analysis: risk (HR) of attaining the endpoints against the reference category (*).



Age at onset of progression	Time from onset of progression to DSS 6			Time from onset of progression to DSS 8		
	mean [95% CI] years (median)	HR	p value	mean [95% CI] years (median)	HR	p value
≤ 35	6.4 [5.4-7.5] (4)	1.01	0.91	16.2 [14.4-18.0] (13)	1.04	0.71
36-45	6.7 [5.9-7.5] (5)	0.95	0.66	16.8 [15.3-18.3] (14)	0.92	0.52
> 45 *	6.2 [5.4-7.0] (5)			15.1 [13.8-16.4] (15)		

5.3.3 The current age

Current age (age at last visit) was known for 1004 patients and ranged between 18 and 94 year. At last assessment, in the total population the mean and the median age were 54.9 (95% CI 54.1-55.7) and 55 years, respectively.

The logistic regression analysis demonstrated that the probability of accumulating disability increased proportionally with ageing. Growing older associated with a significantly higher risk of attaining DSS 3 (OR per additional year = 1.08, $p < 0.001$), DSS 6 (OR per additional year = 1.06, $p < 0.001$) and DSS 8 (OR per additional year = 1.04, $p < 0.001$). Similar effect was exerted by the length of the disease course, which increased proportionally the probability of attaining the endpoints (OR per additional year: DSS 3 = 1.08, $p < 0.001$; DSS 6 = 1.06, $p < 0.001$; DSS 8 = 1.03, $p < 0.001$). However, when the variables were tested in the multivariate model, the effect of current age remained unchanged, while the predictive effect of the disease duration became not significant for all endpoints (Table 5.12). In addition, the impact of ageing on the outcomes was tested in patients stratified by the length of the disease course (short ≤ 15 years; intermediate 16-25 years; long > 25 years). In each subgroup, the effect of current age remained significant and its size did not substantially vary (Table 5.13).

Among relapsing onset patients, growing older increased the probability of converting to SP MS (OR = 1.06, $p < 0.001$) and of developing severe disability (DSS 6 OR = 1.06, $p < 0.001$; DSS 8 OR = 1.04, $p < 0.001$). The multivariate analysis left its effect unchanged, while the impact of the disease duration on the risk of attaining DSS 6 and DSS 8 became not significant (Table 5.14). Current age was assessed as continuous variable, in order to estimate the probability of requiring a walking aid and of attaining SP at specific ages. The relative risk for reaching both endpoints was very low at the age of 20 (OR = 3.1 and 3.3, respectively) and increased proportionally with ageing, becoming about 6 folds higher at the age of 50 (OR =

17.2 and 20.0, respectively), and about 20 times higher at the age of 70 (OR = 54.0 and 66.0, respectively) (Figure 5.19).

In striking contrast, current age did not exert any significant impact on the attainment of the disability endpoints, among PP patients (DSS 3 OR = 0.93, p = 0.52; DSS 6 OR = 1.01, p = 0.70; DSS 8 OR = 1.01, p = 0.27), and among SP patients (DSS 3 OR = 1.06, p = 0.47; DSS 6 OR = 1.01, p = 0.25; DSS 8 OR = 1.00, p = 0.23) (Table 5.15).

Table 5.12 Binary logistic regression univariate and multivariate analyses in the total population: probability (OR) of attaining disability endpoints according to the current age and the disease duration.

	Probability (OR [95%CI]) of attaining		
	DSS 3	DSS 6	DSS 8
	Univariate analysis		
Current age	1.08 [1.06-1.10]; p < 0.001	1.06 [1.05-1.08]; p < 0.001	1.04 [1.03-1.05]; p < 0.001
Disease duration	1.08 [1.06-1.11]; p < 0.001	1.06 [1.04-1.07]; p < 0.001	1.03 [1.02-1.05]; p < 0.001
	Multivariate analysis		
Current age	1.07 [1.04-1.09]; p < 0.001	1.06 [1.04-1.08]; p < 0.001	1.04 [1.02-1.05]; p < 0.001
Disease duration	1.02 [0.99-1.05]; p = 0.18	1.00 [0.98-1.02]; p = 0.74	1.00 [0.98-1.01]; p = 0.99

Table 5.13 Binary logistic regression analysis in the total population, stratified according to the disease duration (short, intermediate, long): probability (OR) of attaining disability endpoints according to the current age.

	Disease duration ≤ 15 years		**Disease duration 16-25 years**		**Disease duration > 25 years**	
Probability of reaching	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
DSS 3	1.07 (1.03-1.12)	< 0.001	1.07 (1.04-1.11)	< 0.001	1.04 (0.99-1.10)	0.09
DSS 6	1.07 (1.03-1.05)	< 0.001	1.06 (1.04-1.09)	< 0.001	1.05 (1.02-1.08)	0.001
DSS 8	1.04 (1.02-1.07)	0.001	1.04 (1.02-1.06)	< 0.001	1.03 (1.01-1.05)	0.001

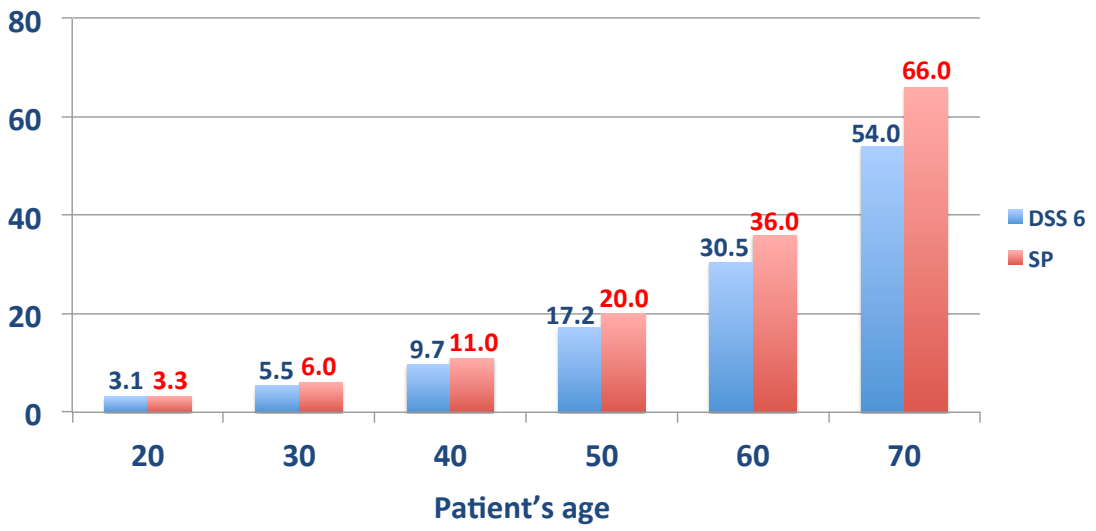
Table 5.14 Binary logistic regression univariate and multivariate analyses in the relapsing onset population: probability (OR) of attaining SP and disability endpoints according to the current age and the disease duration.

	Probability (OR [95%CI]) of attaining		
	SP	DSS 6	DSS 8
	Univariate analysis		
Current age	1.06 [1.04-1.07]; p < 0.001	1.05 [1.04-1.07]; p < 0.001	1.03 [1.02-1.05]; p < 0.001
Disease duration	1.07 [1.05-1.09]; p < 0.001	1.06 [1.04-1.08]; p < 0.001	1.04 [1.02-1.05]; p < 0.001
	Multivariate analysis		
Current age	1.04 [1.02-1.06]; p < 0.001	1.04 [1.02-1.06]; p < 0.001	1.03 [1.01-1.04]; p < 0.001
Disease duration	1.03 [1.00-1.05]; p = 0.18	1.02 [0.99-1.04]; p = 0.69	1.01 [0.99-1.03]; p = 0.23

Table 5.15 Binary logistic regression analysis in the SP and PP populations assessed separately: probability (OR) of attaining disability endpoints according to the current age.

	Probability (OR [95%CI]) of attaining		
	DSS 3	DSS 6	DSS 8
	Univariate analysis		
PP MS	0.93 [0.76-1.14]; p = 0.52	1.01 [0.94-1.08]; p = 0.72	1.01 [0.98-1.04]; p = 0.27
SP MS	1.06 [0.89-1.25]; p = 0.47	1.01 [0.98-1.04]; p = 0.25	1.00 [0.99-1.02]; p = 0.23

Figure 5.19 Binary Logistic regression analysis in RR patients. Probability of reaching DSS 6 (regression coefficient = 0.057, OR per additional year = 1.05; p < 0.001) and SP (regression coefficient = 0.060, OR per additional year = 1.06; p < 0.001) according to the current age: risk (ORs on y-axis) of requiring a walking aid and of converting to SP MS according to patients' age (x-axis). ORs are indicated on top of each column.



5.3.4 The age at disability landmark

For the total population of 1023 patients the mean age at DSS 3 was 41.6 years (95% CI 40.8 - 42.4), at DSS 6 was 49.0 years (95% CI 48.0 - 50.0), at DSS 8 was 58.7 years (95% CI 57.5 - 59.9) and at DSS 10 was 74.9 (95% CI 72.9-76.5) (Table 5.16). By the end of the observation period, among patients who had required a walking cane, the age at the attainment of the endpoint ranged from 16 to 85 years (median age = 48 years); the youngest 25% reached DSS 6 by the age of 37 and the oldest 25% after the age of 53.

Table 5.16 Kaplan Meier analysis from birth to disability endpoints in the total population. Mean and median ages at DSS 3, 6, 8 and 10.

Time from birth to disability endpoints	
	mean [95% CI] years (median)
DSS 3	41.6 [40.8-42.4] (41)
DSS 6	49.0 [48.0-50.0] (48)
DSS 8	58.7 [57.5-59.9] (58)
DSS 10	74.9 [72.9-76.5] (78)

5.3.4.1 Factors affecting the age at disability levels

The Kaplan Meier analysis from birth demonstrated men attained the endpoints at modestly younger age than women (3 mean years difference) (Table 5.17). In addition, groups younger at the disease onset took longer times to reach disability outcomes (Table 5.4), however they were younger when attaining the DSS levels (Table 5.17 and Figure 5.20). Among relapsing onset and progressive onset patients assessed separately, males and groups younger at onset became severely disabled at younger age (Table 5.18). In addition, patients older at the onset of the RR phase were older at the onset of the SP phase (Table 5.19).

Table 5.17 Kaplan Meier analysis: time from birth to DSS 6 and DSS 8 (age at disability levels) in the total population grouped by sex, age at disease onset and type of clinical presentation. Cox regression: risk (HR) of attaining the endpoint from birth among categories.

	Time from birth to DSS 6			Time from birth to DSS 8		
	Mean (95% CI) [median] age at DSS 6	HR	p	Mean (95% CI) [median] age at DSS 8	HR	p
Total population	49.0 [48.0-50.0] (48)			58.7 [57.5-59.9] (58)		
Gender						
Males	47.0 [45.5-48.5] (46)	1.27	0.02	56.8 [54.9-58.7] (56)	1.22	0.03
* Females	50.1 [48.8-51.3] (50)			59.6 [58.1-61.2] (59)		
Age at onset						
*< 20	40.9 [37.3-44.4] (38)			50.8 [46.4-55.3] (48)		
20-30	45.8 [44.2-47.4] (43)	0.68	0.005	55.3 [53.3-57.2] (54)	0.69	0.02
31-40	50.6 [49.1-52.0] (48)	0.47	< 0.001	60.4 [58.6-62.3] (59)	0.44	< 0.001
41-50	56.6 [54.9-58.3] (55)	0.32	< 0.001	64.9 [63.0-66.9] (64)	0.35	< 0.001
> 50	65.5 [62.5-68.5] (63)	0.18	< 0.001	73.0 [69.7-76.4] (72)	0.19	< 0.001
Type of symtpoms at disease						
Motor						
absent	49.1 [47.9-50.3] (48)	0.93	0.46	59.0 [57.5-60.3] (58)	0.87	0.24
* present	49.0 [47.3-50.7] (49)			58.3 [56.3-60.4] (58)		
Sensory						
absent	49.4 [48.1-50.7] (49)	0.88	0.18	58.7 [57.2-60.2] (58)	0.86	0.15
* present	48.6 [47.1-50.0] (47)			58.6 [56.8-60.5] (58)		
Cerebellar						
absent	49.3 [48.3-50.3] (48)	0.74	0.06	59.1 [57.9-60.4] (59)	0.59	0.003
* present	44.6 [40.9-48.2] (45)			51.7 [47.2-56.1] (53)		
Brainstem						
absent	49.3 [48.2-50.3] (49)	0.84	0.13	59.3 [58.0-60.6] (59)	0.68	0.006
* present	47.7 [45.2-50.2] (47)			55.1 [52.1-58.2] (55)		
Optic						
absent	49.0 [47.9-50.0] (48)	0.95	0.69	58.5 [57.2-59.8] (58)	0.99	0.98
* present	48.9 [46.5-51.2] (50)			59.6 [56.8-62.4] (60)		

Figure 5.20 Kaplan Meier analysis from birth (age at) and from disease onset to DSS 8 in the total population, grouped by the age at the disease onset.

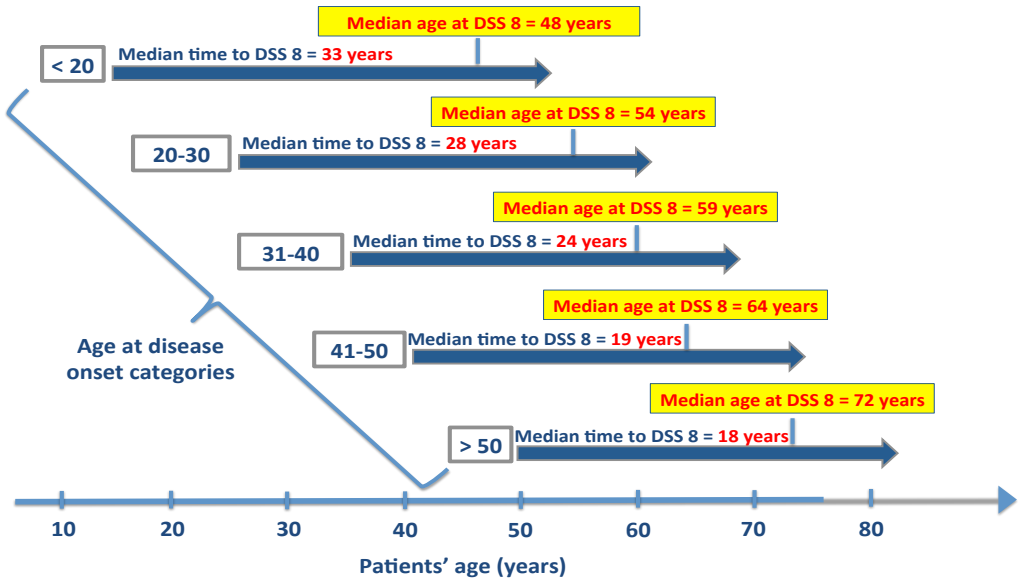


Table 5.18 Kaplan Meier analysis: time from birth to DSS 6 (age at DSS 6) in the relapsing onset and in the progressive onset group, stratified by sex and by the age at the disease onset. Cox regression: risk (HR) of attaining the endpoint from birth among categories.

	Time from birth to DSS 6					
	Relapsing onset patients			**Progressive onset patients**		
	Mean (95% CI) [median] age at DSS 6	**HR**	**p**	**Mean (95% CI) [median] age at DSS 6**	**HR**	**p**
Gender						
Males	47.9 [46.0-49.8] (46)	1.22	0.03	45.4 [43.0-47.8] (46)	1.46	0.08
* Females	50.6 [49.0-52.2] (49)			49.8 [47.6-52.1] (51)		
Age at onset						
*< 20	42.0 [38.2-45.7] (42)			28.1 [21.7-34.5] (25)		
20-30	46.5 [45.0-48.0] (45)	0.68	0.008	35.5 [31.9-39.0] (33)	0.18	< 0.001
31-40	52.4 [50.7-54.2] (50)	0.45	< 0.001	45.3 [43.6-47.0] (45)	0.11	< 0.001
41-50	60.7 [57.5-63.9] (57)	0.28	< 0.001	53.8 [52.4-55.2] (53)	0.05	< 0.001
> 50	70.1 [64.1-76.1] (67)	0.15	< 0.001	62.8 [60.0-65.7] (61)	0.02	< 0.001

Table 5.19 Kaplan Meier analysis: time from birth to SP (age at SP) in relapsing onset patients stratified by the age at the onset of the RR phase. Cox regression analysis: risk of attaining SP from birth against the reference (*) category.

Age at onset of RR phase	**Time from birth to SP (age at SP)**		
	mean [95% CI] years (median)	**HR**	**p value**
≤ 20	42.9 [39.2-46.6] (39)	2.24	< 0.001
21-30	45.9 [44.0-47.7] (42)	1.59	< 0.001
> 30 *	53.3 [51.4-55.2] (50)		

5.3.4.2 The age at disability and the clinical phenotype

The number of patients reaching DSS levels increased proportionally with age; the rate at which disability accumulated while growing older was similar between PP and SP groups. By the age of 55, 70.9% (n = 154) of PP patients and 74.7% (n = 399) of SP patients had reached DSS 6. These percentages increased to 89.4% (n = 194) and to 85.9% (n = 459), respectively, by the age of 65 and to 92.1% (n = 200) and to 88.0% (n = 470), respectively, by the age of 75 (Figure 5.21).

The Kaplan Meier analysis from birth demonstrated that relapsing onset and progressive onset patients attained DSS 3 (41 versus 43 median years, p = 0.82), DSS 6 (48 versus 49 median years, p = 0.05), DSS 8 (58 versus 58 median years, p = 0.44) and DSS 10 (77 versus 78 median years, p = 0.55) at remarkably similar ages (Figure 5.22). The age at disability milestones was unaffected by the type of the disease course even among males (median age at DSS 6: RR/SP = 46 versus PP = 46, p = 0.09), and among females (median age at DSS 6: RR/SP = 49 versus PP = 51, p = 0.35), assessed separately.

Figure 5.21 SP and PP patients assessed separately: cumulative percentages (y-axis) attaining DSS 6 at different ages (x-axis).

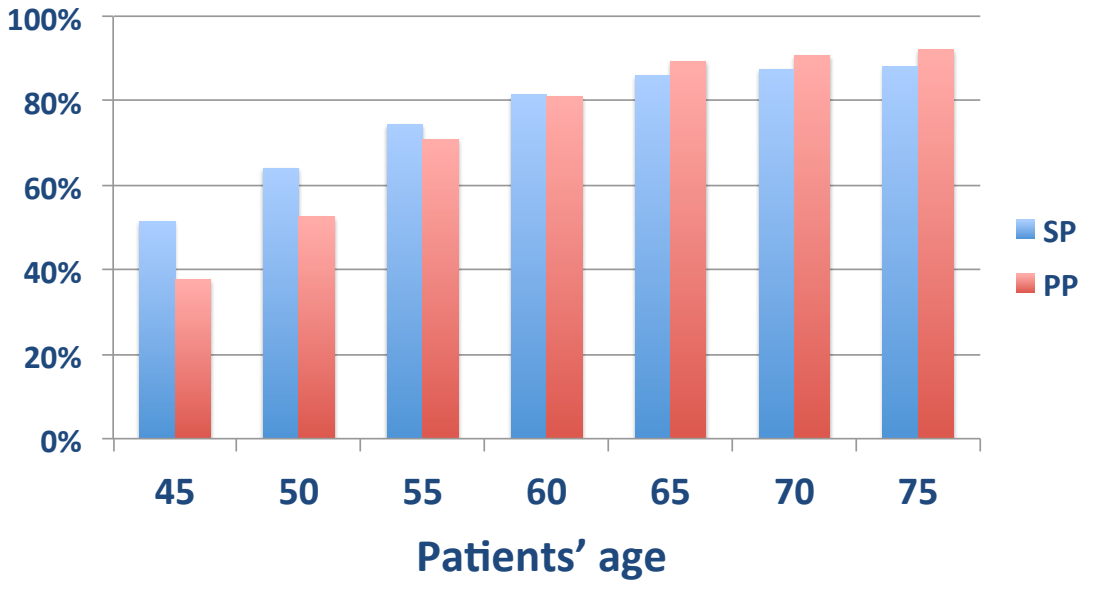
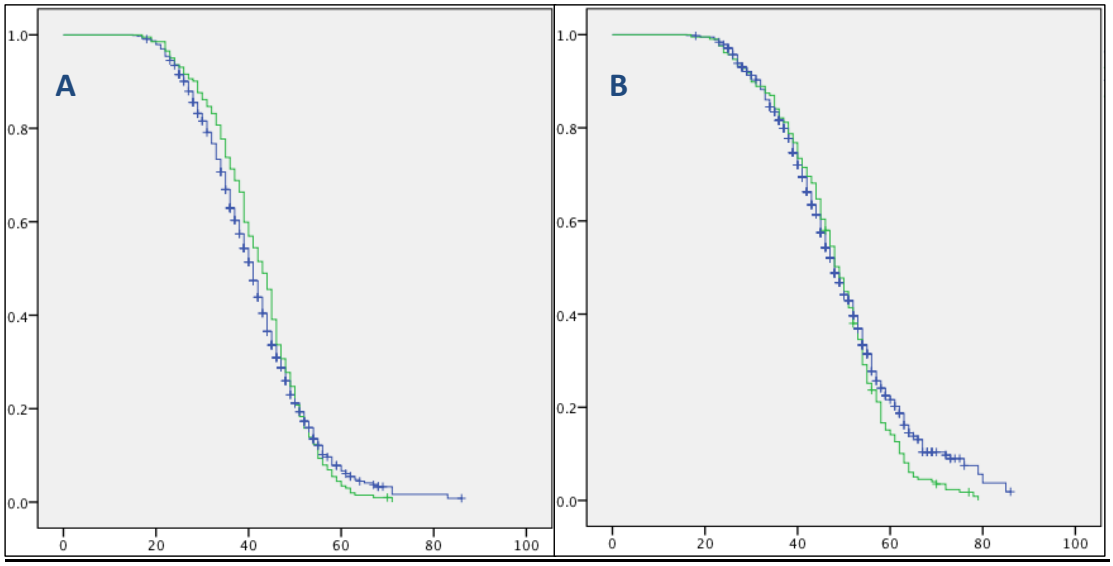
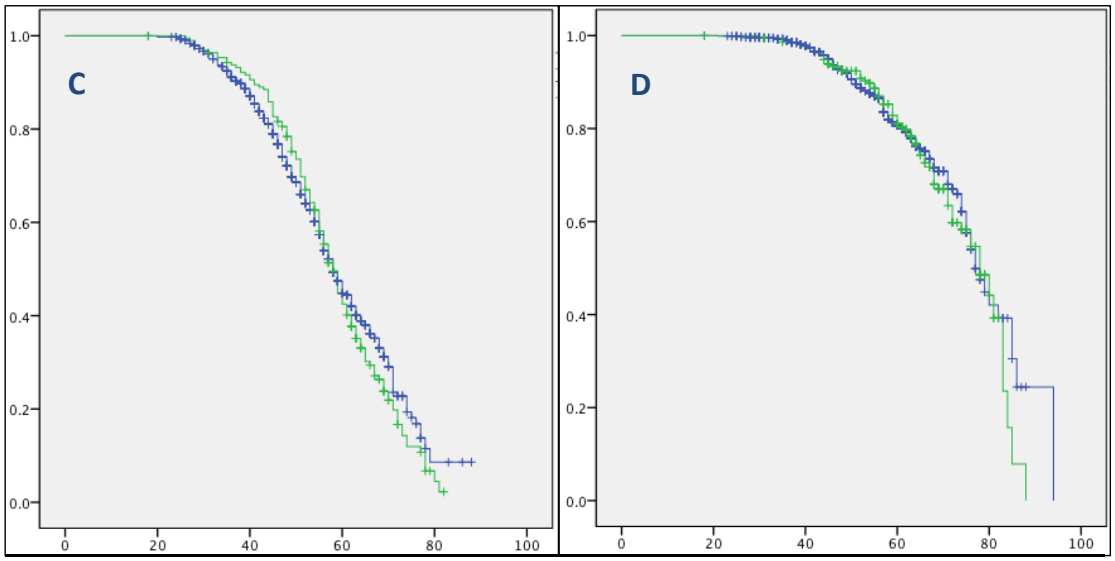


Figure 5.22 Kaplan Meier analysis from birth to DSS 3 (A), DSS 6 (B), DSS 8 (C) and DSS 10 (D): comparison between relapsing onset (blue lines) and progressive onset (green lines) groups. P values were obtained with Log Rank test.



	Mean (95% CI) [median] years at DSS 3	p	Mean (95% CI) [median] years at DSS 6	p
RR/SP	41.6 [40.6-52.6] (41)	0.82	49.7 [48.5-51.0] (48)	0.05
PP	42.3 [40.8-43.7] (43)		48.0 [46.3-49.7] (49)	



	Mean (95% CI) [median] years at DSS 8	p	Mean (95% CI) [median] years at DSS 10	p
RR/SP	59.2 [57.6-60.8] (58)	0.44	75.7 [73.3-78.2] (77)	0.55
PP	58.4 [56.4-60.3] (58)		73.2 [71.0-75.4] (78)	

5.4 Discussion

One of the most puzzling features of MS is its heterogeneous clinical course. The disability accumulates at different rate, among patients, and the disease evolution follows a variable pattern, characterized either by periods of acute worsening (relapses) or by a gradual steady deterioration, or by a combination of both. According to an international consensus, the classification of the disease course is based of these clinical features, and particularly on their points of departure (Lublin and Reingold, 1996). The distinction of clinical phenotypes is convenient and allows to better defining the disability accumulation over time. However, different disease phases are not always discretely separable and do not necessarily mirror different pathological mechanisms, occurring sequentially.

It is generally accepted that the inflammation plays a dominant role in the development of focal demyelinating lesions, but the axonal injury and loss are the major determinants of the unremitting disability (Trapp and Nave, 2008). Both pathological processes are known to occur independently of their clinical counterpart, highlighting a "biological-clinical paradox", which contributes to the difficulties of understanding mechanisms driving the disease evolution. From MRI studies, it is well established that relapses are just the "filtered" clinical expression of an underlying inflammatory activity (Barkhof, 2002). The axonal loss and the degeneration were shown to take place early in the disease course (Filippi et al., 2003; Trapp et al., 1998), when permanent disability has not developed. The assumption that the pathological processes driving the RR and the SP phases are causally related could be simply a misconception of distinguishing separate clinical stages, and it is not easily applicable to PP MS patients.

The reason why the progressive phase can start "de novo" or following inflammatory attacks remain largely debated, but it is unlikely that PP MS represents a separate

clinical entity (Antel et al., 2012; Rice et al., 2013). Indeed, its pathological features are similar to SP MS (Frischer et al., 2009; Revesz et al., 1994), implying that the disability accumulation during the progressive phase might be driven by common mechanisms, among all subtypes. This is also supported by evidence from natural history studies, which demonstrated that the disease evolution from moderate disability (Confavreux and Vukusic, 2006a; Debouverie et al., 2008; Leray et al., 2010) and from onset progression (Kremenchutzky et al., 2006a) is not influenced by the initial clinical course. The role of the inflammation on the development of severe disability remains ambiguous. Early relapses were shown to significantly affect the attainment of late outcomes, however late inflammatory attacks did not influence the latency to progression and the long-term disease evolution (Chapter 4). This suggests that, after an early watershed is reached, focal inflammatory mechanisms probably disconnect from those leading to the onset of the progressive phase.

Age might account for this dissociation and might explain the variable clinical phenotype, among patients. The changes of the disease course over time could be determined by the different ages at which pathological mechanisms become clinically evident. Indeed, progressive MS patients share the same age at onset of progression (Confavreux and Vukusic, 2006a; Koch et al., 2007; Kremenchutzky et al., 2006a; Tutuncu et al., 2013), and the age at the attainment of DSS levels is only marginally affected by the type of the disease course (Confavreux and Vukusic, 2006b; Leray et al., 2010). Overall this data lend support to the unifying concept of the disease evolution (Confavreux and Vukusic, 2006a) and suggest that age related mechanisms might play an important role in the chronic diffuse neurodegeneration, which is the likely pathological substratum of the clinical progression (Confavreux and Vukusic, 2006b). The analyses of the LO database presented in this study allowed to further address the complex interaction between age, inflammatory attacks and the evolution of the progressive phase, attempting to better elucidate factors responsible for the variable clinical pattern among patients.

5.4.1 Age and the long-term disease evolution

5.4.1.1 The total population

As largely reported by previous studies (Table 5.1), the analysis confirmed an association between older age at the disease onset and a worse outcome. Among the LO total population, patients older at the clinical onset attained disability levels in significantly shorter times (Table 5.4). Age groups exhibited a remarkably different rate of disability accumulation (Figure 5.4). Patients aged 20-30 at onset, compared to those aged more than 50, required a walking cane and became bedbound 12 and 10 median years later, respectively. These differences mainly reflected the distribution of the clinical phenotypes, among age categories. Most of the progressive onset patients had the disease onset between the age of 30 and 50 (Figure 3.11, Chapter 3) and, therefore, their faster disease evolution largely accounted for the worse outcome, among groups older at onset in the total population. Indeed, the probability of experiencing the PP course increased with the age at first symptoms (OR = 1.10 per additional year at onset) and peaked between the age of 40 and of 50 (Figure 5.3 B). However, the age at the disease onset did not affect the times from moderate disability (DSS 3) to DSS levels (Table 5.5). This is in line with previous studies (Confavreux and Vukusic, 2006a; Debouverie et al., 2008; Leray et al., 2010), it further emphasizes DSS 3 as the clinical watershed separating two independent phases, and supports the amnestic nature of the late disease evolution, which is largely unaffected by factors influencing the early disease stage.

The analysis of the effect of current age on the attainment of late outcomes further elucidated the relationship between age and the disease course. Growing older in the total population necessarily associated with a higher probability of developing severe disability. The hazard of attaining the DSS levels increased proportionally with the current age (OR per additional year: DSS 3 = 1.08, DSS 6 = 1.06, DSS 8 = 1.04). However, the disease duration also influenced the attainment of the endpoints (ORs per additional year: DSS 3 = 1.08, DSS 6 = 1.06, DSS 8 = 1.03), similarly to ageing.

Importantly, when the two variables were tested in the same multivariate model, the length of the disease course did not significantly affect the outcomes, while the effect of current age remained significant (Table 5.12). In addition, the probability of developing unremitting disability increased proportionally by growing older even among groups of patients with a short disease duration (Table 5.13). These results indicate that ageing is a strong determinant of the outcome and suggest that the accumulation of disability is affected by age related mechanisms, independently of the length of the disease course.

5.4.1.2 The relapsing onset and the progressive onset subgroups

When progressive and relapsing onset patients were assessed separately, the analysis highlighted a largely different effect of age on the two clinical phenotypes. The age at the onset of PP MS did not impact on the attainment of disability endpoints (Table 5.11). In contrast, an older age at the onset of the RR phase associated with a significantly faster disease progression. Groups aged > 30 at the first attack, compared to those aged ≤ 20, reached much earlier DSS 6 (11 median years difference) and DSS 8 (9 median years difference) (Table 5.7). This effect was limited to the evolution of the RR phase and disappeared once the SP phase supervened. Times from DSS 3 and from SP to disability endpoints were remarkably similar among age groups, demonstrating that the evolution of the SP phase was not influenced by the age at which the RR phase started (Table 5.8). These results are not in line with previous analyses from the British Columbia database, which showed a faster attainment of EDSS 8 from the onset of SP, among patients younger at the onset of the RR phase (Tremlett et al., 2008a). Although the number of patients reaching the endpoint was unmentioned, the Kaplan Meier estimate was calculated only for 25% of British Columbia SP patients (Tremlett et al., 2008a), indicating that the proportion of censored information was large. This probably explains the discrepancy between the two databases.

Among LO patients older at the onset of the RR phase, the faster attainment of disability endpoints was secondary to the higher probability of converting to SP MS

and to the shorter latency to progression (Figure 5.6). The age at onset strongly influenced the hazard of becoming progressive. The risk of entering the SP phase increased proportionally with the age at the first attack, and doubled and tripled for onset at the age of 40 (OR = 4.22) and of 50 (OR = 6.04), respectively, compared to onset at the age of 20 (OR = 2.04) (Figure 5.5). Accordingly, groups older at the disease onset reached SP more rapidly (Table 5.6). However, when patients were matched by the duration of the RR phase (short, intermediate, long), in each subgroup the age at the disease onset did not significantly affect the attainment of DSS levels (Figure 5.7). This further demonstrated that an older age at onset of the RR phase affected the outcome mainly by increasing the probability of converting to SP MS. Importantly, similarly to the current age, the effect of the age at first attack on the risk of entering the SP phase was independent of the disease duration and of the number of early relapses (Table 5.9).

Current age was shown to influence the disease evolution also among relapsing onset patients. The probability of converting to SP MS (OR per additional year = 1.06) and to attain DSS 6 and DSS 8 (OR per additional year = 1.06 and 1.04, respectively) increased proportionally by growing older, independently of the disease duration (Table 5.14). The relative risk of requiring a walking aid and of entering the SP phase changed dramatically according to age, over a lifetime. It was obviously very low at young age (age 20 OR for DSS 6 = 3.1 and for SP = 3.3; age 30 OR for DSS 6 = 5.5 and for SP = 6.0) and it became extremely high at older ages (age 60 OR for DSS 6 = 30.5 and for SP = 36.0; age 70 OR for DSS 6 = 54.0 and for SP = 66.0) (Figure 5.18). In striking contrast, the effect of ageing on the disease evolution did not apply to PP and SP MS (Table 5.15).

These results indicated that the effect of current age on the disease progression, in the total population, was limited to the relapsing onset group. In addition, the lack of impact of current age on the evolution of the PP and of the SP phases further suggested that, among relapsing onset patients, growing older affected prognosis primarily by promoting the onset of the progressive course, but did not influence the accumulation of disability during the progressive phase.

5.4.2 The age at the onset of progression

Being younger at the onset of the SP phase associated with a worse outcome. The negative regression coefficient yielded by the logistic regression analysis indicated that the probability of attaining DSS 6 and DSS 8 decreased modestly and proportionally with the age at the onset of SP (for both endpoints OR per additional year was 0.96). Accordingly, groups younger at the conversion to SP MS attained disability endpoints, from the disease onset, in significantly shorter times (Table 5.10). This was expected, and was secondary to the shorter latency to progression, which associated with a faster disability accumulation (Chapter 6, Figure 6.7).

However, the evolution of the progressive phase was largely unaffected by the age at its onset. This was seen in both the SP and the PP subgroups. Times from the onset of progression to disability levels were similar, among patients grouped by the age at the onset of the PP phase, and among patients grouped by the age at the onset of the SP phase (Table 5.11). Although there was a pattern indicating a slightly faster evolution of the SP phase, among groups younger at the onset of progression, differences did not reach statistical significance (Table 5.11). In contrast, Tremlett et al. concluded that being younger at onset of SP associated with a more rapid disability accumulation during the progressive phase; the group aged < 30 attained EDSS 8, from the onset of progression, 10 mean years earlier than the group aged > 50 (Tremlett et al., 2008a). In the British Columbia database, the use of different stratification (5 age categories) and the high proportion of censored information probably accounted for such a large difference between age groups.

Indeed, when LO SP and PP patients were pooled together, the analysis further indicated that the evolution of the progressive phase was largely unaffected by the age at its onset. Progressive patients, starting to progress at young (≤ 35 years), intermediate (36-45 years) or old (> 45 years) age, attained disability endpoints from the onset of progression in remarkably similar times (Figure 5.17).

5.4.3 The age at disability milestones

Kaplan Meier analysis from birth calculated that 50% of the LO MS total population required a walking aid by the age of 48 years (Table 5.16). However, the age at the attainment of DSS 6 largely varied from 16 to 85 years, with the youngest 25% reaching the endpoint by the age of 37 and the oldest 25% after the age of 53. In line with analyses from other databases (Confavreux and Vukusic, 2006b; Leray et al., 2010; Tremlett et al., 2006), males were modestly but significantly younger than women when reaching the endpoints (3 mean years difference for both DSS 6 and DSS 8) and the type of the clinical presentations did not significantly influence the age at the disability milestones (Table 5.17). Only cerebellar and brainstem symptoms at onset were found to be associated with a younger age at DSS 8 (Table 5.17).

For the first time, this study provided a complete account of the mean/median ages at all milestones disability levels in relapsing onset and progressive onset patients. As already suggested by the analyses in the Lyon and Rennes databases (Confavreux and Vukusic, 2006b; Leray et al., 2010), the type of the disease course did not affect the times to endpoints from birth. RR/SP and PP groups attained DSS 3 (41 versus 43 years), DSS 6 (48 versus 49 years), DSS 8 (58 versus 58 years) and DSS 10 (77 versus 78) at remarkably similar median ages (Figure 5.22).

Taken together, the analyses further demonstrated a remarkably similar outcome, among all progressive patients. Primary and secondary progressive MS started at the same ages (Figure 5.13). The evolution of the progressive phase was not influenced by the age at its onset (Figure 5.18). The disability accumulated by growing older at similar rate, between the two disease phenotypes (Figure 5.21), and patients attained disability landmarks at similar ages (Figure 5.22). These results question the widespread belief that an initial progressive course affects the outcome negatively. In addition, they further emphasize the primary role of age on the accumulation of disability, irrespective of the clinical phenotype and, therefore, independent of the

focal inflammatory mechanisms underlying the disease course before the onset of progression (Figure 5.23).

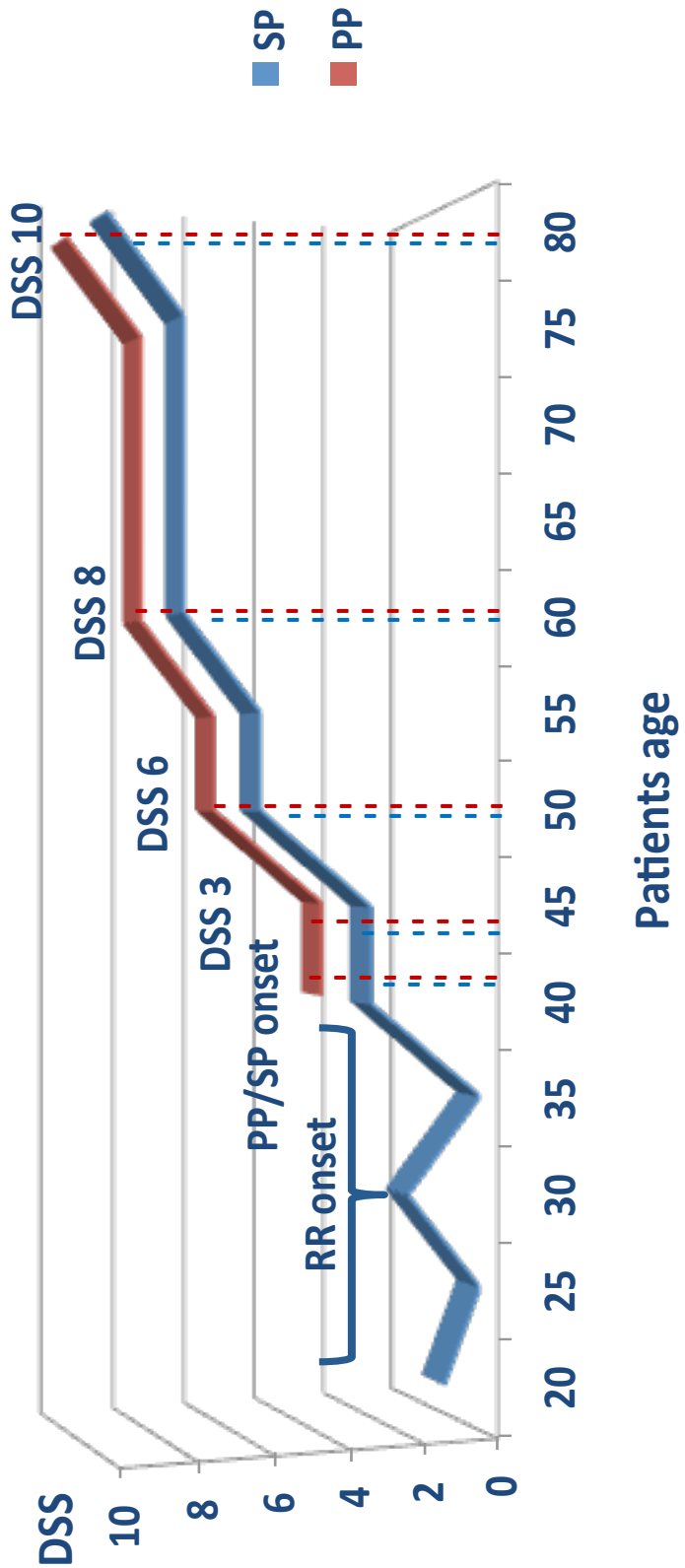


Figure 5.23 Kaplan Meier analysis from birth (age at) to progression, DSS 3, DSS 6, DSS 8 and DSS 10: comparison between PP and SP patients

5.4.4 Age and prognosis

The analyses in this study redefined age as an important prognostic factor, affecting the attainment of late outcomes, independently of the disease duration. The accumulation of severe disability over time is influenced by patient's age, suggesting that different age-related rates of progression might be identified among individuals and within the same patient, during the disease course.

The effect of age is primarily exerted by increasing the probability of experiencing the progressive course, further highlighting the onset of the progressive disease as the overwhelming determinant of the long-term prognosis. Older age at the disease onset associated with a higher risk of experiencing a primary progressive course and, among RR patients, influenced the disease evolution by increasing the hazard of converting to SP MS. Similarly, growing older affected the disease evolution, in relapsing onset patients only, by heralding the progressive course. The disability accumulation during the progressive phase was reported to be homogeneous among all patients (Kremenchutzky et al., 2006a) and independent of factors preceding its onset (Confavreux et al., 2003; Leray et al., 2010). The analyses in this thesis demonstrated that the evolution of progressive MS is largely unaffected by either the age at onset of the RR phase or by the age at onset of progression or by ageing. Therefore, the outcome is mainly determined before the onset of the progressive phase, and it is strongly influenced by age related mechanisms, leading to the onset of progression.

In the context of the long term outcome at population level, the almost identical ages at the onset of progression and at the attainment of disability levels, among clinical phenotypes, minimizes the significance of the clinically evident inflammatory attacks and of the duration of the disease course, and emphasizes the role of the subclinical pathological processes, independent of their clinical counterpart. Nevertheless, it would be an oversimplification to consider the disability accumulation strictly age dependent. Patients older at the disease onset, compared

to those younger, attained endpoints more rapidly, however they were older when they became progressive (Table 5.19) and severely disabled (Figure 5.20). This indicates that the age at disease onset also plays a relevant role, and highlights the complexity of the age related mechanisms affecting the disease evolution.

From the patients' prospective, the prognostic effect of age is rather controversial. Over a lifetime, those older at onset had a longer "disease free" period and were older when they became disabled. Therefore, although a younger age at onset has been largely reported as a predictor of a better prognosis, reaching disability levels in longer time but at an earlier age might be perceived as a negative outcome.

5.4.5 Age and the clinical phenotype

5.4.5.1 Age and progressive MS

A large body of evidence suggests that progressive MS is an age dependent disease. The PP course occurs very infrequently in children and, even when the disease starts during childhood, the SP course develops in adult life (mean age 41 years) (Renoux et al., 2007). The age at onset influences the probability of experiencing a progressive course and, more importantly, the progressive phase starts at remarkably similar ages, among progressive patients. This was consistently demonstrated by comparing the SP and the PP groups (Confavreux and Vukusic, 2006a; Fog and Linnemann, 1970; Koch et al., 2007; Minderhoud et al., 1988b), and by comparing different progressive phenotypes (Table 5.2 and Figure 5.1) (Kremenchutzky et al., 2006a; Tutuncu et al., 2013). The almost identical ages when the progressive phase, starting "de novo" or following the RR phase, becomes clinically evident, further indicates that degenerative processes are already active much before their clinical counterpart (De Stefano et al., 2003; Filippi et al., 2003; Trapp et al., 1998). These observations have supported the unifying concept of the disease course. The changes of the clinical phenotypes might be an effect of the age,

rather than the effect of changing pathogenic mechanisms (Confavreux and Vukusic, 2006a).

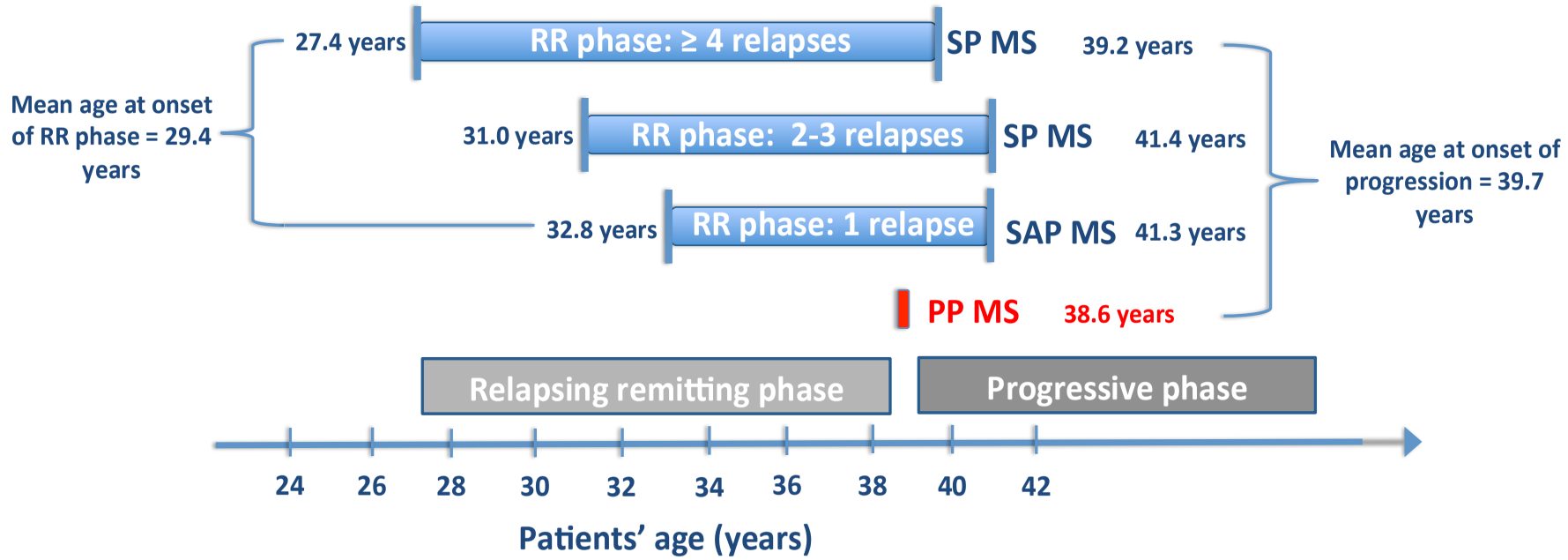
In this study, the Kaplan Meier analysis from birth confirmed a very similar age at the onset of progression between 534 SP patients and 217 PP patients (39 versus 40 median years; $p = 0.09$), despite a 12 years span in the age at the disease onset (28 versus 40 median years; $p < 0.001$) (Figure 5.13). It has been suggested that the exclusion from the analysis of RR patients at risk of developing the SP course represents a methodological caveat that might bias results (Tremlett et al., 2009b). Indeed, among all relapsing onset patients, the estimated age at the onset of secondary progression was 8 mean years older, compared to when only SP patients were analysed (48.3 versus 40.2 years, respectively) (Figure 5.16). The analysis calculated that 50% of the RR/SP group is expected to enter the progressive phase by the age of 45, rising to 65% by the age of 75 (Figure 5.17). As reported in the British Columbia database (Tremlett et al., 2009b), when censored information (patients who did not convert to SP MS) were considered, the estimated mean age at the onset of the SP phase was much older than the mean age at the onset of the PP phase (48.3 versus 38.6 years; $p < 0.001$). However, comparing patients at risk of progression (RR MS) versus those who are progressive already (PP MS) is also methodologically incorrect. In the first group the analysis allowed an estimation of what the age would be, taking into account those patients who have not reached the endpoint, while in the second group it calculated what the actual age was. A correct approach would imply analysing patients in the pre-progressive course, at risk of developing PP MS, which is practically impossible. In addition, although RR MS might be regarded as “a disease that has not grown older yet” (Confavreux and Vukusic, 2006a), and most of the patients eventually attain progression over time, the progressive disease does not necessarily supervene. In the British Columbia population 42% of patients did not convert to SP MS after 20 years of follow up (Tremlett et al., 2009b). Among patients from the Mayo Clinic, 45% had not developed progression after 35 years from onset and 38% were still in the RR phase by the age of 75 (Tutuncu et al., 2013). Even after 50 years of disease course 14% of the Gothenburg population remained progression free, decreasing to 6.5% after 60

years (Skoog et al., 2012). Similarly, in the LO database, by the end of the observation period, 33% of RR patients had not entered the progressive phase. This subgroup was observed for 20 mean years: in 25% (n = 68) progression had not occurred after ≥ 24 years from the disease onset and for some (n = 13) not even after ≥ 40 years. Those who remained in the RR phase had a mean age of 47 years at the last assessment, and, by this age, 75% of all progressive patients had already started to progress (Figure 5.12). In addition, during the RR phase patients may die: this occurred in 9 cases from the Gothenburg population (Skoog et al., 2012), in 9 cases from the Mayo Clinic database (Tutuncu et al., 2013) and in 20 cases from the LO database (Chapter 3). Therefore, it can be assumed that some of the RR patients would have eventually converted to SP MS, if observed for longer time, but probably not all of them. The comparison with the geographically ascertained subgroup from Middlesex County (Figure 3.16, Chapter 3) demonstrated that little bias affected the ascertainment of the LO MS population, which can be considered thoroughly representative of patients at risk of both secondary and primary progressive MS. Therefore, from the analysis presented in this thesis it can be reliably concluded that the age at which progression started is not affected by the type of the disease course.

This was further demonstrated by comparing progressive patients stratified by the total number of relapses during the pre-progressive phase (Figure 5.14) and stratified by the number of early relapses (Figure 5.15). Groups with no (PP MS), 1 (SAP MS), 2-3 (SP MS) and ≥ 4 (SP MS) attacks before progression differed by the age at the disease onset but shared very similar age at the onset of the progressive phase, which ranged only by 2.8 mean years among the 4 subgroups (Figure 5.14). This was also observed among patients grouped by the number of early relapses, attaining progression at similar age, compared to PP patients (Figure 5.15). Only the group with frequent early attacks (≥ 3) diverged from the other progressive subtypes and became progressive at significantly younger age (35 mean years). These resulted highlighted, again, *frequent early relapsers,* as a specific subgroup of patients with an unusually aggressive disease course.

Overall, the age at which progression started was not influenced by the clinical phenotype, but was also unaffected by the number of relapses before the progressive phase started (Figure 5.23). This further emphasizes the age as the cardinal factor, which influences when the relentless accumulation of disability becomes clinically evident, independently of inflammatory attacks. However, the age at the onset of the progressive phase remains extremely unpredictable at individual level. Among LO patients, it spanned from 14 to 83 years; 50% of progressive patients started to progress by the age of 39 and 75% by the age of 47 (Figure 5.12). In addition, being older at the onset of the RR phase associated with older age at the conversion to SP MS (Table 5.19), partially explaining the variability of the long-term clinical outcome.

Figure 5.24 Kaplan Meier analysis from birth to the disease onset and to the onset of progression: comparison among SP patients with 1, 2-3, ≥ 4 total attacks and PP patients



5.4.5.2 Age and relapsing MS

The conspicuous fact that SP subgroups, stratified by the number of total relapses, had a statistically significant different age at the disease onset (Figure 5.14) warrants further comments. Despite sharing almost the same age at the onset of progression, patients with a larger number of total attacks (≥ 4) were the youngest (27.4 mean years) at clinical presentation, followed by patients with 2-3 attacks (31.0 mean years) and by patients with only a single attack (32.8 mean years) (Figure 5.24). This indicated that the age at the disease onset influenced the frequency of relapses. Indeed, although the number of early relapses was not affected by the age at the clinical onset ($r = -0.05$; $p = 0.13$), being older at the first attack associated with a significantly lower number of late ($r = -0.26$; $p < 0.001$) and total ($r = -0.24$; $p < 0.001$) relapses (Figure 5.8). This was in line with previous reports, showing that the annualized relapse rate decreases by growing older (Tremlett et al., 2008b) (Figure 4.2, Chapter 4). The total number of attacks recorded during the RR phase reduced proportionally with age, from 1000 attacks, among patients with onset between the age 31-40, to only 29 attacks, among those with onset after the age 50 (Figure 5.9). The same pattern was observed when patients were stratified in small groups according to the number of total relapses. Those with a higher total relapse frequency were younger at onset (Figure 5.10). It is worth noticing that women, compared to men, were slightly younger at the onset of the RR phase (27.9 versus 29.7 mean years; $p = 0.01$) and experienced a slightly higher number of total attacks (4.2 versus 3.8 mean attacks; $p < 0.001$). However, these differences are small and can only partially explain the higher total relapse frequency observed among patients younger at the clinical presentation.

These results cast further light on the evolution of the RR phase and on the complex interaction between the age at its onset and the total number of attacks during its course. The time to the onset of SP was longer among patients younger at the disease onset (Figure 5.6) and also longer among patients with a higher attacks frequency before progression (Figures 4.21, 4.22, Chapter 4). Therefore, groups older at onset took a shorter time to enter the SP phase and had less relapses before

progression. The converse relationship between the latency to progression and the number of total attacks is largely accounted for by the age at onset, which affects the relapse frequency. As already suggested by imaging studies (Granberg et al., 2013), inflammatory mechanisms are already active before the clinical onset and age seems to influence when they first become clinically evident. Alternatively, the association between an older age at onset and a lower relapse frequency, among patients sharing the same age at the onset of progression, implies a yet undetermined interaction between age related degenerative processes and factors determining the frequency of inflammatory attacks. With ageing the subtle degenerative process gradually prevails over the inflammatory mechanisms, possibly suppressing or masking the clinically evident inflammatory activity.

5.4.6 Conclusions

The data presented in this study confirmed that the clinical onset of the progressive phase is strongly determined by the age rather than by the pre-progression disease course. Also, age affects the variable presenting pattern of inflammatory attacks, by influencing the clinical onset and by affecting the evolution of the relapsing course. These results further defined MS as a one-stage disorder, where the complex balance between inflammatory and neurodegenerative processes, and their clinical manifestations, changes over time according to the age. Therefore, the clinical phenotype is strictly age dependent and does not necessarily mirrors changes in mechanisms driving the different phases of the disease.

Chapter 6

The onset and the evolution of secondary progressive MS

6.1 Introduction

The outcome's severity in MS is extremely variable. The disease evolution ranges widely, and while some patients remain without significant disability after 10-15 years from onset (Hawkins and McDonnell, 1999; Ramsaransing and De Keyser, 2006; Sayao et al., 2007), others become severely disabled within a short period of time (Lublin and Reingold, 1996). Groups at risk of poor outcome can be defined, however the factors accounting for the large variability of the disease severity at the individual level are still unclear. The unpredictable clinical course makes the task of monitoring the disease activity (Rio et al., 2009b) and of testing the efficacy of treatments (Rieckmann, 2008) particularly problematic.

Among RR patients, the onset of the SP phase represents a crucial milestone in the disease course. Inflammatory attacks during the RR phase can contribute to the development of moderate disability (Lublin et al., 2003), but only rarely are a direct cause of permanent severe disability (Bejaoui and Rolak, 2010). The attainment of hard outcomes, such as requiring a walking aid or worse, almost invariably results from the relentless, steady accumulation of disability, which occurs during the progressive phase (Kremenchutzky et al., 2006a; Tutuncu et al., 2013). Indeed, most of the LO patients converted to SP MS when they were scored at DSS ≤ 3 (Figure 4.9, Chapter 4). Previous analyses identified DSS 3/DSS 4 as the clinical watersheds between two independent phases (Confavreux and Vukusic, 2006a; Debouverie et al., 2008; Leray et al., 2010). Once the moderate disability develops, the further disease evolution appears largely uninfluenced by baseline prognostic factors and by the type of the initial disease course (Confavreux et al., 2003; Confavreux and Vukusic, 2006a; Leray et al., 2010) (Figure 3.22, Chapter 3). Similarly to the LO patients, among patients from the French cohorts, the attainment of moderate disability probably heralded the SP phase in most of the cases. Overall, the results support the homogeneity of the progressive course, among progressive patients. Therefore, the conversion to SP MS is undoubtedly the key determinant of the long-

term outcome and the true clinical watershed between two disease phases, mostly independent of each other.

In the LO database, MS progressive subtypes shared almost the same rate of disability accumulation during the progressive phase (Kremenchutzky et al., 2006a). In addition, the evolution of the SP phase was mainly unaffected by relapses preceding its onset (Figure 4.6, Chapter 4) or occurring during its course (Figures 4.7, 4.8, Chapter 4), it was similar among patients grouped by the age at the onset of the RR phase (Figure 5.6, Chapter 5) and by the age at the onset of progression (Figure 5.18, Chapter 5), and it was not influenced by the current age (Table 5.15, Chapter 5). The predictive effect of early relapses (Figure 4.10, Chapter 4) and of age (Figure 5.5, Chapter 5) was primarily exerted by increasing the probability of converting to SP MS. Even among patients selected for the adverse feature of frequent early inflammatory attacks, the occurrence of the SP course differentiated groups with a benign or an aggressive disease course, and accounted for the severity of the final outcome (Figure 4.11, Chapter 4).

Taken together these data indicate that the severity of the outcome is mainly accounted for by mechanisms driving the evolution of the RR phase and leading to the conversion to SP MS. Therefore, the onset of the progressive phase is a robust surrogate marker for late disability and a major therapeutic target. Factors affecting the probability of entering the SP phase should be used to optimize the individual therapeutic management and to improve the design of RCTs.

6.1.1 Factor affecting the evolution of the RR phase

A relatively low number of natural history studies focused specifically on SP MS. This probably reflects the difficulty of reliably pinpointing when the SP phase occurs. It has been suggested that SP MS can be regarded as “RR MS that has to grow older” (Confavreux and Vukusic, 2006a). Indeed, the number of patients entering the SP phase increases over time, and parallels the duration of the disease course (Confavreux et al., 1980; Mc and Compston, 1952; Weinshenker et al., 1989b).

Consequently, among natural history cohorts, the time to SP largely varies, based on the variable proportion of censored information. Overall, 19 median years can be considered a reliable estimate of the duration of the RR phase (Figure 3.4, Chapter 3).

The most extensive analysis of the disease evolution up to the onset of the secondary progression was carried out in the British Columbia database. Two separate studies included 2485 patients (58% converting to SP MS) (Tremlett et al., 2008a), and subsequently 5162 patients (35% converting to SP MS) (Koch et al., 2010). In the first study, the Kaplan Meier estimated time to SP was 18.9 median years from the disease onset, and 49.0 median years from birth (age at the onset of progression) (Tremlett et al., 2008a). Being male and older at onset associated with a shorter time to SP. Among SP patients (n = 1415), the information on the EDSS score at the onset of progression was available for 913 (64.5%); 24% reached SP at ≤ EDSS 3, 80% at ≤ EDSS 6 and 99% at ≤ EDSS 8 (Tremlett et al., 2008a). This was in contrast with the LO population, where only approximately 30% of patients attained progression after DSS 3 (Figure 4.9, Chapter 4).

In the Lyon and Lorraine databases, males, groups older at onset and with a longer first inter-attack interval had a higher probability of entering the SP phase and a shorter latency to progression (Debouverie et al., 2008; Vukusic and Confavreux, 2003). Interestingly, in the Gothenburg database, men and women exhibited the same risk of converting to SP MS (Eriksson et al., 2003). Bergamaschi et al. used a different statistical approach and developed a Bayesian model, which confirmed older age at onset, and male sex as unfavourable prognostic factors (Bergamaschi et al., 2001). Other studies reported a higher probability of becoming SP, among patients with multiple symptoms at the clinical onset (Amato et al., 1999; Debouverie et al., 2008; Phadke, 1990). However, the type of the initial clinical manifestations has little effect on the time to SP (Tremlett et al., 2008a; Trojano et al., 1995; Vukusic and Confavreux, 2003).

6.1.2 Factor affecting the evolution of the SP phase

Results from MS cohorts converge in demonstrating that most of the factors, affecting the evolution of the RR phase, do not exert any impact on the slope of the SP phase. In the Lyon (Vukusic and Confavreux, 2003), the British Columbia (Tremlett et al., 2008a) and the Gothenburg (Eriksson et al., 2003) databases, gender and the type of symptoms at the clinical presentation were found not to influence the times from the onset of SP to disability endpoints. In the LO database, the age at the onset of the RR phase and the age at the onset of progression did not impact on the evolution of the SP phase (Figures 5.6, 5.18, Chapter 5). In contrast, among patients from British Columbia, a younger age at the onset of the RR phase and an older age at the onset of the SP phase associated with a slower rate of disability accumulation during the progressive phase (Tremlett et al., 2008a).

6.1.3 Latency to SP and the evolution of the SP phase

Only three studies assessed the time to SP as a predictor of the disease evolution during the SP phase. In the Gothenburg database, a latency to progression ≤ 10 years, compared to > 10 years, associated with an increased risk (2.4 time higher) of attaining EDSS 7 from the onset of SP (Eriksson et al., 2003). In the Lyon cohort, patients who converted to SP MS within or after 8 years were compared. A longer duration of the RR phase predicted slightly longer times from SP to DSS 6 (1.4 mean years difference) and to DSS 7 (1 mean year difference) (Vukusic and Confavreux, 2003). The British Columbia patients were stratified in 3 categories, according to the latency to progression (short < 10; intermediate 10 to < 20; long ≥ 20). The analysis demonstrated that the group with short, compared to the groups with intermediate and long time to SP, reached EDSS 8 from the onset of progression in faster times (2.8 and 5.5 mean years difference, respectively) (Tremlett et al., 2008a). However, the proportion of patients becoming bedbound was low, as shown by the Kaplan Meier estimate, which was calculated for 25% only. In addition, although a shorter duration of the RR phase associated with a shorter evolution of the SP phase, those older at the onset of the RR phase converted more quickly to SP MS, but took longer

times to reach DSS 8 from onset of progression (Tremlett et al., 2008a). These contradicting results were not addressed by the authors.

6.1.4 Aim of the study presented in this chapter

Thanks to the high number of patients who had converted to SP MS, by the end of the observation period, the LO database offered the opportunity to investigate in details factors affecting the disease course before and after the onset of the secondary progressive phase. This allowed to further elucidate the relationship between mechanisms driving the evolution of the RR phase and the accumulation of disability in the long-term outcome.

6.2 Methods

Among relapsing onset patients, the Kaplan Meier analysis was used to estimate the time to the onset of the SP phase and, among SP patients, to estimate the time to attain DSS 8, from the onset of progression. Patients were grouped according to baseline clinical and demographic features, in order to test the predict effect of the following variables: gender, the age at the disease onset, the number of relapses during the first two years (early relapses), the type (motor, sensory, cerebellar, brainstem, optic) and the number (1, > 1) of neurological systems affected at the disease onset. The Log Rank test assessed the differences among groups. Patients not reaching DSS 8 or not reaching SP, but followed for known periods, were right censored. In the analyses of the time to the onset of SP and of the time to DSS 8 from the onset of SP, 37 and 60 patients, respectively, were excluded because of missing information on the disease duration or on the time to the endpoint.

Multiple Cox proportional hazard analysis was used to investigate the risk of developing secondary progressive MS and of reaching DSS 8 from the onset of progression, according to the concomitant effect of all clinical and demographic features. Proportional hazards assumption was checked by visual inspection of Schoenfeld residual plots and corresponding statistical tests. In addition, within the SP MS group, the Kaplan Meier analysis was used for assessing (1) times from the disease onset and from the onset of SP to DSS 6 and to DSS 8, in patients stratified by the duration of the RR phase (latency to SP: short=1–5 years; intermediate=6–12 years; long ≥ 13 years); (2) the time to DSS 6 and to DSS 8, from the disease onset, in patients stratified by the duration of the RR phase and by the number of total relapses during the RR phase (low = 1–2; intermediate = 3–4; high ≥ 5 attacks) and in patients stratified by the duration of the RR phase and by the number of early relapses (low = 1; intermediate = 2; high ≥ 3 attacks). Groups were computed in order to maintain, to the possible extent, similar numbers in each category. However, the analyses were also carried out among patients with different stratifications, for confirming results.

Finally, the binary logistic regression analysis was used to calculate the independent predictive effect of the disease duration, the frequency of early relapses and the age at the disease onset on the probability of entering the SP phase, expressed by OR. The multiple binary logistic regression analysis allowed to build up models predicting the variation, according to the disease duration, of the probability of converting to SP MS, influenced by the age at the disease onset and by the frequency of early relapses.

6.3 Results

Table 6.1 Clinical and demographic features of patients stratified by the type of the disease course at the end of the observation period (RR and SP MS). * Chi Square test; ** Wilcoxon rank test.

	RRMS	**SPMS**	p
N	272	534	
Females; n (%)	208 (76.5)	346 (64.8)	0.001*
Males; n (%)	64 (23.5)	188 (35.2)	
F/M ratio	3.2	1.8	
Mean (median) age at onset	26.8 (25) years	29.4 (28) years	< 0.001**
Mean (median) disease duration	20.3 (20) years	26.5 (26) years	< 0.001**
Monosymptomatic onset; n (%)	173 (63.6%)	362 (67.8%)	0.19*
Polysymptomatic onset; n (%)	97 (35.7%)	165 (30.9%)	
Info not available; n (%)	2 (0.7%)	7 (0.3%)	
Type of initial presentation; n (%)			
Motor	40 (14.7%)	105 (19.7%)	0.08*
Sensory	159 (58.5%)	279 (52.2%)	0.09*
Cerebellar	16 (5.9%)	35 (6.6%)	0.71*
Brainstem	59 (21.7%)	108 (20.2%)	0.63*
Optic	56 (20.6%)	118 (22.1%)	0.62*
Bowel/Bladder	9 (3.3%)	16 (3.0%)	0.81*
Mean relapse rate in year 1-2 (median)	0.97 (0.75) attack/year	0.91 (0.5) attack/year	0.18**
Mean duration of RR phase (median)		10.7 (9) years	
Mean DSS score at onset of SP (median)		2.9 (3)	
Kaplan Meier estimated mean (median) time from disease onset to			
DSS 6		15.7 (14) years	
DSS 8		26.5 (24) years	
DSS 10		44.8 (45) years	
Kaplan Meier estimated mean (median) time from SP to			
DSS 6		5.3 (3) years	
DSS 8		15.2 (12) years	
DSS 10		32.5 (30) years	

Among relapsing onset patients (n = 806), by the end of the observation period, 66.3 % (n = 534) had converted to SP MS and 33.7% (n = 272) had remained in the RR

phase (Table 6.1). In the RR group, compared to the SP group, there was a larger percentage of women (76.5% versus 64.8%; $p < 0.001$), patients were slightly younger at the disease onset (26.8 versus 29.4 mean years; $p < 0.001$) and had shorter disease duration (20.3 versus 26.5 mean years; $p < 0.001$). However, in both groups, the frequency of early relapses was similar (0.97 versus 0.91 attacks/year; $p = 0.18$) and most of the patients presented with a mono-symptomatic onset, which was mainly characterized by sensory disturbances. Among those with SP MS, 92% reached DSS 6, 68% reached DSS 8 and 23% reached DSS 10; the times to the endpoints were 14, 24 and 45 median years from disease onset and 3, 12 and 30 median years from the onset of SP, respectively (Table 6.1).

6.3.1 The attainment of the secondary progressive phase

The rate of conversion to SP MS increased proportionally over time. The percentage of patients entering the SP phase was larger in the groups observed for longer time (Figure 6.1). Accordingly, the binary logistic regression analysis calculated that the probability of becoming progressive became 9% higher every 5 years of the disease duration (OR = 1.07 per additional year; $p < 0.001$). The hazard of attaining the secondary progression doubled (OR = 1.99, probability = 43.9%) and quadrupled (OR = 3.97, probability = 61.1%) after 10 and 20 years from the disease onset, respectively (Figure 6.2). This effect was independent of the age at the disease onset (Chapter 5).

The Kaplan Meier analysis allowed to calculate the latency to progression in the relapsing onset group; 37 patients were excluded from the analysis because of missing information on the disease duration or on the time to SP. Among the remaining 769 patients, the estimated mean time to enter the SP phase was 21.4 (95% CI: 19.5-23.1) years. By 6 and 15 (median time) years from onset, 25% (n = 181) and 50% (n = 387), respectively, had become SP MS. Few additional patients, to a final total of 66%, attained progression in the following 20 years (Figure 6.3). Accordingly, the rate of conversion to SP MS was much higher during the first 15 years (25.8 patients/year) of disease duration than during the following 15 years (8.1

patients/year). The comparison of the time to SP, between the total population and the geographically ascertained subgroup from Middlesex County, did not show any statically significant difference (Figure 6.4).

Figure 6.1 Percentages (x-axis) of patients converting to SP MS in each disease duration category (y-axis). Exact value is indicated next to each bar.

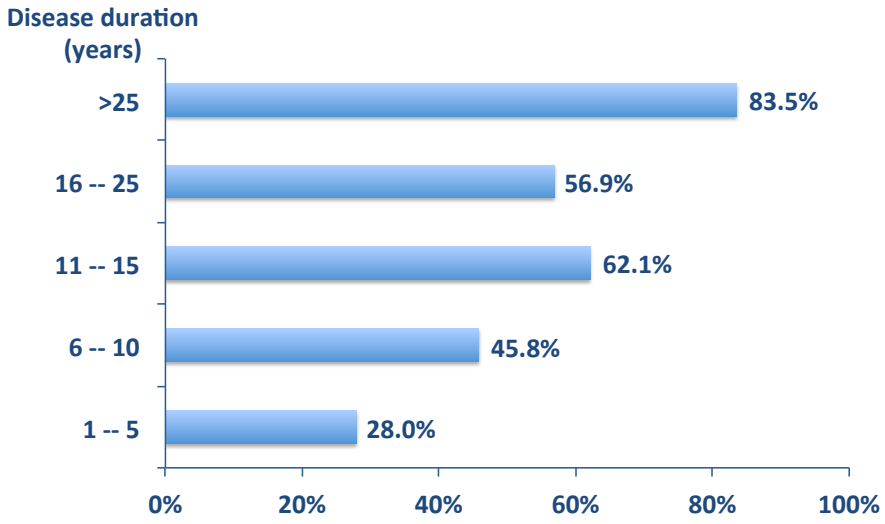


Figure 6.2 Binary logistic regression analysis: calculated risk (ORs on y-axis) of converting to SP MS according to the disease duration (x-axis): OR per additional year = 1.07. The probabilities of SP for each value of disease duration were calculated from the logistic model.

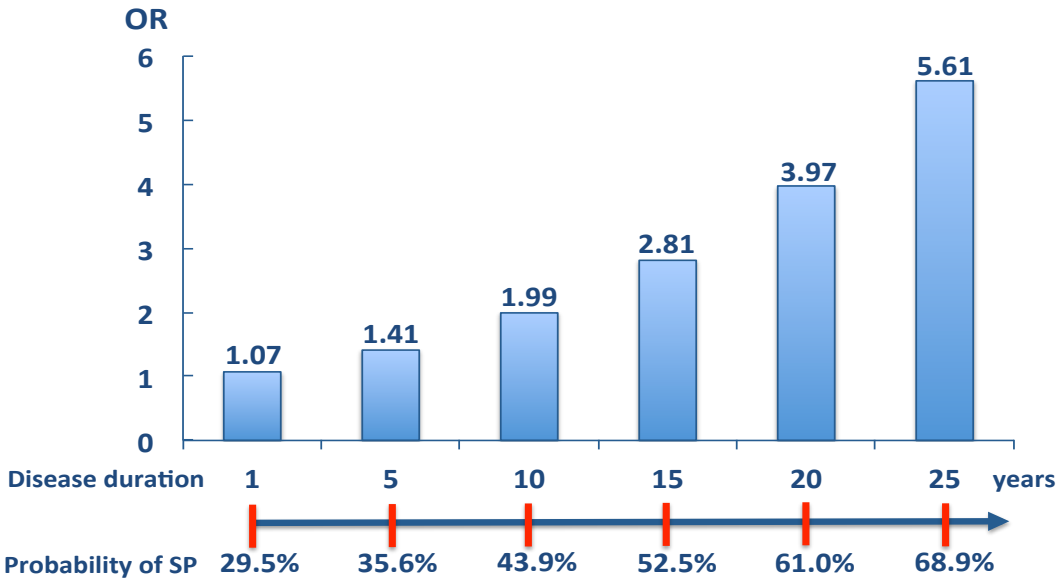


Figure 6.3 Kaplan Meier analysis of time to SP: cumulative percentage (y-axis) of patients, in the total population, entering the SP phase over time (x-axis).

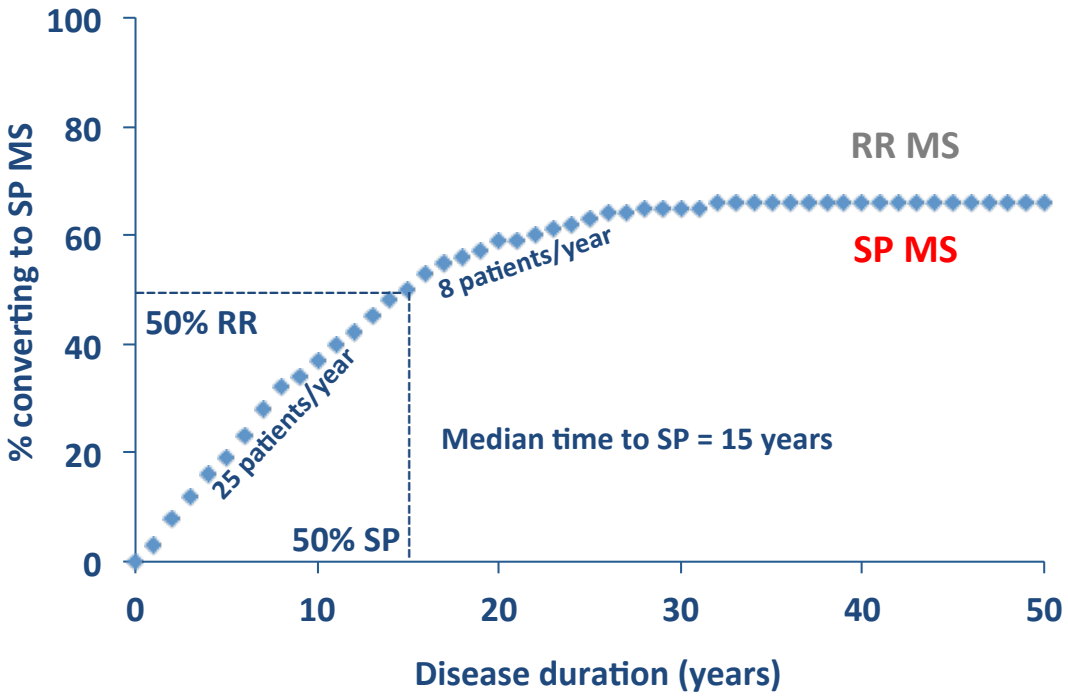
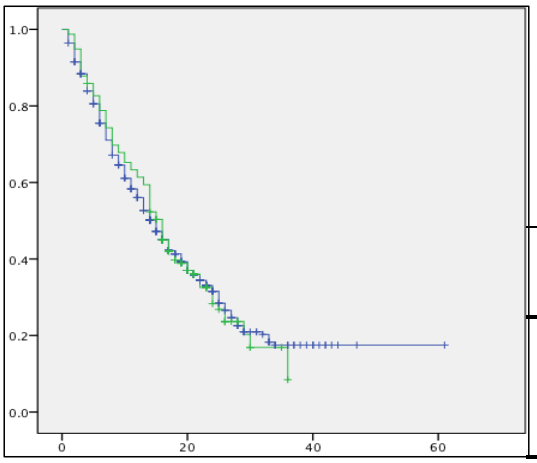
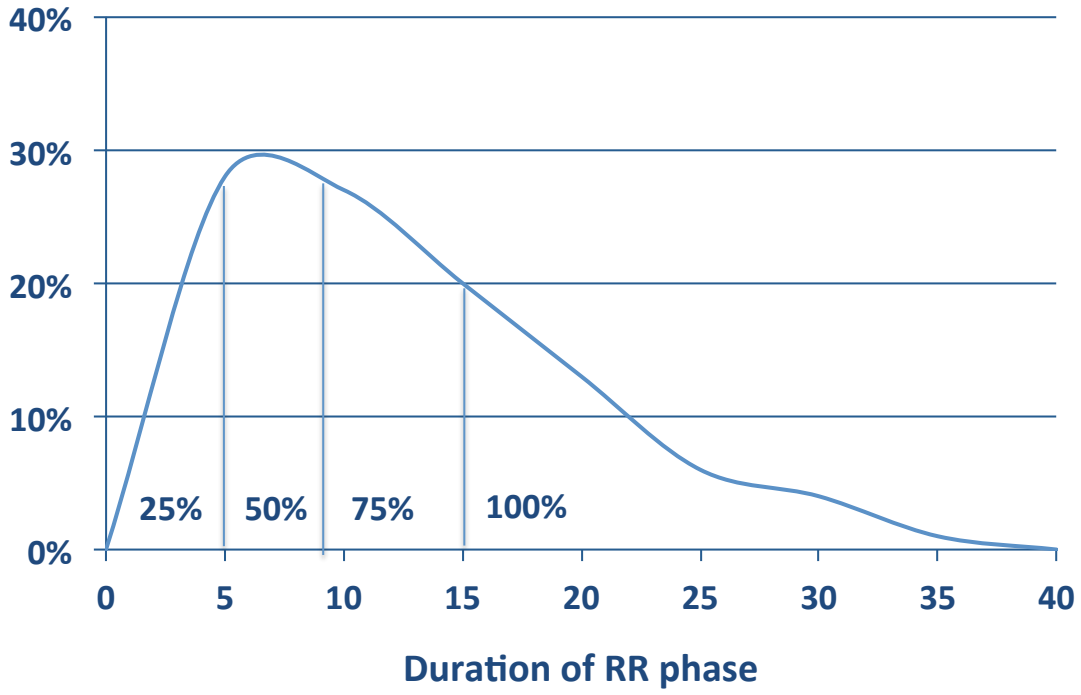


Figure 6.4 Kaplan Meier analysis of the time to SP: comparison between the total population and the Middlesex County subgroup. P value was obtained with Log Rank test.



	Mean [95% CI] (median) years from disease onset to SP	
Total population	21.4 [19.5-23.1] (15)	p = 0.95
Middlesex County	17.4 [15.4-19.3] (16)	

Figure 6.5 Percentages (y-axis) of SP patients entering the progressive phase at different time from onset (x-axis): percentages are calculated within each disease duration group (0-5; 6-10; 11-15; 16-20; 21-25; 26-30; 31-35; 36-40). Percentiles of time from the disease onset to the onset of SP are also indicated.



At end of the observation period, within the group of RR patients (n = 272), 50% had been observed for 20 years (median disease duration). However, in 26% (n = 71) progression did not supervene after ≥ 24 years of disease duration, and for some (n = 13) of them not even after ≥ 40 years. Among SPMS patients (n = 534), the latency to progression widely varied, from 1 to 36 years; it ranged from 5 years in the quickest 25%, to more than 15 years in the slowest 25% (Figure 6.5). The mean time to enter the progressive phase was 10.7 years (95% CI = 10.0-11.3): by 5, 9 (median time) and 15 years, 25%, 50% and 75%, respectively, converted to SP MS (Figure 6.5).

6.3.2 The latency to progression and the late outcomes

The association between the latency to progression and the risk of developing severe disability was examined using the binary logistic regression analysis. The analysis yielded negative regression coefficients, which indicated a converse

relationship between the time to SP and the probability of reaching the DSS levels. A longer duration of the RR phase correlated with a proportionally lower probability of attaining DSS 6 (regression coefficient = -0.055; OR = 0.95 [95% CI 0.90-0.98] per additional year; p = 0.01) and DSS 8 (regression coefficient = -0.055; OR = 0.95 [95% CI 0.92-0.97] per additional year; p < 0.001). Among patients free from progression for 5, 10 and 15 years, the hazard of requiring a walking aid was reduced by 24% (OR = 0.76), 43% (OR = 0.58) and 56% (OR = 0.44), respectively (Figure 6.6).

The attainment of disability endpoints from the disease onset and from the onset of SP was assessed among patients grouped by the duration of the RR phase (short = 1-5 yeas; intermediate = 6-12 years; long ≥ 13 years). The Kaplan Meier analysis demonstrated that groups with a shorter latency to progression reached the DSS levels, from the onset of the disease, in significantly shorter times. There was a difference of 15.6 and 16.4 mean years for requiring a walking aid and for becoming bedbound, respectively, between patients with a short and long time to SP (Figure 6.7). These differences disappeared once the SP phase supervened. Times to DSS 6 and to DSS 8, from the onset of SP were very similar, among groups, and were largely unaffected by the duration of the RR phase (Figure 6.8)

Figure 6.6 Binary logistic regression analysis: calculated risk (ORs on y-axis) of attaining DSS 6 according to the duration of the RR phase (x-axis).

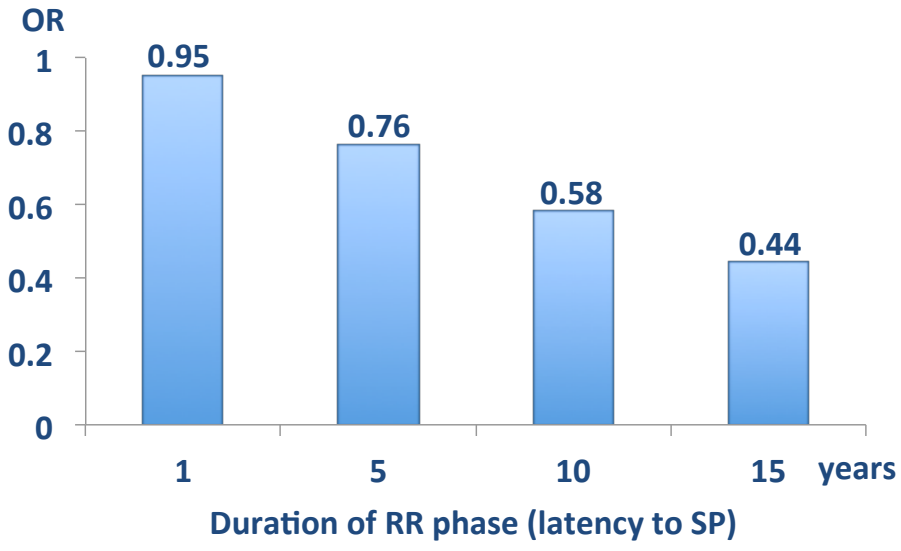
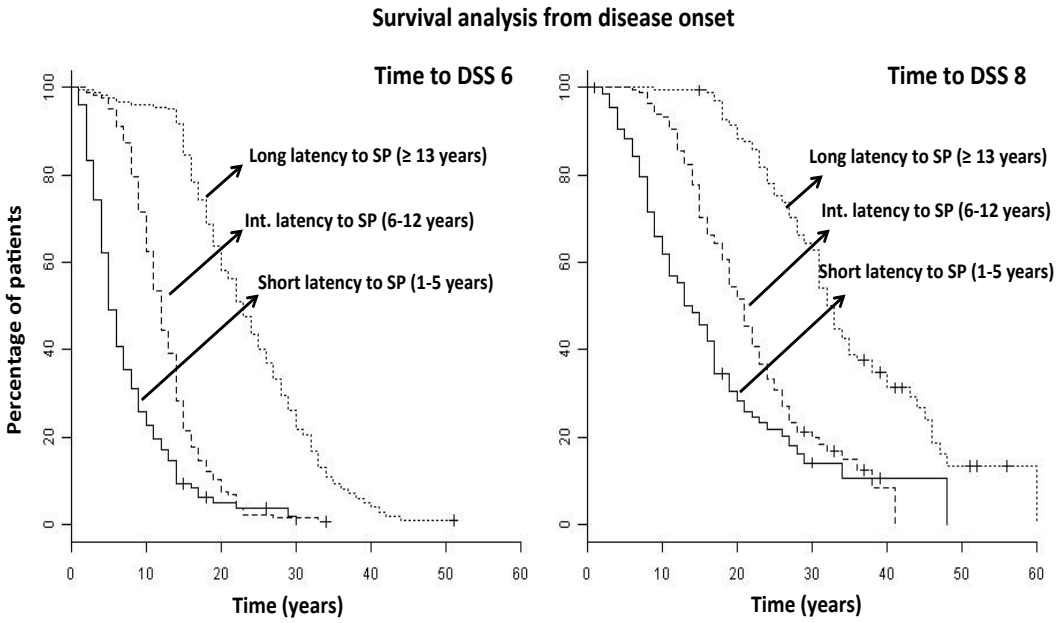
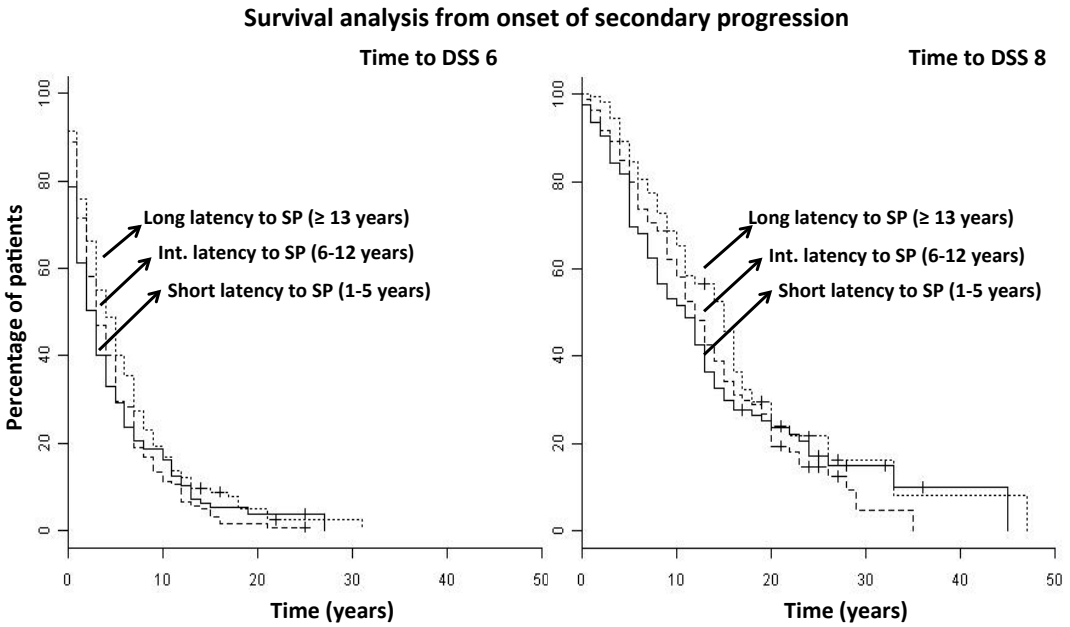


Figure 6.7 Kaplan Meier analysis: times from the disease onset to DSS 6 and to DSS 8, among patients stratified by the duration of the RR phase (short, intermediate and long). Percentiles of the estimated times to disability endpoints in each group are indicated. P values were obtained with Log Rank test by comparison with the reference category (*).



		Mean [95% CI] years from disease onset to DSS 6				
Duration of RR phase	**N (% censored)**		p	**25%**	**50%**	**75%**
1--5 years	139 (5%)	7.6 [6.4-8.5] years	< 0.001	9 years	6 years	3 years
6--12 years	171 (4%)	12.6 [11.9-13.6] years	< 0.001	15 years	12 years	9 years
≥ 13 years*	183 (10%)	23.2 [22.4-25.0] years		30 years	23 years	17 years
Total	493 (7%)					
		Mean [95% CI] years from disease onset to DSS 8				
1--5 years	137 (25%)	17.5 [14.8-19.9] years	< 0.001	21 years	14 years	8 years
6--12 years	170 (28%)	22.2 [20.6-23.8] years	< 0.001	27 years	21 years	15 years
≥ 13 years*	169 (45%)	33.9 [33.0-38.0] years		45 years	33 years	26 years
Total	476 (33%)					

Figure 6.8 Kaplan Meier analysis: times from the onset of SP to DSS 6 and to DSS 8, among patients stratified by the duration of the RR phase (short, intermediate and long). Percentiles of the estimated times to disability endpoints in each group are indicated. P values were obtained with Log Rank test by comparison with the reference category (*).



		Mean [95% CI] years from onset of SP to DSS 6				
Duration of RR phase	**N (% censored)**		p	**25%**	**50%**	**75%**
1--5 years	133 (5%)	4.8 [3.6-5.7] years	0.04	6 years	3 years	1 years
6--12 years	158 (4%)	4.7 [4.0-5.6] years	0.04	7 years	3 years	1 years
≥ 13 years*	168 (11%)	6.0 [5.0-7.1] years		8 years	4 years	2 years
Total	459 (7%)					
		Mean [95% CI] years from onset of SP to DSS 8				
1--5 years	137 (25%)	14.4 [11.9-16.9] years	0.03	19 years	11 years	5 years
6--12 years	170 (28%)	13.7 [12.2-15.3] years	0.10	20 years	12 years	6 years
≥ 13 years*	167 (45%)	16.7 [14.1-20.2] years		20 years	15 years	8 years
Total	474 (33%)					

The predictive effect of the latency to progression was also assessed among patients grouped by the number of early (Table 6.3) and total (Table 6.4) relapses. Even when patients shared the same number of attacks during the first 2 years or the same number of total attacks before the onset of progression, a shorter duration of the RR phase associated with significantly shorter times from the disease onset to the disability endpoints.

Table 6.2 Kaplan Meier analysis from the disease onset to DSS 6 and to DSS 8, among patients stratified by the duration of the RR phase (short, intermediate and long). The analysis was carried out in subgroups selected for the same number of attacks during the first 2 years (1, 2, ≥ 3). P values were obtained with Log Rank test by comparison with the reference category (*).

Duration of RR phase	**Early relapses = 1**			**Early relapses = 2**			**Early relapses ≥ 3**		
	Mean (median) years from disease onset to DSS 6								
	n (% censored)		p	**n (% censored)**		p	**n (% censored)**		p
1--5 years	55 (7%)	9.5 (7) yrs	< 0.001	30 (10%)	9.2 (8) yrs	< 0.001	47 (0%)	4.2 (4) yrs	< 0.001
6--12 years	82 (5%)	14.0 (13) yrs	< 0.001	54 (2%)	11.9 (12) yrs	< 0.001	32 (3%)	10.1 (10) yrs	< 0.001
≥ 13 years*	125 (8%)	23.6 (23) yrs		39 (13%)	21.7 (23) yrs		16 (25%)	16.1 (19) yrs	
Total	262 (7%)			123 (7%)			95 (5%)		
	Mean (median) years from disease onset to DSS 8								
1--5 years	53 (26%)	19.7 (17) yrs	< 0.001	30 (30%)	18.0 (16) yrs	< 0.001	46 (22%)	11.7 (9) yrs	< 0.001
6--12 years	82 (28%)	23.6 (21) yrs	< 0.001	53 (30%)	21.2 (22) yrs	< 0.001	32 (22%)	18.0 (18) yrs	< 0.001
≥ 13 years*	113 (41%)	34.4 (33) yrs		38 (58%)	29.3 (32) yrs		15 (40%)	27.3 (29) yrs	
Total	248 (34%)			121 (39%)			93 (25%)		

Table 6.3 Kaplan Meier analysis from the disease onset to DSS 6 and to DSS 8, among patients stratified by the duration of the RR phase (short, intermediate and long). The analysis was carried out in subgroups selected for the same number of total attacks before progression (1-2, 3-4, ≥ 5). P values were obtained with Log Rank test by comparison with the reference category (*).

Duration of RR phase	Tot. Relapses = 1-2			Tot. Relapses = 3-4			Tot. Relapses ≥ 5		
	Mean (median) years from disease onset to DSS 6								
	n (% censored)		p	**n (% censored)**		p	**n (% censored)**		p
1--5 years	58 (8.6%)	9.9 (8) yrs	<0.001	32 (0%)	5.7 (5) yrs	<0.001	31 (0%)	5.0 (5) yrs	<0.001
6--12 years	52 (1.9%)	14.3 (14) yrs	<0.001	47 (0%)	12.5 (12) yrs	<0.001	52 (7.7%)	10.6 (10) yrs	<0.001
≥ 13 years*	39 (12.8%)	23.3 (24) yrs		51 (9.8%)	24.7 (23) yrs		75 (12.0%)	22.9 (22) yrs	
Total	149 (7.4%)			130 (3.8%)			154 (8.2%)		
	Mean (median) years from disease onset to DSS 8								
	n (% censored)		p	**n (% censored)**		p	**n (% censored)**		p
1--5 years	57 (28%)	20.0 (17) yrs	<0.001	30 (20%)	13.7 (10) yrs	<0.001	31 (25%)	15.1(11) yrs	<0.001
6--12 years	50 (26%)	25.0 (23) yrs	<0.001	48 (31%)	21.5(21) yrs	<0.001	52 (23%)	18.9 (19) yrs	<0.001
≥ 13 years*	37 (35%)	34.0 (33) yrs		45 (40%)	34.2 (31) yrs		71 (54%)	34.7 (33) yrs	
Total	144 (29%)			123 (31%)			154 (38%)		

6.3.3 Factors affecting the onset of SP MS

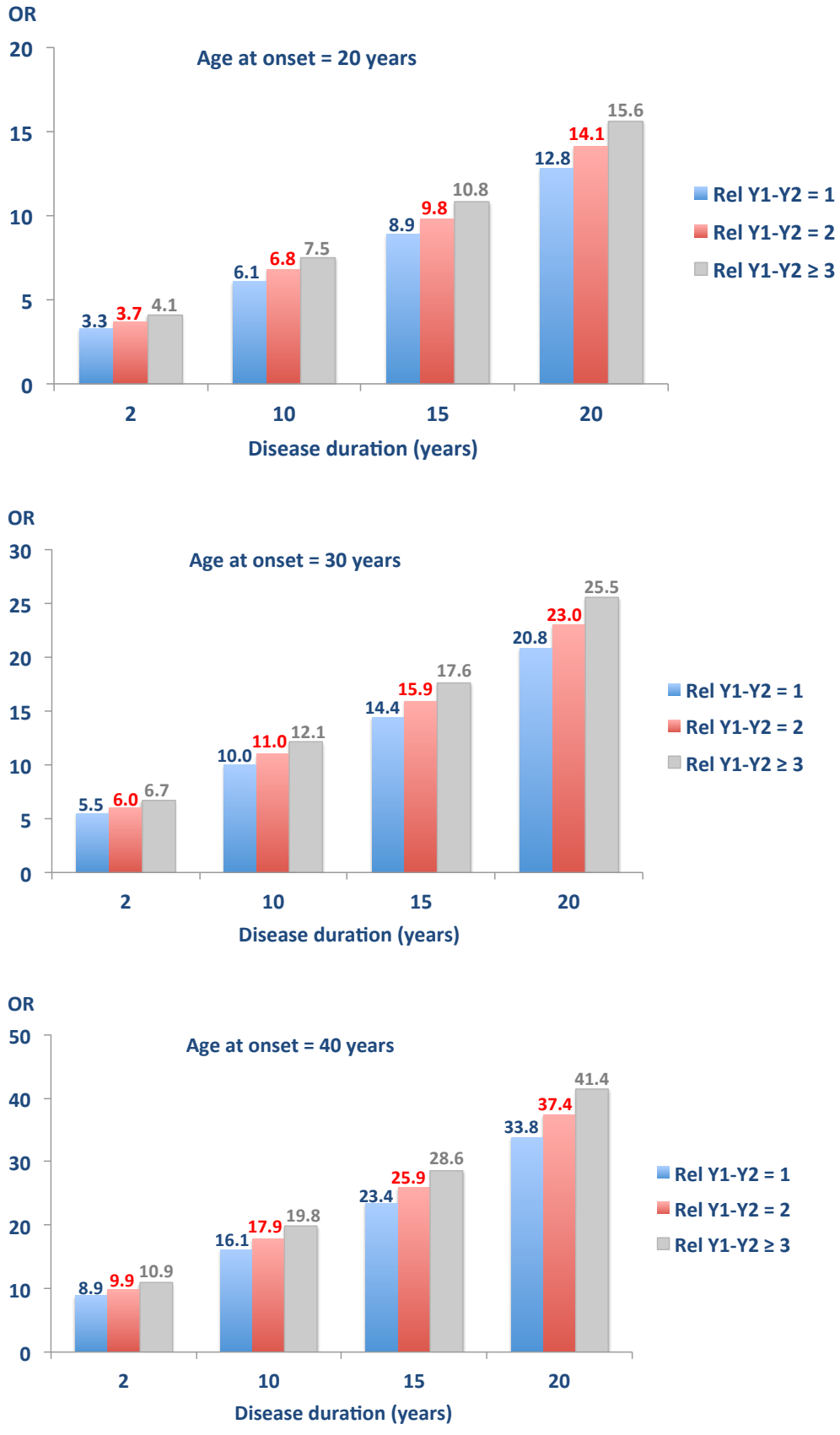
The Kaplan Meier survival analysis demonstrated that male sex, an older age at the disease onset and a higher early relapse frequency associated with a significantly shorter time to the onset of SP (Table 6.4). There was a difference of 6.5 mean years between males (15.9 years) and females (22.4 years) for becoming progressive. As extensively discussed in Chapter 4 and 5, a high early relapses frequency and an older age at the onset of the RR phase predicted a shorter latency to progression. The type and the number of symptoms at the clinical onset did not significantly affect the duration of the RR phase. The multiple Cox regression analysis, which assessed the concomitant predictive effect of all baseline features, confirmed a higher probability of becoming SPMS in males (HR = 1.41 versus females), in those older at the disease onset (HRs: age ≤ 20 versus age > 30 = 0.52; age 21-30 versus age > 30 = 0.65) and in those with a high early relapse frequency (HRs: 1 attack versus ≥ 3 attacks = 0.63, 2 attacks versus ≥ 3 attacks = 0.75) (Table 6.4).

The multiple binary logistic regression analysis allowed to calculate the probability of becoming progressive based on the number of early relapses (regression coefficient = 0.101; OR = 1.11 [95%CI = 0.96-1.27] per additional relapse) and based on the age at disease onset (regression coefficient = 0.049; OR = 1.05 [95%CI = 1.02-1.07] per additional year), and its variation according to the disease duration (regression coefficient = 0.074; OR = 1.08 [95%CI = 1.05-1.09] per additional year). By combining the effect of the 3 variables, the risk of converting to SP MS was computed to simulate hypothetical clinical scenarios (Figure 6.9). Two years after the disease onset, 3 relapses (grey columns) yielded OR 4.1, 6.7, 10.9 among patients aged 20, 30 or 40 at first symptom, respectively. In each subgroup, the probability of becoming SP MS increased with the disease duration and became double at 10 years (OR 7.5, 12.1, 19.8, respectively), approximately 5-fold higher at 20 years (OR 15.6, 25.5, 41.4, respectively) and 6-fold higher at 25 years (OR 22.6, 36.9, 60.0, respectively), from the disease onset.

Table 6.4 Kaplan Meier analysis: estimated times from the disease onset to the onset of the SP phase, among patients grouped by clinical and demographic features. Multiple Cox regression analysis: risk (HR) of converting to SP MS according to the variables.

	Survival analysis from disease onset				
		Kaplan Meier analysis		Cox Regression multiple analysis	
	n (%) censored	Mean years to onset of SP (95% CI)	p	Risk of converting to SPMS; HR (95% CI)	p
Total population	769 (33.8%)	21.4 (19.5-23.1)			
Gender					
Males	240 (24.6%)	15.9 (13.4-17.1)	< 0.001	1.41 (1.17-1.71)	0.001
* Females	529 (38.0%)	22.4 (21.4-26.1)			
Age at onset					
≤ 20	145 (41.1%)	25.8 (21.7-29.9)	< 0.001	0.52 (0.40-0.68)	< 0.001
21-30	371 (38.1%)	20.2 (18.2-21.7)	< 0.001	0.65 (0.53-0.80)	< 0.001
* > 30	285 (24.6%)	15.3 (13.3-17.3)			
Relapses during year 1-2					
1	380 (28.7%)	19.9 (18.3-21.5)	0.01	0.63 (0.49-0.80)	< 0.001
2	179 (30.2%)	16.7 (14.6-18.9)	0.38	0.75 (0.57-0.99)	0.041
* ≥ 3	149 (34.2%)	15.1 (12.8-17.4)			
No of symptoms at disease onset					
1	515 (32.6%)	20.3 (19.2-23.6)	0.75	1.19 (0.92-1.54)	0.17
* > 1	245 (36.7%)	19.9 (16.6-21.1)			
Type of symtpoms at disease onset					
Motor					
present	139 (28.8%)	18.0 (14.1-18.7)	0.18	1.22 (0.93-1.61)	0.15
* absent	630 (34.9%)	20.0 (19.7-23.8)			
Sensory					
present	419 (35.6%)	20.7 (17.7-21.2)	0.41	0.99 (0.75-1.30)	0.93
* absent	350 (31.7%)	19.7 (18.2-23.4)			
Cerebellar					
present	46 (30.4%)	18.7 (12.9-20.9)	0.62	1.16 (0.79-1.71)	0.44
* absent	723 (34.0%)	19.7 (19.5-23.3)			
Brainstem					
present	154 (36.4%)	19.2 (16.3-24.3)	0.75	1.12 (0.83-1.52)	0.44
* absent	615 (33.2%)	20.1 (17.4-19.8)			
Optic					
present	170 (32.4%)	20.3 (16.2-20.7)	0.86	1.04 (0.78-1.39)	0.77
* absent	599 (34.2%)	19.5 (19.0-23.0)			

Figure 6.9 Multiple binary Logistic regression analysis: calculated probability (ORs on y-axis) of converting to SP MS, according to the concomitant effect of the age at onset (20, 30 and 40 years), the number of early relapses (1, 2 and ≥ 3 attacks: blue, red and grey columns, respectively) and the disease duration (2, 10, 15, 20 years) (x-axis). ORs are indicated on top of each column.



6.3.4 Factors affecting the evolution of SP MS

Table 6.5 Kaplan Meier analysis: estimated times from the onset of SP to DSS 8 among patients grouped by clinical and demographic features. Multiple Cox regression analysis: risk (HR) of attaining DSS 8 from onset of progression according to the variables.

	Survival analysis from onset of progression				
		Kaplan Meier analysis		Cox Regression multiple analysis	
	n (%) censored	Mean years to DSS 8 (95% CI)	p	Risk of reaching DSS 8; HR (95% CI)	p
Total population	474 (33.1%)	15.2 (13.7-16.5)			
Gender					
Males	165 (33.9%)	15.5 (12.8-16.0)	0.92	1.03 (0.81-1.31)	0.80
* Females	309 (32.7%)	14.7 (13.3-16.7)			
Age at onset					
≤ 20	80 (35.0%)	13.9 (10.2-17.7)	0.11	1.55 (1.10-2.18)	0.01
21-30	207 (30.0%)	15.3 (13.3-17.4)	0.44	1.15 (0.89-1.48)	0.29
* > 30	187 (35.8%)	15.0 (13.4-16.6)			
Relapses during year 1-2					
1	246 (34.1%)	16.4 (14.5-18.2)	< 0.001	0.47 (0.35-0.63)	< 0.001
2	121 (38.8%)	14.2 (12.5-15.9)	< 0.001	0.53 (0.38-0.74)	< 0.001
* ≥ 3	93 (24.7%)	9.6 (8.2-11.1)			
No of symptoms at disease onset					
1	321 (35.2%)	15.1 (13.7-16.3)	0.17	1.00 (0.72-1.40)	0.98
* > 1	146 (28.1%)	13.9 (11.9-16.7)			
Type of symtpoms at disease onset					
Motor					
present	89 (28.1%)	15.6 (12.5-18.7)	0.87	1.12 (0.78-1.61)	0.55
* absent	385 (34.3%)	15.1 (13.5-16.7)			
Sensory					
present	255 (32.9%)	15.3 (13.8-17.6)	0.36	1.01 (0.71-1.41)	0.97
* absent	219 (33.3%)	13.9 (12.3-15.4)			
Cerebellar					
present	30 (16.7%)	9.5 (6.1-11.3)	0.001	2.21 (1.40-3.37)	< 0.001
* absent	444 (34.2%)	14.8 (14.0-17.0)			
Brainstem					
present	91 (23.1%)	11.0 (8.8-13.0)	< 0.001	1.91 (1.32-2.79)	<0.001
* absent	383 (35.5%)	15.7 (!4.5-17.8)			
Optic					
present	110 (38.2%)	14.9 (12.9-16.7)	0.22	0.90 (0.62-1.30)	0.58
* absent	364 (31.6%)	14.4 (13.3-16.4)			

The Kaplan Meier analysis estimated 15.2 (95% CI: 13.7-16.5) mean years as the time for reaching DSS 8, from the onset of progression. Half of the patients attained the endpoint within 12 years (median time), from the conversion to SPMS. Although they predicted the time SPMS, gender and age at disease onset were found not to affect the time from SP to DSS 8 (Table 6.5). As previously shown (Chapter 4), a high early relapse frequency associated with a faster evolution of the progressive phase. In addition, those who presented with brainstem and cerebellar symptoms at onset attained more rapidly DSS 8, from the onset of progression (Table 6.5). These results were confirmed by the Cox regression multiple analysis, which also showed a slightly higher risk (HR= 1.55) of becoming bedbound, from the onset of progression, among those younger at the disease onset (≤ 20 years).

6.4 Discussion

Among MS patients, the attainment of severe outcomes (DSS 6 or worse) almost invariably results from the relentless accrual of disability, which characterizes the progressive course. Undoubtedly, the onset of the progressive phase is the key determinant of the long-term disease evolution. The number of early inflammatory attacks and the age at the disease onset, which are the strongest predictors of prognosis, affect the attainment of late disability by increasing the probability of entering the SP phase and by shortening the latency to progression (Figure 4.10, Chapter 4 and Figure 5.5, Chapter 5). However, the rate of the disability accumulation during the progressive phase was shown to be uninfluenced by late (Table 4.17, Chapter 4) and superimposed relapses (Confavreux et al., 2000; Kremenchutzky et al., 1999a), to be homogeneous among progressive subtypes (Kremenchutzky et al., 2006a) and to be independent of factors preceding its onset (Confavreux et al., 2003; Eriksson et al., 2003; Leray et al., 2010; Tremlett et al., 2008a). Therefore, the outcome is largely determined before the onset of progression, by the mechanisms driving the evolution of the RR phase, and the conversion to SP MS is a robust marker for late disability.

Although factors affecting the probability of converting to SP MS were previously investigated in other cohorts, results were not always consistent. The LO database is advantaged by a long follow up, which allowed to observe a large proportion of relapsing onset patients entering the SP phase. This offered the opportunity to further investigate clinical and demographic features affecting the occurrence of the progressive course and to elucidate the relationship between the evolution of the RR phase and the attainment of late outcomes.

6.4.1 Defining the onset of SP MS

Defining the onset of the SP phase requires retrospective assessments and it is rarely straightforward. Remission from inflammatory attacks can occur even after several months, potentially clouding the clinical onset of the progressive phase (Kremenchutzky et al., 2006a). Determining exactly when the transition from RR to SP MS takes place can be particularly challenging in patients who start to progress, but continue to relapse. In these cases the accrual of irreversible disability can be caused either by the lack of recovery from the relapses or by the progressive phase, and the two mechanisms are not easily discernable. This is less problematic if progression supervenes after only few attacks or when the motor disability has not developed yet (Kremenchutzky et al., 2006a). Despite this, evidence suggests good inter-examiner reliability in assigning the time to enter the progressive phase (Amato et al., 2004b). In the EVALUED study, involving 180 patients from 6 European centres, the 2 assessors from each clinic had substantial agreement (k = 0.76) when deciding on the development of secondary progression (both examiners agreed within 1 years in 72% of cases) (Amato et al., 2004b).

Given its relevance as clinical outcome, the information on the onset of progression, among LO patients, was extensively audited. In order to conform to the latest international consensus on the classification of the disease course (Lublin and Reingold, 1996), clinical data of those cases previously defined as relapsing progressive MS were carefully checked (Kremenchutzky et al., 1999a). Few of them were categorized as RR MS, because they had a relapsing disease with no progression between relapses. The remaining patients were allocated in the SP, PP and PR groups. In view of the similar demographic and clinical features and of the same long-term outcome, those with PR MS were incorporated into the PP category (Kremenchutzky et al., 1999a). According to the new definition, the progressive disease, preceded or not by a RR phase, had to be characterized by at least 1 year of continuous deterioration, regardless the rate of worsening. In the relentless progressive course temporary improvements were allowed, although steady accumulation of disability was the rule (Kremenchutzky et al., 1999a; Kremenchutzky

et al., 2006a). This definition offered high reliability of a correct classification of progressive patients, as the likelihood of remission after such a long interval is very low. In addition, the yearly assessments, in most of the cases, facilitated the retrospective determination of the onset of the progressive phase (Kremenchutzky et al., 1999a).

As extensively outlined in methods (Chapter 2), during the latest data review of the LO database special attention was paid to address the problem of the overlapping between the relapsing and the progressive phase. Information on the onset of progression were audited and researcher focused on patients who have had multiple relapses but with complete recovery, making the insidious onset of the SP phase identifiable at an early stage (DSS 2), when they typically complained of exercise-induced impairment of the ambulation. With the advantage of frequent neurological evaluations, in few cases the onset of progression could be even identified at DSS 1, by documenting the asymptomatic emergence of long tract signs. Overall, majority of SP patients attained the progressive course at or before DSS 3 (Figure 4.9), and the great majority (84.8%) of SAP patients at or before DSS 2 (Kremenchutzky et al., 2006a). The onset of the progressive course was predominantly characterized by a distal central motor dysfunction and the progressive deficit was almost exclusively localized to the distal lower extremity corticospinal tract fibres, and manifested with ambulation impairment (Kremenchutzky et al., 2006a).

This extensive review of the clinical data, along with the long follow up, allowing to observe more than 65% of relapsing patients entering the progressive phase, makes the LO database particularly suitable for addressing in details the onset and the evolution of SP MS.

6.4.2 The conversion to SP MS

Unsurprisingly, the analysis confirmed that the number of patients converting to SP MS increases over time. More than 80% of patients observed for more than 25 years had entered the progressive phase (Figure 6.1). Accordingly, the binary logistic

regression analysis demonstrated that the disease duration is a strong predictor of the risk of becoming progressive (OR = 1.07, for each additional year of disease duration). This increased by 9% every 5 years; at 10 and 20 years from onset, patients had 2 (OR = 1.99) and 4 (OR = 3.97) times higher probability to covert to SP MS, respectively (Figure 6.2). Importantly, the predictive effect of the length of the disease course was shown to be independent of the age at onset of the RR phase (Chapter 5). This has relevant implication for the patients' therapeutic management, as groups with a longer disease duration are necessarily at higher risk of entering the progressive course.

In the relapsing onset group, the Kaplan Meier estimated mean time to SP was 21.4 years. Half of patients entered the progressive phase in 15 years (median time), and for 25% progression supervened very rapidly (6 years). It has been previously suggested that the rate of conversion from a remitting to a progressive course is constant over time (Broman et al., 1981; Confavreux et al., 1980; Mc and Compston, 1952). Nevertheless, in the LO population, 25 patients per year became progressive during the first 15 years from onset, and, past this time point, only 8 patients per year (Figure 6.3). This obviously reflected the different disease duration, among patients, but also depended on the different severity of the outcome. Indeed, among all SP patients, the latency to progression largely varied from 1 to 36 years (median time = 9 years). The progressive course occurred within 5 years in the quickest quartile and in more than 15 years in the slowest quartile (Figure 6.5).

By the end of the observation period, about one third (n = 272) of the relapsing onset group had not entered the progressive phase, despite their median disease duration of 20 years (Table 6.1). Although most of the patients presumably eventually developed the SP course, a minority (n = 13; 5% of RR patients) did not experience progression even after 40 years or more from onset. This is consistent with the recently reported proportion (5%) of patients with benign MS, free from SP after 50 years of follow up (Skoog et al., 2012).

As observed for late disability, among patients from the LO database, the median time to convert to SP MS (15 years) was slightly shorter (19 years), compared to other cohorts (Figure 3.4, Chapter 3). There are several possible explanations. The proportion of censored information is unlikely to account for these differences, as the number of patients attaining the SP phase was above 50% in most of the other databases. The ascertainment bias might have been responsible for the recruitment of more severe cases among LO patients, however this seems improbable. The comparison between the total population and the geographically ascertained subgroup from Middlesex County demonstrated no difference in the time to SP (Figure 6.4). It remains possible that the LO patients exhibited a more aggressive disease course. Nevertheless, in none of the other databases the clinical information on the onset of progression were specifically reviewed, or at least this has not been documented. A higher accuracy when pinpointing the clinical occurrence of the steady progression results in an earlier detection of the progressive phase, which might be mistakenly interpreted as a more rapid evolution of the RR phase, compared to other cohorts. Indeed, the British Columbia patients had accumulated an unusually high level of disability before starting to progress; more than 50% were scored between EDSS 3 and EDSS 6 and 19% were scored between EDSS 6 and EDSS 8 (Tremlett et al., 2008a). This might indicate that the onset of progression was detected late and the time to SP was overestimated.

6.4.3 The predictive value of the latency to progression

The analysis demonstrated that the latency to progression dictates the "*tempo*" of the long-term disease evolution. As widely evident from the clinical practice, an earlier conversion to SP MS associated with a worse prognosis. The binary logistic regression analysis indicated that the probability of attaining the disability endpoints, from the disease onset, decreased proportionally with the duration of the RR phase. Remaining free of progression for 10 and 15 years reduced the risk of requiring a walking aid by 43% (OR = 0.58) and by 56% (OR = 0.44), respectively (Figure 6.6). Accordingly, when patients were matched by the duration of the RR phase, the group with a shorter latency to progression attained DSS 6 and DSS 8,

from the disease onset, in significantly shorter times (Figure 6.7). Importantly, the differences among groups disappeared once the progressive phase occurred. Those with short, intermediate and long time to SP reached the disability endpoints, from the onset of progression, in similar times, demonstrating that the evolution of the SP phase is largely unaffected by the duration of the RR phase. This might appear in contrast with the mild predictive effect exerted by the time to DSS 3 (Table 4.25, Chapter 4). Among relapsing onset patients, a shorter time to moderate disability associated with modestly shorter times from DSS 3 to higher DSS levels. The differences between the two analyses are probably explained by the selection bias, as some of the patients who reached DSS 3 never attained higher levels of disability, while SP patients are selected for a more aggressive disease course.

Overall, these results further reinforce the concept that the late disease evolution is amnestic of the initial disease course (Confavreux and Vukusic, 2006a) and the outcome is largely determined before the onset of progression, which represents the true clinical watershed, separating two stages largely independent of each other stages. The mechanisms, leading to the transition from relapsing to progressive disease, account for the large variability of the clinical outcome among patients. Interestingly, this was also observed when patients were matched by the number of early and total relapses (Table 6.2 and 6.3), further indicating a partial dissociation between the focal inflammatory attacks and the mechanisms driving the evolution of the RR phase. In each group, sharing the same attacks frequency, an earlier conversion to SP MS predicted shorter times from the disease onset to DSS levels, and the latency to progression differentiated the severity of the disease course. Even among those with frequent early relapses (≥ 3 attacks) the outcome largely varied, according to the duration of the RR phase. There was a difference of 15 median years for reaching DSS 6, between the group with short and the group with long latency to progression (Table 6.2).

6.4.4 Predicting the evolution of the RR phase and of the SP phase

The analysis confirmed that a higher number of early relapses and an older age at the disease onset are the two strongest predictors of the evolution of the RR phase. Their predictive effect was also validated by the multivariate analysis, which assessed the concomitant impact of all baseline variables on the probability of converting to SP MS. In line with previous reports, the model also confirmed that men had a higher (HR = 1.41) risk of becoming progressive and took shorter time to the onset of SP, compared to women (6.5 mean years difference; $p < 0.001$). In addition, the features of the first attack did not influence the risk of SP and the latency to progression. In contrast with some previous studies (Amato et al., 1999; Bergamaschi et al., 2001; Debouverie et al., 2008; Eriksson et al., 2003; Phadke, 1990), the type and the number of symptoms involved at the clinical onset did not affect the probability of MS becoming progressive.

As previously reported (Confavreux et al., 2003; Kremenchutzky et al., 2006a; Leray et al., 2010; Tremlett et al., 2008a), this study demonstrated that the evolution of the SP phase is largely unaffected by factors preceding its onset. Among the predictors of the evolution of the RR phase, only early relapses frequency influenced the times from the onset of progression to DSS levels. In addition, patients presenting with cerebellar and brainstem symptoms at onset had faster accumulation of disability during the progressive phase. This might indicate that when the degenerative process is more severe, brainstem and cerebellar dysfunctions are more likely to emerge at clinical onset.

6.4.5 The conversion to SP MS as outcome measure in clinical trials

The results in this study further highlighted the onset of the SP phase as the overwhelming determinant of the long-term prognosis and, therefore, as the only robust surrogate marker for late disability. Factors affecting the evolution of the RR phase strongly influence the attainment of late outcomes. Among patients with poor prognosis, the faster attainment of DSS levels is secondary to the rapid conversion to

SP MS (Figure 6.7). A longer time free of progression associates with a proportionally lower risk of developing severe disability (Figure 6.6). Therefore, the delay or prevention of the SP phase is the most relevant therapeutic target for future treatments, and the RR phase represents the only plausible window of therapeutic opportunity, at least with currently available DMTs.

Predictive models based exclusively on the frequency of relapses are not sufficiently reliable for detecting a potential effect of treatments on late outcomes. The number of late and total relapses is not a good indicator of the evolution of the RR phase, leading to the onset of progression (Figures 4.16 and 4.21, Chapter 4). Based on the risk of experiencing the SP course, groups destined to have a faster disease course should be identified for testing the risk/benefit ratio of more aggressive treatments. The multiple binary logistic model provided some useful information that could be used for designing future RCTs, assessing the potential effect of therapies on the onset of SP. From hypothetical baseline clinical scenarios, in which patients are stratified according to the number of early relapses and according to the age at onset, the model allowed to calculate the variation over time of the probability of becoming progressive (Figure 6.9).

In groups experiencing the clinical onset at older age and having a high number of attacks during the first two years, the risk of converting from relapsing to progressive MS increased dramatically in a short time from onset (Figure 6.9). For instance, among patients with high early relapse frequency (≥ 3 attacks) and aged 40 at onset, the baseline risk of entering the SP phase (OR = 10.9) doubles after 8 years (OR = 19.8), and it is more than 2 times higher than in patients with the same relapse frequency and disease duration, but younger (20 years) at onset (OR = 7.5). Therefore, those experiencing disease onset at older age (≥ 40 years) and with a high frequency of attacks during the early phase of the disease (≥ 3 relapses during the first 2 years) are expected to convert very rapidly to SP MS and can be recruited in small trials, assessing the potential impact of therapies on the probability of entering the progressive phase. In addition, the frequency of early relapses and the age are information, available early in the disease course, which can be used for selecting

patients, who might benefit from an induction therapy with a strong immunosuppression. Furthermore, the analysis demonstrated that the window of therapeutic opportunity narrows over time, as the probability of entering the progressive phase increases proportionally with the disease duration. This implies that a therapeutic intervention is likely to exert most of its efficacy in the earliest stage of the disease, supporting an early use of aggressive treatments. The number of early attacks, the patients' age and the length of the disease course should be regarded as important factors in the therapeutic decision making algorithm, and should be considered as eligibility criteria in the randomization scheme of the clinical trial using the onset of SP as endpoint.

These results also emphasize the importance of improving the definition of progressive disease, which remains ambiguous. The diagnosis of SP MS necessarily requires retrospective assessments for satisfying the criterion of 1 year of continuous deterioration. The current definition does not specify what type of functional impairment should be observed, among patients at the clinical onset of the progressive phase. Natural history studies showed that progression, both in SP and PP patients, predominantly presents with a distal central motor dysfunction, almost exclusively localized to the lower extremities, and causing ambulation difficulties. Therefore, the onset of the progressive course might be more appropriately defined as an insidious walking impairment, progressively worsening for at least 1 year. In this context, the T25FW test (paragraph 1.6.2) holds promise for evaluating clinically relevant ambulation deterioration in trials, using the of onset of SP as clinical endpoint.

Chapter 7

General discussion

7.1 Mechanisms driving the disease evolution

It is generally accepted that the focal inflammation plays a dominant role in the formation of the demyelinated areas, which are the pathological substrate of the clinical attacks (Lucchinetti et al., 2000). However, the axonal loss (Bjartmar and Trapp, 2001), is considered the major determinant of the permanent disability (Lassmann et al., 2007; Trapp and Nave, 2008). Evidence strongly suggests that the inflammation and the axonal damage are connected (Frischer et al., 2009; Trapp and Nave, 2008), but it is unclear which process is the initial trigger in this complex interaction. After decades of research, mechanisms driving the disease evolution are still widely debated (Chaudhuri and Behan, 2004; Lassmann, 2007; Stys et al., 2012; Trapp and Nave, 2008).

7.1.1 The outside-in and inside-out hypotheses

The "outside-in" model of MS is based on the hypothesis that the pathophysiology of the disease begins with a systemic dysregulation of the immune system, which then targets the CNS. Therefore, the primary autoimmune inflammatory process is considered the essential cause of the axonal damage. However, it has been speculated that the inflammatory mechanisms might not be the driver of the disease evolution, but rather an epiphenomenon of a primary degenerative disorder. The "inside-out" hypothesis proposes that the pathological insult initially targets the oligodendrocytes and the myelin, presumably many years before the clinical onset. The host reacts with an aberrant immune response to the release of highly autoantigenic components, caused by the underlying degenerative process (Chaudhuri and Behan, 2004; Stys et al., 2012).

7.1.2 The white matter pathology

The central role played by the immune system in the disease pathogenesis is indisputable (Sospedra and Martin, 2005). Reactive CD4+ Th1 cells are probably

activated in the peripheral immune system and subsequently migrate across the BBB into the CNS, where they damage the myelin components (Sospedra and Martin, 2005). Expanded pro-inflammatory CD8+ T-lymphocytes are present in the peripheral blood and infiltrate the brain lesions (Annibali et al., 2011). In addition, B-lymphocytes and antibodies are also considered important players in the pathological mechanisms of the disease (Barnett et al., 2009; Freedman et al., 2005). Pathology studies indicate that inflammatory mechanisms are closely involved in the progression of the disease, both during the early and late stages. The white matter lesions are heavily infiltrated by T-lymphocytes and macrophages, which tend to spill into the surrounding parenchyma (Lassmann et al., 2001; Lucchinetti et al., 2000). However, autopsy studies of tissue obtained during the very early phase of the disease demonstrated only sparse inflammatory cells and little evidence of T and B cells infiltration in the lesions, before the demyelination occurred (Barnett and Prineas, 2004; Gay et al., 1997; Henderson et al., 2009; Marik et al., 2007). This suggests that the formation of focal white matter lesions is initially characterized by "apoptosis-like" cells death, accompanied by limited inflammation, and then by a massive lymphocytes infiltration, when the myelin falls apart (Lassmann, 2010).

It has been speculated that the degeneration might occur before the demyelination, explaining the relative sparse inflammatory cells in the lesions at the very early stage. The formation of immune infiltrates in the chronic inflammatory plaques might be secondary to the release of immunogenic debris, resulting from the myelin destruction (Chaudhuri and Behan, 2004; Stys et al., 2012). Indeed, myelin-antigen T cells are not specific for MS only, as they increase in the blood and in the CSF of patients suffering from an acute stroke, suggesting that they might be just an indicator of the brain tissue injury (Wang et al., 1992). The hypothesis that the white matter degeneration can cause a secondary inflammatory disease finds support in the pathological changes occurring among patients affected by the very rare Harding's syndrome. This is described as an association between the Leber's hereditary optic neuropathy (LHON), which is a mitochondrial genetic disease, and RR MS (Kovacs et al., 2005). The coexistence of the two pathologies supports the plausibility of a disease model where the inflammatory reaction in the CNS develops

as consequence of an underlying degenerative process (Stadelmann, 2011; Stys et al., 2012; Trapp and Nave, 2008).

A large body of evidence suggests that the degeneration can take place in the absence of inflammation. The axonal damage was shown to occur in the NAWM, independently of the active demyelination (Bjartmar et al., 2001; DeLuca et al., 2006; Evangelou et al., 2000; Frischer et al., 2009; Lovas et al., 2000; Seewann et al., 2009; Trapp et al., 1998). In the spinal cord, which is ubiquitously involved during the progressive phase of the disease, the extent of the axonal loss was found to correlate poorly with the degree of demyelination (DeLuca et al., 2006; Lovas et al., 2000). The axonal loss is already present in the early course of the disease, and does not correlate with the baseline white matter inflammatory lesions volume (De Stefano et al., 2003; Filippi et al., 2003). In addition, evidence of axonal damage was recently found in a cohort of subjects with RIS (Stromillo et al., 2013), indicating that the subtle degenerative process starts even before the initial clinical manifestation of the disease. It has been suggested that the axonal injury might results from the increased expression of sodium channels in the demyelinated axonal membranes, which triggers an intracellular, calcium-mediated, mitochondrial dysfunction, eventually leading to the neurodegeneration ("the mitochondrial theory") (Dutta et al., 2006; Su et al., 2009; Waxman et al., 2004). Indeed, a sodium-channel blocker (phenytoin) was shown to preserve axons in EAE (Lo et al., 2003), in line with this hypothesis.

However, the presence of a diffuse axonal transection, within the acutely demyelinated plaques (Kuhlmann et al., 2002; Trapp et al., 1998) and in the context of the chronic demyelination (Frischer et al., 2009; Lovas et al., 2000), lend strong support to a causal relationship between the inflammatory processes and the degeneration. The extent of the axonal damage was found to correlate significantly with the number of inflammatory cells and with the activated microglia in the surrounding parenchyma (Ferguson et al., 1997; Kuhlmann et al., 2002; Trapp et al., 1998). The residual disability following the relapses is probably caused by the axons transection within the active lesions (Trapp et al., 1998). Inflammatory cells might

have a direct toxic effect on axons, by releasing proteolytic enzymes, cytokine and oxidative products (Smith et al., 2001; Trapp and Nave, 2008; Weiner, 2009). Despite the early occurrence of the axonal loss, only little permanent disability normally accumulates during the initial stage of the RR phase. This is possibly explained by the compensatory effect of the remyelination, which restores the axonal function after the acute demyelination (Franklin and Kotter, 2008; Irvine and Blakemore, 2008), and by the adaptive functional reorganization of the brain function (Tomassini et al., 2012). The attainment of late clinical outcomes almost invariably results from the relentless disability accumulation, which characterizes the disease course during the progressive phase, when the focal inflammation gradually becomes less prominent (Revesz et al., 1994; Trapp and Nave, 2008). This might indicate that, in late stages, the disease evolves independently of inflammatory mechanisms. However, a profound diffuse inflammation was also found in the brains of patients with advanced, progressive disease (Frischer et al., 2009). Both in the slowly expanding lesions and in the NAWM there was a strong correlation between inflammatory cells and the axonal injury (Frischer et al., 2009). Although this correlation does not necessarily implies that the neurodegenerative process is driven by the inflammation (Lassmann, 2010), the data seems to support a causal relationship between the two mechanisms. It remains possible that the primary degeneration generates a secondary, persistent, diffuse inflammatory response during the late stage of the disease (Stys et al., 2012).

7.1.3 The grey matter pathology

MRI studies have also greatly contributed to characterize the inflammatory nature of the disease. The inflammation in the CNS is a dynamic process, which is accompanied by a breakdown of the BBB (Thompson et al., 1992) and can occur independently of the clinical activity (Barkhof, 2002; Miller et al., 1996). However, the association between the inflammatory white matter lesions load and the development of late disability is poor (2008; Calabrese et al., 2013; Fisniku et al., 2008a). More recently, the cortical demyelination was suggested as a potential pathological driver of the disease progression (Calabrese et al., 2013; Geurts et al.,

2012; Kutzelnigg et al., 2005). Several studies have demonstrated that the grey matter damage (Calabrese et al., 2007b; Calabrese et al., 2009b; Calabrese et al., 2013; Roosendaal et al., 2009) and the grey matter atrophy (Bakshi et al., 2001; De Stefano et al., 2003; Fisniku et al., 2008b; Roosendaal et al., 2011; Tedeschi et al., 2005) are good predictors of the accumulation of disability in the long term. As the disease advances, the grey matter damage accumulates and probably accounts for the transition from relapsing to progressive MS (Calabrese et al., 2013). In contrast to the white matter lesions, in the context of the cortical plaques, little inflammation is detected and the cause of the demyelination is still unknown (Bo et al., 2007; Lassmann, 2012). Cortical lesions occur in the absence of BBB disruption and do not correlate with the extent of the focal inflammation (Bo et al., 2007; Kutzelnigg et al., 2005). However, a recent large biopsy study demonstrated variable degree of cortical demyelination, with diffuse inflammatory infiltrates, in nearly 40% of patients during the early stage of the disease (Lucchinetti et al., 2011), implying that the cortex might be an early target of the focal inflammatory mechanisms. It remains unclear if the cortical pathology might precede the white matter damage, and therefore the focal demyelination might be secondary to the neuronal pathology (Geurts et al., 2012). Indeed, the grey matter atrophy was shown to occur during the very early stage of the disease (Calabrese et al., 2007a; Chard et al., 2002), even in subjects with RIS (Giorgio et al., 2011).

Other studies indicate that, over the disease course, inflammation becomes increasingly compartmentalized in the perivascular spaces, behind a relatively intact BBB (Meinl et al., 2008), and aggregates in follicle-like structures at the level of the meninges (Magliozzi et al., 2007). In both PP and SP MS, the demyelination in the grey matter was found to be associated with profound meningeal inflammation and microglia activation (Choi et al., 2012; Frischer et al., 2009; Howell et al., 2011; Magliozzi et al., 2007; Magliozzi et al., 2010; Serafini et al., 2004). This indicates that the cortical damage and demyelination are possibly caused by the inflammatory cells within the meninges, which infiltrate the underlying parenchyma (Lassmann, 2010; Lassmann, 2012). In support of this , among SP MS patients, the presence of

meningeal lymphatic follicles was found to associate with a more severe disease course (Magliozzi et al., 2007).

7.2 The progressive phase

7.2.1 Primary and secondary progression: evidence from the pathology studies

In the context of the controversial relationship between the inflammation and the neurodegeneration, whether PP and SP MS share common pathogenesis or not, becomes a particularly relevant topic. Imaging studies demonstrated that PP patients have a lower inflammatory lesion load (Rovaris et al., 2000; Thompson et al., 1990) and lower levels of BBB damage (Thompson et al., 1991). In some PP cases, the diffuse abnormalities in the brain and in the spinal cord occurs in the absence of focal inflammatory lesions, suggesting a complete lack of the adaptive immune response (Zwemmer et al., 2008). Pathology assessments provided contradicting evidence. Both subtypes are characterized by brain inflammatory lesions, more prominently in SP patients, nonetheless suggesting that also PP MS is an inflammatory disease (Revesz et al., 1994). It has been shown that the two progressive subtypes share the same pattern of the brain tissue injury, with demyelination, remyelination, loss of oligodendrocytes, and axonal damage (Frischer et al., 2009; Prineas et al., 2001). However, studies comparing the extent of the axonal loss between PP and SP MS are discordant. The axonal damage was found to be similar between the two subtypes (Frischer et al., 2009; Prineas et al., 2001) or, within the demyelinated areas, to be more pronounced, among SP MS patients, and very limited among PP MS patients (Bitsch et al., 2000). In addition, in the cervical cord, the extent of the axonal loss was shown to be larger in PP patients, compared to SP patients, despite the higher degree of white and grey matter demyelination in the SP group (Tallantyre et al., 2009). Interestingly, meningeal B cells follicles, which are found in a large percentage of SP MS patients and are known to correlate with a more severe disease course (Howell et al., 2011; Magliozzi et al., 2007), were not observed in PP MS (Choi et al., 2012). Nevertheless, in both progressive subtypes it

has been demonstrated a large degree of diffuse meningeal inflammation, which associates with extensive axonal loss in the cortical tissue (Choi et al., 2012; Frischer et al., 2009; Howell et al., 2011). It remains undetermined whether these data indicate that the inflammatory mechanisms are a causative factor of the grey matter damage or a simple concomitant (Lassmann, 2010).

7.2.2 Primary and secondary progression: evidence from the natural history studies

Natural history studies consistently showed remarkable similarities between primary and secondary progressive MS. Among progressive patients, the progressive phase starts at similar ages (Confavreux and Vukusic, 2006a; Koch et al., 2007; Kremenchutzky et al., 2006a; Tutuncu et al., 2013) and evolves at similar rate (Kremenchutzky et al., 2006b), irrespective of whether it is preceded by the RR phase or not. In the LO database, progressive patients with one (SAP), many (SP MS) or no (PP MS) relapses before progression attained disability endpoints from the onset of progression in almost the same time (Kremenchutzky et al., 2006a). In the 3 French EDMUS cohorts (Confavreux and Vukusic, 2006a; Debouverie et al., 2008; Leray et al., 2010), times from DSS 3 and from DSS 4 to higher disability levels were similar between PP and RR/SP, indicating that, once established disability occurs, the disease evolution is not influenced by the clinical phenotype. As DSS 3/DSS 4 probably heralded the progressive phase for most of the relapsing onset patients, these data confirmed a similar rate of late disability accumulation between the two progressive subtypes.

The work described in this thesis provided further evidence that progressive and relapsing onset patients share much more than they differ. Both subgroups took almost the same mean times to advance from one disability hallmark to the following (Figure 3.14 B, Chapter 3). In addition, the Kaplan Meier estimated times from DSS 3 to DSS 6 and to DSS 8 differed little (3 mean years difference) between RR/SP and PP groups (Figure 3.21, Chapter 3), and were remarkably similar between the PP and SP groups (Figure 3.22, Chapter 3). Furthermore, the age at which the progressive phase started ranged by only 2.8 mean years among SP patients with 1,

2-3 and ≥ 4 total attacks during the RR phase and PP patients (Figure 5.14, Chapter 5). Finally, the evolution of progressive phase was similar among progressive patients stratified by the age at the onset of progression (Figure 5.18, Chapter 5), the disability accumulated while growing older at similar rate between PP and SP groups (Figure 5.21, Chapter 5) and the ages at the attainment of DSS levels were not affected by the type of the disease course (Figure 5.23, Chapter 5).

Taken together, these data suggest common mechanisms driving the accumulation of severe disability during the progressive phase and question the contribution of the focal inflammatory processes, occurring during the RR phase, to the late disease evolution. The similar outcome among all MS progressive subtypes might indicate that the disease course evolves in two stages, independent of each other (Leray et al., 2010). Biologically, the accumulation of moderate disability, probably by heralding the onset of the progressive course, might mark the failure of compensatory mechanisms (threshold effect), initially abating the axonal injury. The inflammatory mechanisms seem to influence the accumulation of disability during the early phase, but have no impact on the evolution of progressive MS. It remains possible that, once the progressive course supervenes, the focal inflammation gradually becomes compartmentalized in the meninges, and drives both primary and secondary progression, by causing the cortical damage (Choi et al., 2012; Frischer et al., 2009; Howell et al., 2011; Lassmann, 2010). This would justify common pathophysiological mechanisms, accounting for the similar evolution of the progressive phases. However, this notion is not easily applicable to those patients lacking the RR phase and experiencing a progressive course at onset.

7.3 The role of the inflammation

7.3.1 The therapeutic suppression of the inflammation

The therapeutic suppression of relapses and of MRI inflammatory lesions, was shown to prevent the disability progression during the RR phase (1993; 1998a; Coles

et al., 1999; Jacobs et al., 1996a; Johnson et al., 1995; Kappos et al., 2006a; Polman et al., 2006). However, the limited impact of DMTs on the long term disease evolution (Bermel et al., 2010; Ebers et al., 2010; Goodin et al., 2012; Kappos et al., 2006c; Shirani et al., 2012) and in the progressive forms of MS (La Mantia et al., 2012), cast doubt on the contribution of the focal inflammatory processes in mechanisms driving the accumulation of late disability. It has been suggested that the trapped diffuse inflammation behind a closed or repaired BBB, and therefore not easily targetable, explains the poor efficacy of DMTs during the late phase of the disease (Lassmann, 2010). Alternatively, the early focal inflammation might set up the brain for the degenerative process, which occurs later and is largely independent of the initial focal inflammatory insult (Coles et al., 2006). In support of this hypothesis, a profound immunosuppression with a monoclonal antibody (Alemtuzumab), compared to treatment with IFN, exerted a dramatically different effect, among patients with early or advanced disease. The marked reduction of the inflammatory activity had no impact on the disease progression in SP patients (Coles et al., 2006), but prevented the short-term disability accumulation among early active patients (Coles et al., 2006; Coles et al., 2008). In addition, the impact of Alemtuzumab on the disease progression was shown to be larger among patients with shorter (2.1 mean years) (Cohen et al., 2012a), compared to those with a longer disease duration (4.5 mean years) (Coles et al., 2012b). Similarly, the treatment with haemapoietic stem cell transplantation did not exert any significant beneficial effect in progressive patients during the advance stage of the disease (Burt et al., 2003). In contrast, in a group of RR patients early in the disease course (5 median year disease duration), autologous haemapoietic stem cell transplantation associated with a reversal of the neurological disability and with an improvement of the EDSS score (Burt et al., 2009a)

7.3.2 Relapses and the disease progression

By assessing the relationship between clinical relapses and the long-term disease evolution, natural history studies can help to further elucidate the role of the focal inflammatory mechanisms on the disease pathogenesis. Nevertheless, the partial

dissociation between the biological activity and the clinical events, as highlighted by imaging studies (Barkhof, 2002; Filippi et al., 2003), limit the power to draw definitive conclusion.

7.3.2.1 The focal inflammatory activity early in the disease course

Results from different natural history cohorts, obtained with different methodology, consistently demonstrated that patients with a large number of attacks during the first two (Leray et al., 2010; Weinshenker et al., 1989c) and five (Confavreux et al., 2003; Debouverie et al., 2008; Eriksson et al., 2003; Kantarci et al., 1998; Tremlett et al., 2009a) years, experience a faster disease evolution (Table 4.2, Chapter 4). More relapses during the initial stage of the disease correlated with a significantly higher risk of reaching the SP phase and the DSS levels, and with shorter times to the endpoints, suggesting that the early focal inflammatory activity contributes significantly to the attainment of late disability. Interestingly, the strong association between early clinical relapses and late outcomes partially diverges from MRI studies, which showed only a limited correlation between the extent of the baseline inflammatory lesions and the long-term disease evolution (2008; Calabrese et al., 2013; Fisniku et al., 2008a). This might be due to the limited pathological specificity of the white matter lesions detected by the MRI T2 sequences (Filippi et al., 2012).

The analysis in this thesis confirmed the strong association between attacks occurring during the first two years and the development of late disability. Patients with a larger number of early relapses reached severe outcomes more rapidly, secondary to the shorter conversion to SP MS (Table 4.5, Chapter 4). Previous studies demonstrated that the disease evolution from DSS 3/DSS 4 was not influenced by the number of early inflammatory attacks, in line with the amnestic nature of the late disease progression (Confavreux et al., 2003; Debouverie et al., 2008; Leray et al., 2010). In contrast, among LO database patients, the frequency of early attacks mildly influenced the disease evolution from moderate disability and from the onset of progression (Table 4.8, Chapter 4). Therefore, the relapses during the first two years affected the attainment of late outcomes primarily by increasing

the risk of converting to SP MS, by shortening the latency to progression and, to lesser degree, by influencing the slope of the SP phase. The results confirm that the outcome is mainly determined by mechanisms driving the evolution of the RR phase and leading to the conversion to SP MS. In addition, they underline the very early phase of the disease as the crucial time when pathological changes, which will affect the accumulation of severe disability in the long term, take place. Imaging studies demonstrate that the biological onset of the disease might precede the clinical onset (Granberg et al., 2013; Stromillo et al., 2013). Most likely, the factors responsible for determining when the asymptomatic inflammatory activity becomes first clinically evident are also implicated in the cascade of events, occurring early in the disease course, that largely influences the individual outcome in the long term.

The analyses of early relapses indicate that the early focal inflammation plays a direct role in the pathological processes responsible for promoting the occurrence of the progressive course and for driving the evolution of the progressive phase. This suggests that the initial focal inflammatory white matter pathology might be involved in the biological events leading to the axonal damage (Ferguson et al., 1997; Kuhlmann et al., 2002; Trapp et al., 1998). In addition, the mild impact of early relapses on the slope of the progressive phase supports the notion that the early focal inflammatory activity contributes to set up the brain for a faster late disease evolution (Coles et al., 2006). However, once the initial tissue injury is established, the role of focal inflammation on the disease progression becomes less relevant. The axonal degeneration, occurring during the progressive phase, might result from the loss of trophic support, as a consequence of the focal inflammatory insult during the initial stage of the disease (Coles et al., 2006). Alternatively, the RR and the SP phases are possibly linked by inflammatory mechanisms, which are initially focalized and gradually become diffuse. The demyelinated areas, where the axons are already damaged during the early disease stage (Kuhlmann et al., 2002; Trapp et al., 1998), might be the vulnerable niche where the focal inflammation initially becomes compartmentalized and subsequently determines the cortical pathology, which drives the disease evolution during the progressive phase (Frischer et al., 2009; Howell et al., 2011). However, it can be speculated that this unifying mechanism

would also imply similarities in the clinical pattern before and after the onset of progression. Indeed, the SP phase is predominantly characterized by motor disturbances, irrespective of the type of symptoms occurring during the RR phase (Confavreux and Vukusic, 2006a; Kremenchutzky et al., 2006a). Nevertheless, this does not necessarily exclude that the diffuse inflammation is involved in the evolution of the progressive phase. The cortical damage was shown to be strongly associated with the EDDS score in the short term (Calabrese et al., 2012), suggesting that the motor disability is more related to the grey matter than to the white matter pathology. Therefore, among progressive patients, the abundant cortical lesions (Calabrese et al., 2007b), potentially linked to the meningeal inflammation, would explain the predominance of motor symptoms during both primary and secondary progressive MS.

The LO database offered the unique possibility of analysing the long-term evolution, among patients with ≥ 3 relapses during the first two years ("*frequent early relapsers*"), who were the main driver of the association between early relapses and late outcomes. This allowed to further address the question whether early inflammatory attacks are a causative factor for the late disability, developing years later. Unexpectedly, in the group with early adverse clinical features, the outcome was not homogeneous, but widely variable, ranging from one extreme to the opposite of the disability spectrum. More than 60% had the expected aggressive disease course: they converted to SP MS in 5 median years and attained DSS 6 and DSS 8, from the disease onset, in 7 and 17 median years, respectively, and from the onset of progression, in 1 and 9 median years, respectively (Table 4.11, Chapter 4). These results further indicate that a very active early inflammation is responsible for determining a remarkably fast disability accumulation, by accelerating the onset of the progressive phase and its evolution. This lends further support to the hypothesis that the initial focal inflammatory insult creates a pathological substrate, which promotes the rapid degeneration of the axons (Coles et al., 2006). However, among *frequent early relapsers*, more than 30% of patients did not convert to SP MS and had a remarkable benign disease course, despite the long disease duration (Figure 4.11, Chapter 4). The opposite outcome in the two distinct subgroups, sharing the

same high early inflammatory activity, was differentiated by whether the SP phase occurred or not. This demonstrated that frequent early relapses do not necessarily associate with a faster accumulation of severe disability, and suggested that additional factors, other than the early inflammatory frequency, might be implicated in the development of the progressive course.

These results need confirmation in other suitable populations. A recent imaging study analysed the disease course of 334 relapsing onset patients observed for 5 years. Almost 20% (n = 66) rapidly converted to SP MS (4 median year) and had a significantly higher baseline T2 white matter lesions volume, compared to those who remained in the RR phase (Calabrese et al., 2013). However, the number of contrast enhancing lesions was lower in the SP group, both at baseline and at 5 years, and, after adjusting for the patients' age and disease duration, the best predictor of the probability of entering the SP phase was the volume of the cortical lesions (Calabrese et al., 2013). In view of the association between early relapses and late outcomes it can be speculated that the same factors, accounting for the transition from RIS to clinically isolated syndrome (CIS), might control the outcome severity in the long term, by promoting the progressive course. The first attack may not be occurring randomly but rather be intrinsically involved in mechanisms initiating the clinical disease by targeting the grey matter, as shown by a recent CSF study in CIS patients (Schutzer et al., 2013). In addition, the cortical damage might represent the common pathological substrate between early relapses and age, both affecting the disease evolution by promoting the onset of the progressive course.

Overall, the extent of the cortical pathology was shown to be the strongest determinant of the risk of entering the SP phase, independently of the inflammatory activity (Calabrese et al., 2013). Similarly, the results from the analysis of the frequent early relapses group suggest that the axonal vulnerability or its resistance to the focal inflammation might be regulated by other factors. Indeed, the extent of the axonal damage in the demyelinated plaques widely varies, ranging from nearly normal to a loss of 80%, and averaging 60-70% (Bjartmar and Trapp, 2001; Mews et al., 1998), highlighting different degrees of susceptibility among axons. It has been

hypothesized that the clinical outcome could be influenced, to some extent, by genetic factors. Indeed, it has been demonstrated that location (Mowry et al., 2013a) and severity (Mowry et al., 2013b) of early clinical manifestations may be influenced by the MS susceptibility polymorphisms. In addition, patients carrying the allele HLA-DRB1*15 were recently shown to have a more pronounced inflammation in the spinal cord, suggesting a higher predisposition to develop inflammatory attacks (Deluca et al., 2013). The HLA-DRB1*01 allele was found to be underrepresented among patients with an aggressive disease course, compared to patients with a benign outcome, suggesting that it might exert a protective effect (DeLuca et al., 2007). However, its axonal relevance has never been demonstrated.

7.3.2.2 The focal inflammatory activity late in the disease course

Natural history studies consistently demonstrated that inflammatory attacks occurring during the primary (Andersson et al., 1999; Kremenchutzky et al., 1999a) and the secondary (Confavreux et al., 2000; Vukusic and Confavreux, 2003) progressive phase do not affect the development of disability. This confirms that when the tissue damage is established, the focal inflammation has not detectable impact on the mechanisms driving the disease evolution, probably explaining the lack of efficacy of DMT in the progressive forms of the disease (La Mantia et al., 2012; Wolinsky et al., 2007).

The relationship between relapses during the late stage of the RR phase and late outcomes was previously investigated in the British Columbia database. One attack, occurring between 5 to 10 years after onset, modestly increased the hazard of reaching SP (HR = 1.23) and EDSS 6 (HR = 1.31) within this period of time. However, this effect was smaller than the effect of early relapses, and also smaller when the two endpoints were attained after 10 years from onset (HR = 1.07 and 1.06, respectively). The authors concluded that late relapses pose an immediate risk on the disease progression but their impact on the endpoints diminishes with time. As outlined in Chapter 4 (paragraph 4.4.2.2), this analysis was affected by some methodological caveats.

In contrast, among LO SP patients, relapses occurring from the third year up to the onset of progression did not influence negatively the disease evolution. Unexpectedly, a larger number of attacks after year 2 associated with a longer latency to progression and with longer times to disability endpoints (Table 4.16, Chapter 4), and did not affect the evolution of the progressive phase (Table 4.17, Chapter 4). Therefore, the analysis highlighted a dichotomy between early and late relapses, which related to the outcomes in an opposite way. After an early watershed is reached, focal inflammatory attacks disconnect from mechanisms driving the evolution of the RR and the SP phases. This reversal seems to take place after year 2 however, methodological differences (paragraph 4.4.5, Chapter 4) make early and late relapses difficult to compare, and it remains possible that the timing of the watershed was slightly affected by the modality of the data collection. Late relapses were counted, among SP patients, during the variable period of time from year 3 up to the onset of progression, while early relapses were counted, among relapsing onset patients, during the fixed first two years of the disease. However, the analysis indisputably demonstrated that groups with a larger number of late relapses had longer latency to progression and, consequently, took longer times to disability endpoints (Figure 4.16, Chapter 4). In addition, SP patients with a larger number of total attacks before the onset of progression, had a longer duration of the RR phase (Figure 4.21, Chapter 4).

These results challenge the hypothesis that the transition to progressive MS occurs as a consequence of serial exacerbations, after compensatory mechanisms fail. If the focal inflammatory insult, initially abated by the remyelination and by the brain plasticity (Franklin and Kotter, 2008; Irvine and Blakemore, 2008; Tomassini et al., 2012), causes the changing course of MS over time, a larger number of attacks would be expected to correlate with a faster attainment of SP. Nevertheless, the early disconnection between mechanisms underlying the inflammatory attacks and those driving the evolution of the RR phase could mirror a gradual increment of a more diffuse inflammatory activity, involving the NAWM and microglia (Frischer et al.,

2009; Lassmann et al., 2012). This would imply that the compartmentalization of the inflammation (Frischer et al., 2009) occurs much earlier than initially hypothesized.

As highlighted by the analysis of early relapses (Figure 4.10, Chapter 4), the pathological changes, determining the development of the progressive course, most likely take place during the early phase of the disease. Indeed, the axonal damage is known to occur already during the initial disease stage (De Stefano et al., 2003; Filippi et al., 2003; Stromillo et al., 2013) and the cortical pathology was shown to gradually take over the white matter pathology, during the transition to progressive MS (Calabrese et al., 2013). Therefore, the dissociation between late relapses and the disease evolution suggests that, the degenerative process, already set up early in the disease course, slowly prevails on the focal inflammation and subtly becomes clinically evident, hindering the clinical inflammatory attacks. It remains unclear which factors cause the changes in this complex interaction between focal inflammation and degeneration, and shifts the balance in favour of the latter. These results imply a yet undetermined interaction between the development of the progressive course and the suppression, or the masking, of relapses, possibly analogous to what occurs in PP MS. Among patients with worse prognosis, the anticipated and rapid onset of the progressive phase overlaps the episodic acute clinical attacks, explaining why a lower number of late and total relapses associates with a shorter duration of the RR phase (Figures 4.16 and 4.21, Chapter 4). Indeed, the multivariate analysis of early and late relapses further demonstrated that, when the disease is less aggressive and the occurrence of the progressive course is delayed, the late inflammatory activity is clinically more prominent, and a higher late attack frequency is a concomitant of a longer disease evolution (Figure 4.23, Chapter 4).

In addition, SP patients grouped by the number of total attacks during the RR phase had remarkably similar outcome (Figure 4.21, Chapter 4). These results are probably explained by early and late relapses neutralizing each other, among SP groups. Those with high early attacks frequency, which associated with a fast disease evolution and a short latency to progression, are counterbalanced by those with high late attacks

frequency, which associated with a slower evolution and a longer time to progression. Overall, the analysis of total relapses further confirmed that the focal inflammation, after the initial disease stage, does not contribute to the development of late disability.

7.4 Age and the disease progression

7.4.1 Age and the progressive course

The analysis of relapses further underlined the complex interaction between the focal inflammatory activity and the degenerative process. The balance between the two mechanisms and their clinical counterparts changes over time, and other factors might influence the disease course. The transition of the clinical phenotype, from relapsing to progressive, could be determined by the evolution of the initial pathological processes, implying separate clinical stages causally related and occurring sequentially. However, it remains unclear why the progressive phase can start “de novo” or following inflammatory attacks. The similar pattern of the brain tissue injury (Frischer et al., 2009; Prineas et al., 2001) and the similar disease evolution (Kremenchutzky et al., 2006b), among progressive subtypes, make unlikely that PP MS is a separate clinical entity (Antel et al., 2012; Rice et al., 2013). In view of the strikingly similar ages at the onset of progression, between PP and SP patients (Table 3.6, Chapter 3) (Confavreux and Vukusic, 2006a; Koch et al., 2007; Kremenchutzky et al., 2006a; Tutuncu et al., 2013), it has been suggested that age related degenerative mechanisms might determine the emergence of the progressive phase and drive its evolution (Confavreux and Vukusic, 2006b; Kremenchutzky et al., 2006a). Indeed, progressive MS only rarely is observed during childhood (Renoux et al., 2007). This leads to a unifying concept of the disease course, characterized by a predefined pattern of accrual of disability during the progressive phase, not influenced by relapses preceding its onset. The clinical phenotype might be mainly a function of the different ages at which the pathological

processes become clinically evident, rather than an effect of changing pathogenic mechanisms (Confavreux and Vukusic, 2006a).

The analyses presented in this thesis demonstrated that age is an important driver of the disability accumulation and affects the attainment of late outcomes by influencing the evolution of the RR phase and by promoting the occurrence of the progressive course. Among relapsing onset patients, being older at the disease onset and growing older increased the probability of entering the SP phase, independently of the disease duration (Figures 5.5, 5.19 and Table 5.12, Chapter 5). An older age at the onset of the RR phase associated with a shorter latency to progression (Table 5.6, Chapter 5). Among all progressive subtypes, the age at the onset of the progressive phase was unaffected by the clinical phenotype (Figure 5.13, Chapter 5), and not influenced by the total relapses occurring during the RR phase (Figure 5.14, Chapter 5). In addition, the age at the onset of progression was only marginally influenced by the number of early relapses (Figure 5.15, Chapter 5). However, considering progressive MS exclusively an age-dependent disease would be an oversimplification, as demonstrated by the widely variable age at the onset of progression, among individuals (Figure 5.12, Chapter 5).

Overall, these data strongly suggest that age related mechanisms are primarily involved in the pathological processes responsible for the changes of the clinical phenotype, over time. Age is the major determinant of the transition from relapsing to progressive MS, and influences when the progressive courses becomes clinically evident, irrespective of the disease duration. The almost identical ages when patients entered the progressive phase, preceded or not by the RR phase, indicated that the biological mechanisms, causing the permanent axonal damage and leading to the onset of the progressive course, are active much before its clinical occurrence. This is line with results from imaging studies, which demonstrated evidence of axonal loss early in the disease course (De Stefano et al., 2003; Filippi et al., 2003; Stromillo et al., 2013). In addition, the lack of impact of relapses on the age at onset of SP MS (Figures 5.14, 5.15 Chapter 5) confirmed that the development of the progressive course is largely independent of the focal inflammatory pathology,

occurring during the RR phase. However, the group with high early relapse frequency (≥ 3 attacks) diverged from the other progressive patients and attained progression at significantly younger age (Figure 5.15, Chapter 5). This reinforced the concept that a pronounced early inflammatory activity directly contributes to the mechanisms responsible for accelerating the occurrence of the progressive course.

Results from a recent longitudinal MRI study underlined the close interaction between age and the grey matter damage, influencing the conversion from relapsing to progressive MS. The multivariate model identified age and the volume of the cortical pathology as the strongest predictors of the onset of the progressive phase, and confirmed the limited role of the white matter damage in the late disability progression (Calabrese et al., 2013). The grey matter damage was shown to occur in the early phase of the disease (Calabrese et al., 2007b; Lucchinetti et al., 2011), to increase over time (Calabrese et al., 2010) and to become diffuse during the SP phase (Calabrese et al., 2007b). It is reasonable to assume that the failure of compensatory mechanisms marks the attainment of a critical threshold, after which further cortical damage determines the transition to progressive MS. Age related mechanisms could directly contribute to the pathological processes driving the grey matter pathology, or being indirectly involved in the occurrence of the progressive phase, by affecting the efficiency of the mechanisms initially abating the damage.

The remyelination by oligodendrocytes is known to protect the axons from the injury (Irvine and Blakemore, 2008; Kornek et al., 2000). It is a reparatory process, which actively occurs during the RR phase (Prineas et al., 1993), but becomes less efficient during the progressive phase (Lassmann et al., 2012; Patrikios et al., 2006). A large body of evidence indicates that rate of remyelination decreases with age (Rist and Franklin, 2008; Ruckh et al., 2012; Sim et al., 2002), partially because of changes within the aging olygodendrocytes precursor cells, which gradually loose their ability of differentiating into remyelinating oligodendrocytes (Shen et al., 2008; Sim et al., 2002). This age-dependent pathological substrate might justify why, among patients, the progressive course emerges while growing older, independently of the disease duration. Indeed, oligodendrocytes precursor cells with lost ability to differentiate

into remyelinating oligodendrocytes, were described in the chronically demyelinated lesions (Kuhlmann et al., 2008), which are abundant during the progressive phase (Frischer et al., 2009; Lovas et al., 2000). Interestingly, a recent experimental study demonstrated that the capacity of remyelinate can be restored in aged oligodendrocytes precursor cells, which can, therefore, be a potential target for future remyelination-enhancing therapies (Ruckh et al., 2012).

It has also been hypothesized that the progressive failure of reparatory remyelinating mechanisms might be mediated by an increment of iron deposits in the CNS (Lassmann et al., 2012). Iron and ferritin are stored in the oligodendrocytes of MS brains (Hulet et al., 1999) and, as shown by studies *in vitro*, its accumulation might determine a higher susceptibility of these cells to degenerate, secondary to the oxidative stress induced by the inflammation (Zhang et al., 2005). Following the oligodendrocytes destruction, the accumulated iron is released in the extracellular space, further enhancing the oxidative damage and increasing the susceptibility of the surrounding tissue to the degeneration (Lassmann et al., 2012). Since iron deposits are known to increase in human brain while growing older, and to reach a peak between 40 and 50 years of age (Hallgren and Sourander, 1958), an age-dependent iron accumulation might partially explain the age-related changes of the disease course.

7.4.2 Age and the relapsing phase

Age influences when the progressive course becomes clinically evident, in addition, it affects the presenting pattern of the clinical inflammatory activity. The number of attacks is known to decrease over time, partially due to the regression to the mean (Martinez-Yelamos et al., 2006), proportionally to the patients' age (Tremlett et al., 2008b) (Figure 4.2, Chapter 4). For the first time, the analyses in this thesis defined more precisely the relationship between the age at the disease onset and the frequency of relapses. Interestingly, a dichotomy was again highlighted between early and late attacks, which correlated to the patients' age in an opposite way. Among relapsing onset patients, the number of relapses during the first two years

was not affected by the age at clinical presentation. This confirmed that the age and the early inflammatory activity, which are the strongest predictors of the evolution of the RR phase, exert an independent effect on the mechanisms responsible for promoting the emergence of the progressive course. In contrast, patients older at the first clinical manifestation had a significantly lower number of late and total relapses (Figure 5.8, Chapter 5). The total number of attacks recorded during the RR phase reduced proportionally with the increasing age at disease onset (Figure 5.9, Chapter 5). Despite sharing almost the same age at the onset of progression, the SP group with a high number of relapses, compared to the groups with a low number, were significantly younger at the clinical presentation (Figure 5.24). This might indicate that the focal inflammation decreases while growing older because a gradual compartmentalization (Frischer et al., 2009). Alternatively, the age when the inflammatory activity becomes clinically evident might be controlled by genetic factors, affecting the autoimmune mechanisms. A recent autopsy study demonstrated that patients carrying the allele HLA-DRB1*15, who are known to develop the disease at younger age (Masterman et al., 2000), have significantly more pronounced inflammation in the spinal cord, suggesting that they are more susceptible to episodes of inflammatory attacks (Deluca et al., 2013).

However, the analyses also demonstrated that the latency to progression is shorter, among patients with older age at onset, and among patients with a smaller number of late and total attacks. This highlights the complexity of the mechanisms regulating the duration of the RR phase, the age at its onset and the relapses occurring during its course. Age appears to be involved in the biological processes underlying the opening and the closing of the time window, which spans from the first acute attack to the gradual emergence of the relentless accrual of disability. Age related degenerative mechanisms might be the primary pathological event, which affects the latency to progression, and consequently, the number of relapses. With ageing, the subtle degeneration gradually emerges and prevails over the inflammatory mechanisms, suppressing or masking the clinical attacks. At a population level, among progressive subtypes, the age of 40 is the landmark point in the patients' life when the degenerative process, which remains subclinical for a certain time, is likely

to become clinically evident. Therefore, among patients starting to relapse at older age, close to the clinical occurrence of the progressive phase, the latency to progression is shorter and the number of inflammatory attacks is lower. This unifying hypothesis further defines MS as a disorder, where initially different clinical phenotypes converge into a progressive course, under the dominant influence of age related mechanisms. The age dependent clinical phenotypes, before the onset of the progressive phase, broadly range. On one end of this spectrum are the SP patients, who experience the disease onset at young age, have long duration of the RR phase and a pronounced clinical inflammatory activity. On the opposite end are the SAP patients, who have a single attack, followed by the progressive phase after a short latency, and experience the disease onset at old age, close to the age at onset of PP patients.

In this unifying model of the disease, the primary and secondary progressive patients accumulate disability at similar rate, while growing older (Figure 5.21, Chapter 5), and attain the disability endpoint at similar age (Figure 5.23, Chapter 5). Both subtypes are exposed to a diffuse meningeal inflammation (Choi et al., 2012; Frischer et al., 2009; Howell et al., 2011), which might drive the evolution of the progressive phase. However, it remains unclear why the early focal inflammation would be less disposed to cause symptoms among patients with a progressive onset, compared to patients with a relapsing onset. This is unlikely to be attributed to a different location of the demyelinated lesions, as the spatial distribution is not obviously different (Frischer et al., 2009; Revesz et al., 1994). Nevertheless, the proportion of remyelinated plaques and the overall remyelination capacity was shown to be higher in PP, compared to SP, brains (Bramow et al., 2010), which might be a potential explanation of the different clinical pattern before the onset of progression. In addition, the recovery from clinical attacks is related to a cessation of the conduction block, following the resolution of the focal inflammation (Smith, 1994). Therefore, individual differences in the intensity of the inflammatory insult, and of the compensatory response, might contribute to determine the variability of the clinical inflammatory activity (Smith, 1994).

7.5 Predicting the outcome

Although groups at higher risk of experiencing a faster disease evolution can be identified, the long-term outcome of the disease, at individual level, remains unpredictable. This was demonstrated by the wide variation, among patients, of the time to DSS levels from the disease onset (Figures 3.18, 3.19, Chapter 3) and from the onset of progression (paragraph 6.3.4, Chapter 6), of the time to SP (Figure 6.5, Chapter 6), and of the age at onset of the progressive phase (Figure 5.12, Chapter 5) and at the attainment of disability landmarks (Figure 5.20, Chapter 5). It is worth noticing that in a well-ascertained MS cohort of untreated patients, the rate of disability accumulation was not excessively fast. The results indicated that half of the total population is expected to require a walking aid in 15 years. However, after 15 years from the disease onset, 30% of patients did not even accumulate moderate disability, and after 25 years 38% were still free of severe disability (< DSS 6).

The analyses provided strong evidence indicating that, among relapsing onset patients, the late clinical outcome is determined primarily by the occurrence of the progressive course, and by the latency to progression. Consequently, mechanisms driving the evolution of the RR phase largely account for the disease severity. Early relapses (Figures 4.10, Chapter 4) and the age at onset (Figure 5.5, Chapter 5) affected the attainment of late outcome mainly by promoting the onset of the progressive phase and by shortening the time to SP. A longer latency to progression associated with a proportionally lower risk of becoming disabled (Figure 6.6, Chapter 6). Among patients with poor outcome, the faster accumulation of disability was secondary to the rapid conversion to SP MS (Figure 6.7, Chapter 6). This was also observed among SP patients grouped by the frequency of relapses. Despite sharing the same number of attacks, the rate of the disability accumulation varied according to the latency to progression (Tables 6.2, 6.3, Chapter 6). Similarly, when patients were matched by the duration of the RR phase, the age at disease onset did not exert any effect on the attainment of disability levels (Figure 5.7, Chapter 5).

Unfortunately, predicting the long-term outcome, based on the information available at the onset of the disease, remains challenging. The analyses in this thesis helped to better define the effect on the disease evolution of baseline variables, which were already known to have a predictive value. In line with previous reports, the characteristics of the onset attack (type and number of neurological systems involved) were found to not impact on the attainment of the disability outcomes. However, the predictive effect of the degree of recovery from the first relapse could not be assessed (Chapter 4).

Overall, males had a worse outcome than females, although the differences were rather small and not very significant in the context of the disease spanning over 30-40 years. Men become progressive (Table 6.4, Chapter 6) and reached death (Tables 3.19, 3.21, 3.22, 3.23, 3.24, Chapter 3) in modestly shorter times, and they were slightly younger at death (Table 3.19, Chapter 3). This was partially accounted for by the larger percentage of PP MS cases among males.

As extensively shown in previous paragraphs, although the primary progressive course, compared to the relapsing onset course, associated with a faster accumulation of disability from the disease onset, the two clinical phenotypes differed little in the late disease progression (Figures 3.21, 3.22, Chapter 3). In addition, progressive and relapsing onset patients attained the disability landmarks and died at very similar ages (Figure 5.23, Chapter 5). Interestingly, among dead patients only (not including censoring information), the type of the disease course did not affect the time to death (Tables 3.15, 3.19, Chapter 3) and the probability of dying from causes related to MS (Table 3.17, Chapter 3). However, patients with initial relapsing disease had a worse outcome, as they died at slightly younger age (Tables 3.15, 3.19, Chapter 3).

The frequency of early relapses and the age at the onset of the RR phase were identified as the two strongest predictors of the long-term disease evolution. Patients older at first attack and experiencing a larger number of relapses within two

years from onset were at higher risk of becoming progressive and had a shorter duration of the RR phase. The hazard of converting to SP MS was 1.5 fold higher among the group aged 45 at disease onset, compared to the group aged 30. In addition, the analyses demonstrated that the patients with frequent early relapses (≥ 3 attacks) have a high probability of accumulating disability very rapidly. They converted to SP MS in 9 median years and reached DSS 6 and DSS 8 in 10 and 21 median years from the disease onset, respectively (Table 4.5, Chapter 4), and in 1 and 9 median years from the onset of progression, respectively (Table 4.8, Chapter 4). Importantly, *frequent early relapsers*, who converted to SP MS, took only 4 mean years to reach DSS 3, indicating that the fast attainment of moderate disability predicts a very poor outcome. This information can become available early in the disease course and has relevant implications for the patients' therapeutic management.

Taken together, these data demonstrate that the individual risk of attaining the disability landmarks in shorter or longer times is, to a large extent, determined in the early stage of the disease, by mechanisms tide to the onset of the SP. However, the outcome changes over time, and it is primarily influenced by the patients' age and by the length of the disease course, both affecting the probability of converting to SP MS. The hazard of becoming progressive increases by approximately 9% every 5 years of the disease duration (Figure 6.2, Chapter 6) and proportionally with ageing (Figure 5.19, Chapter 5). Importantly, the disease duration exerted no predictive effect when it was tested along with the patients' current age (Table 5.12, Chapter 5). By growing older, patients necessarily become more likely to experience the progressive course and to accumulate severe disability, independently of the length of the disease course. The probability of reaching DSS 6 was approximately double in a patient at the age of 50, compared to a patient at the age of 40 (Figure 5.19, Chapter 5). Unexpectedly, there was no evidence that the outcome, among SP patient, changed according to the number of late attacks during the RR phase. This discourages the use of the late relapse frequency as predictor of late outcomes.

Only few variables were found to predict the evolution of the SP phase. A larger number of early relapses associated with a slightly faster rate of disability accumulation after the onset of progression. In addition, patients with brainstem and cerebellar symptoms at clinical presentation took shorter times to DSS level from the onset of progression. The analysis demonstrated that the disability accumulation during the SP phase was largely unaffected by the duration of the RR phase (Figure 6.8, Chapter 6). This is in line with the amnestic nature of the late disease course (Confavreux et al., 2003; Leray et al., 2010) and reinforces the concept that the outcome is mainly determined before the onset progression.

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7.6 Clinical indicators of the disease activity

The number of relapses is considered a valid indicator of the disease activity and it is used as a predictor of the response to DMTs (Freedman et al., 2004; Rio et al., 2011). A standardized definition of the treatment response is still lacking and the algorithms used for monitoring the disease evolution (Rio et al., 2011) are based on data collected over a short period of time (Bosca et al., 2008; Rio et al., 2006; Rudick et al., 2004; Sormani et al., 2007).

The analysis of relapses demonstrated that, the number of attacks after year 2 did not affect the time to SP and the times to hard disability outcomes, from the disease onset (Figure 4.16, Chapter 4) and from the onset of progression (Table 4.17, Chapter 4). Similarly, groups with different frequencies of total attacks, before progression, had exactly the same long-term outcome (Figure 4.21, Chapter 4). This was even observed when comparing SAP patients versus those with ≥ 7 attacks (Figure 4.22, Chapter 4). There was no indication that, patients with an increasing number of relapses after the first two years accumulated disability more rapidly. Therefore, the frequency of late attacks is not a reliable indicator of the disease evolution .

These results caution again the use of the number of relapses for monitoring the clinical activity, among patients under treatment (Freedman et al., 2004; Rio et al., 2009b; Rio et al., 2011), and challenge the notion that the attacks frequency, during the short observation time of RCTs, is a valid surrogate marker for late disability (Sormani et al., 2011; Wolinsky and Beck, 2011). However, it remains possible that, in a population of treated patients, inflammatory attacks might relate to the long-term disease evolution (Bermel et al., 2013) in a different way, compared to natural history studies. Among 172 patients from the original IFN-b-1a trial (Jacobs et al., 1996b), the number of relapses and of MRI inflammatory lesions was found to predict the disability score after 15 years from randomization. Nevertheless, these results need further validation, especially because the EDSS score was assessed only by using a questionnaire administered to the patients, potentially affecting the conclusions of the study. The comparison between treated and untreated patients was proven to be difficult (Shirani et al., 2012) and the lack of randomization in observational studies does not allow conclusions to be drawn that are easily applicable to clinical trials (Derfuss and Kappos, 2012). The LO cohort was well ascertained and therefore more representative of the whole MS population compared to RCTs groups, which are inevitably subjected to ascertainment bias towards more severe cases.

7.7 Optimizing the therapeutic approach

Currently available DMTs have different efficacy and different safety profiles, therefore optimizing the therapeutic approach is a crucial aspect of the disease management. Patients at major risk of developing severe disability in rapid time should be targeted with more intense therapies. Patients destined to have a less aggressive disease should not be unnecessarily exposed to potentially toxic drugs.

There are two therapeutic strategies for treating MS patients, the escalating and the induction therapy. The escalating therapy is the most commonly used approach. This implies starting patients on first line DMTs (glatiramer acetate and interferons beta),

which are associated with a good safety profile, and, if clinical response is sub-optimal, switching to more powerful second or third line therapies, though at the cost of more severe adverse effects (Rio et al., 2011). The lack of standardized definition of treatment failure makes problematic to decide when to introduce the next level therapeutic option.

In addition, evidence suggests that the window of therapeutic opportunity is restricted to the early phase of the disease and, once the permanent tissue injury is established, the suppression of inflammation does not impact on the disease evolution (Coles et al., 2006). This was initially suggested by the early trials with Alemtuzumab, showing that the marked reduction of the inflammatory activity exerted no beneficial effect on SP patients, but prevented the disease progression among early active patients (Coles et al., 2006; Coles et al., 2008). The concept was reinforced by the results of the phase III trials, demonstrating that the impact of Alemtuzumab vs IFN on the disability accumulation was larger among patients with shorter (2.1 mean years) (Cohen et al., 2012a), compared to those with a longer disease duration (4.5 mean years) (Coles et al., 2012b). Similarly, the treatment with haemapoietic stem cell transplantation had no beneficial effect in progressive patients (Burt et al., 2003), however it improved the neurological disability in RR patients, early in the disease course (5 median year disease duration) (Burt et al., 2009a).

Based on these observations, despite the potential serious side effects, an initial strong immunosuppression, with drugs normally used as second or third line therapies, is indicated in patients with very active and aggressive disease (Edan and Le Page, 2013). The theoretical advantage of the induction therapy is to tackle the disease hard and early in order to achieve a rapid control of the inflammation, before the neurological function is permanently damaged. However, currently there are no precise criteria for identifying patients requiring a more aggressive treatment regime, making difficult to rationalize the use of the induction therapy, which is limited by the safety concerns (Comi, 2008).

The data presented in this thesis strongly indicate that the prevention or the delay of the conversion to SP MS should be considered the major therapeutic target for future treatments. Results lend support to the notion that inflammatory mechanisms responsible for determining the occurrence of the progressive course are already active in the early phase of the disease. In line with the larger effect on the disease progression exerted by Alemtuzumab in patients with very short disease duration, the disconnection between early and later relapses implies that the window of therapeutic opportunity is confined to the very early stage, and narrows with time, along with the increasing disease duration. A permanent irreversible damage probably occurs within a short time from the disease onset, and, past this point, the role of the focal inflammatory activity on the disease evolution becomes less relevant. This implies that the therapeutic suppression of the focal inflammation is likely to exert most of its efficacy in the earliest stage of the disease and justifies the use of aggressive treatments in virtually all patients, especially in those with high early relapse frequency. The evidence indicates that this specific subgroup, with such an exceptionally severe disease course, can be considered a target for induction therapy. It remains unclear if the strong immunosuppression early in the disease course can prevent the occurrence of the progressive course, and only long term follow up studies will provide the answer.

In addition, the analyses highlighted the importance of including age in the therapeutic algorithm. Patients in young age are more likely to respond to current DMTs, in view of their florid inflammatory activity (Figure 5.24, Chapter 5). In contrast, being older at the disease onset and growing older, independently of the length of the disease course, increase consistently the risk of experiencing a progressive course in a short time and, therefore, should be considered a criteria for implementing a more aggressive therapeutic approach. Overall, among RR patients, the age at the disease onset and the early relapse frequency are important information, which are available early in the disease course and can be used for rationalizing the choice of DMTs. The therapeutic strategy during the disease course should be then optimized by taking into account patients' age and the disease duration.

7.8 Final conclusions

Analyses presented in this thesis addressed in details the puzzling and widely variable course of MS. To my knowledge, this was the most extensive assessment of the relationship between relapses and the attainment of unambiguous, hard clinical outcomes, in a well-ascertained MS cohort. The analyses highlighted a true dichotomy between early and late focal inflammatory activity, influencing the disease evolution in an opposite way. As already suggested by imaging and pathology studies, data indicated that the mechanisms underlying the inflammatory attacks gradually disconnect from those responsible for the attainment of late disability. Importantly, results demonstrated that the dissociation between the focal inflammation and the pathological processes, leading to the onset of the progressive phase, occurs much earlier than initially hypothesized. The crucial cascade of events, determining the individual severity of the outcome, probably takes place during the very early phase of the disease. This implies that the early stage of the RR phase is the only plausible window of therapeutic opportunity, and supports the use of aggressive treatments early in the disease course.

In addition, the work presented in this thesis, for the first time, extensively addressed the complex interaction between age, the disease evolution and the clinical phenotypes. Age affects the outcome, by influencing the evolution of the RR phase and by promoting the onset of the progressive course. Furthermore, age related mechanisms probably account for the changes over time in the balance between inflammation, degeneration and their clinical counterparts. The analyses provided a large body of evidence, which indicated that age is not only implicated in the clinical occurrence of the progressive course, but also influence the clinical phenotype before the onset of progression.

Overall, results highlighted the onset of the SP phase as the overwhelming determinant of the long-term disease evolution. Mechanisms tied to the onset of progression, and driving the evolution of the RR phase, dictate the “tempo” of the

disability accumulation and are responsible for the variability of the clinical outcome, among patients. Therefore, the conversion to SP MS is a robust surrogate marker for late disability and its prevention or delay should be the target of future treatments.

The predictive models have successfully identified clinical features, early in the disease course, than can help to select groups at high risk of experiencing a fast disease progression, and therefore requiring a more aggressive therapeutic approach. This has relevant implications for optimizing the patients' therapeutic management and for rationalizing the use of the induction therapy. In addition, this data can be used for improving the methodology of RCTs, testing the efficacy of drugs with potential neuroprotective effect. Results indicated that the number of attacks after the first two years is not a reliable indicator of the disease evolution, cautioning against the use of the frequency of relapses as clinical endpoints. The age at the disease onset, the early relapse number and the length of the disease course should be utilized in the randomization schemes for selecting patients, who can be targeted with more powerful treatments, based on their probability of becoming progressive.

7.9 Appendix: list of publications that have arisen from this thesis

Scalfari A, Degenhardt A, Neuhaus A, Harner N,Seemueller S, Nicholas R, Muraro P, Daumer M, Ebers G
The Role of Relapses Affecting the Long- Term Disability Outcome in the London Ontario Multiple Sclerosis Database
Neuroepidemiology Aug 2009; 33:131–223 SS3/2

A.Scalfari, A.Neuhaus, A.Degenhardt, G.P.Rice, P.A Muraro, M.Daumer and G.C.Ebers
The natural history of multiple sclerosis, a geographically based study 10: relapses and long-term disability.
Brain 2010 133: 1914-1929

A.Scalfari, R. Nicholas, O. Malik and PA Muraro
Escalating Immunotherapies for highly active multiple sclerosis: reviewing the evidence.
CML-Multiple Sclerosis 2010; 2(3):61-73

Ebers GC, Daumer M, Scalfari A
Predicting a therapeutic opportunity in multiple sclerosis
Brain 2010; 133 (Pt 12): e162

Ebers GC, Daumer M, Scalfari A
Surrogate endpoints for EDSS worsening in multiple sclerosis: a meta-analytic approach: measuring disability in relapsing-remitting MS.
Neurology 2011;76 (11): 1025

A. Scalfari, A. Neuhaus, M. Daumer, G.C. Ebers, and P. A. Muraro
Age and disability accumulation in multiple sclerosis
Neurology 2011. 27;77 (13): 1246-52

Ebers GC, Scalfari A, Daumer M, Lederer C.
Combined MRI lesions and relapses as surrogate for disability in MS
Neurology 2012;78 (17): 1367

A.Scalfari, A. Neuhaus, M. Daumer, GC DeLuca, P. A. Muraro G.C. Ebers,
Early relapses, onset of progression and late outcomes in multiple sclerosis
JAMA Neurology 2013 Feb; 70(2):214-22

A.Scalfari, A. Knapperts V, Cutter G, Goodin DS, Ashton R and G.C. Ebers,
Mortality in patients with multiple sclerosis
Neurology 2013 Jul 9; 81 (2): 184-92

A.Scalfari, A. Neuhaus, M. Daumer, P. A. Muraro G.C. Ebers,
Onset of secondary progressive phase and long term evolution of multiple sclerosis
JNNP 2013 Mar 13 [ahead of printing]

References:

1993. 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. 43**,** 655-61.

1998a. Randomised double-blind placebo-controlled study of interferon beta-1a in relapsing/remitting multiple sclerosis. PRISMS (Prevention of Relapses and Disability by Interferon beta-1a Subcutaneously in Multiple Sclerosis) Study Group. Lancet. 352**,** 1498-504.

1998b. Placebo-controlled multicentre randomised trial of interferon beta-1b in treatment of secondary progressive multiple sclerosis. European Study Group on interferon beta-1b in secondary progressive MS. Lancet. 352**,** 1491-7.

2001a. Randomized controlled trial of interferon- beta-1a in secondary progressive MS: Clinical results. Neurology. 56**,** 1496-504.

2001b. PRISMS-4: Long-term efficacy of interferon-beta-1a in relapsing MS. Neurology. 56**,** 1628-36.

2008. Multiple sclerosis risk after optic neuritis: final optic neuritis treatment trial follow-up. Archives of neurology. 65**,** 727-32.

Abbott, R.D., 1985. Logistic regression in survival analysis. American journal of epidemiology. 121**,** 465-71.

Abramsky, O., Teitelbaum, D., Arnon, R., 1977. Effect of a synthetic polypeptide (COP 1) on patients with multiple sclerosis and with acute disseminated encephalomeylitis. Preliminary report. Journal of the neurological sciences. 31**,** 433-8.

Acevedo, A.R., Nava, C., Arriada, N., Violante, A., Corona, T., 2000. Cardiovascular dysfunction in multiple sclerosis. Acta neurologica Scandinavica. 101**,** 85-8.

Acheson, E.D., 1977. Epidemiology of multiple sclerosis. British medical bulletin. 33**,** 9-14.

Achiron, A., Barak, Y., Gail, M., Mandel, M., Pee, D., Ayyagari, R., Rotstein, Z., 2005. Cancer incidence in multiple sclerosis and effects of immunomodulatory treatments. Breast cancer research and treatment. 89**,** 265-70.

Agranoff, B.W., Goldberg, D., 1974. Diet and the geographical distribution of multiple sclerosis. Lancet. 2**,** 1061-6.

Aharoni, R., Eilam, R., Domev, H., Labunskay, G., Sela, M., Arnon, R., 2005. The immunomodulator glatiramer acetate augments the expression of neurotrophic factors in brains of experimental autoimmune encephalomyelitis mice. Proceedings of the National Academy of Sciences of the United States of America. 102**,** 19045-50.

Ahlgren, C., Toren, K., Oden, A., Andersen, O., 2009. A population-based case-control study on viral infections and vaccinations and subsequent multiple sclerosis risk. European journal of epidemiology. 24**,** 541-52.

Albrecht, P., Frohlich, R., Hartung, H.P., Kieseier, B.C., Methner, A., 2007. Optical coherence tomography measures axonal loss in multiple sclerosis independently of optic neuritis. Journal of neurology. 254**,** 1595-6.

Alonso, A., Hernan, M.A., 2008. Temporal trends in the incidence of multiple sclerosis: a systematic review. Neurology. 71**,** 129-35.

Alshubaili, A.F., Alramzy, K., Ayyad, Y.M., Gerish, Y., 2005. Epidemiology of multiple sclerosis in Kuwait: new trends in incidence and prevalence. European neurology. 53**,** 125-31.

Alter, A., Duddy, M., Hebert, S., Biernacki, K., Prat, A., Antel, J.P., Yong, V.W., Nuttall, R.K., Pennington, C.J., Edwards, D.R., Bar-Or, A., 2003. Determinants of human B cell migration across brain endothelial cells. Journal of immunology. 170**,** 4497-505.

Amato, M.P., Groppi, C., Siracusa, G.F., Fratiglioni, L., 1987. Inter and intra-observer reliability in Kurtzke scoring systems in multiple sclerosis. Italian journal of neurological sciences. Suppl 6**,** 129-31.

Amato, M.P., Fratiglioni, L., Groppi, C., Siracusa, G., Amaducci, L., 1988. Interrater reliability in assessing functional systems and disability on the Kurtzke scale in multiple sclerosis. Archives of neurology. 45**,** 746-8.

Amato, M.P., Ponziani, G., Bartolozzi, M.L., Siracusa, G., 1999. A prospective study on the natural history of multiple sclerosis: clues to the conduct and interpretation of clinical trials. Journal of the neurological sciences. 168**,** 96-106.

Amato, M.P., Bartolozzi, M.L., Zipoli, V., Portaccio, E., Mortilla, M., Guidi, L., Siracusa, G., Sorbi, S., Federico, A., De Stefano, N., 2004a. Neocortical volume decrease in relapsing-remitting MS patients with mild cognitive impairment. Neurology. 63**,** 89-93.

Amato, M.P., Grimaud, J., Achiti, I., Bartolozzi, M.L., Adeleine, P., Hartung, H.P., Kappos, L., Thompson, A., Trojano, M., Vukusic, S., Confavreux, C., 2004b. European validation of a standardized clinical description of multiple sclerosis. Journal of neurology. 251**,** 1472-80.

Andersen, O., Elovaara, I., Farkkila, M., Hansen, H.J., Mellgren, S.I., Myhr, K.M., Sandberg-Wollheim, M., Soelberg Sorensen, P., 2004. Multicentre, randomised, double blind, placebo controlled, phase III study of weekly, low dose, subcutaneous interferon beta-1a in secondary progressive multiple sclerosis. Journal of neurology, neurosurgery, and psychiatry. 75**,** 706-10.

Andersson, J.P., 1991. Clinical aspects on Epstein-Barr virus infection. Scandinavian journal of infectious diseases. Supplementum. 80**,** 94-104.

Andersson, P.B., Waubant, E., Gee, L., Goodkin, D.E., 1999. Multiple sclerosis that is progressive from the time of onset: clinical characteristics and progression of disability. Archives of neurology. 56**,** 1138-42.

Annibali, V., Ristori, G., Angelini, D.F., Serafini, B., Mechelli, R., Cannoni, S., Romano, S., Paolillo, A., Abderrahim, H., Diamantini, A., Borsellino, G., Aloisi, F., Battistini, L., Salvetti, M., 2011. CD161(high)CD8+T cells bear pathogenetic potential in multiple sclerosis. Brain : a journal of neurology. 134**,** 542-54.

Antel, J., Antel, S., Caramanos, Z., Arnold, D.L., Kuhlmann, T., 2012. Primary progressive multiple sclerosis: part of the MS disease spectrum or separate disease entity? Acta neuropathologica. 123**,** 627-38.

Archelos, J.J., Storch, M.K., Hartung, H.P., 2000. The role of B cells and autoantibodies in multiple sclerosis. Annals of neurology. 47, 694-706.
Arnson, Y., Amital, H., Shoenfeld, Y., 2007. Vitamin D and autoimmunity: new aetiological and therapeutic considerations. Annals of the rheumatic diseases. 66, 1137-42.
Asadullah, K., Schmid, H., Friedrich, M., Randow, F., Volk, H.D., Sterry, W., Docke, W.D., 1997. Influence of monomethylfumarate on monocytic cytokine formation--explanation for adverse and therapeutic effects in psoriasis? Archives of dermatological research. 289, 623-30.
Ascherio, A., Munch, M., 2000. Epstein-Barr virus and multiple sclerosis. Epidemiology. 11, 220-4.
Ascherio, A., Munger, K.L., Lennette, E.T., Spiegelman, D., Hernan, M.A., Olek, M.J., Hankinson, S.E., Hunter, D.J., 2001. Epstein-Barr virus antibodies and risk of multiple sclerosis: a prospective study. JAMA : the journal of the American Medical Association. 286, 3083-8.
Ascherio, A., Munger, K.L., 2007. Environmental risk factors for multiple sclerosis. Part I: the role of infection. Annals of neurology. 61, 288-99.
Ascherio, A., Bar-Or, A., 2010. EBV and brain matter(s)? Neurology. 74, 1092-5.
Ascherio, A., Munger, K.L., 2010. Epstein-barr virus infection and multiple sclerosis: a review. Journal of neuroimmune pharmacology : the official journal of the Society on NeuroImmune Pharmacology. 5, 271-7.
Ascherio, A., Munger, K.L., Simon, K.C., 2010. Vitamin D and multiple sclerosis. Lancet neurology. 9, 599-612.
Badovinac, V.P., Tvinnereim, A.R., Harty, J.T., 2000. Regulation of antigen-specific CD8+ T cell homeostasis by perforin and interferon-gamma. Science. 290, 1354-8.
Bagnato, F., Jeffries, N., Richert, N.D., Stone, R.D., Ohayon, J.M., McFarland, H.F., Frank, J.A., 2003. Evolution of T1 black holes in patients with multiple sclerosis imaged monthly for 4 years. Brain : a journal of neurology. 126, 1782-9.
Bahmanyar, S., Montgomery, S.M., Hillert, J., Ekbom, A., Olsson, T., 2009. Cancer risk among patients with multiple sclerosis and their parents. Neurology. 72, 1170-7.
Bakshi, R., Benedict, R.H., Bermel, R.A., Jacobs, L., 2001. Regional brain atrophy is associated with physical disability in multiple sclerosis: semiquantitative magnetic resonance imaging and relationship to clinical findings. Journal of neuroimaging : official journal of the American Society of Neuroimaging. 11, 129-36.
Bakshi, R., Thompson, A.J., Rocca, M.A., Pelletier, D., Dousset, V., Barkhof, F., Inglese, M., Guttmann, C.R., Horsfield, M.A., Filippi, M., 2008. MRI in multiple sclerosis: current status and future prospects. Lancet neurology. 7, 615-25.
Balashov, K.E., Olek, M.J., Smith, D.R., Khoury, S.J., Weiner, H.L., 1998. Seasonal variation of interferon-gamma production in progressive multiple sclerosis. Annals of neurology. 44, 824-8.
Baranzini, S.E., Wang, J., Gibson, R.A., Galwey, N., Naegelin, Y., Barkhof, F., Radue, E.W., Lindberg, R.L., Uitdehaag, B.M., Johnson, M.R., Angelakopoulou, A., Hall, L., Richardson, J.C., Prinjha, R.K., Gass, A., Geurts, J.J., Kragt, J., Sombekke, M., Vrenken, H., Qualley, P., Lincoln, R.R., Gomez, R., Caillier,

S.J., George, M.F., Mousavi, H., Guerrero, R., Okuda, D.T., Cree, B.A., Green, A.J., Waubant, E., Goodin, D.S., Pelletier, D., Matthews, P.M., Hauser, S.L., Kappos, L., Polman, C.H., Oksenberg, J.R., 2009. Genome-wide association analysis of susceptibility and clinical phenotype in multiple sclerosis. Human molecular genetics. 18**,** 767-78.

Barcellos, L.F., Oksenberg, J.R., Begovich, A.B., Martin, E.R., Schmidt, S., Vittinghoff, E., Goodin, D.S., Pelletier, D., Lincoln, R.R., Bucher, P., Swerdlin, A., Pericak-Vance, M.A., Haines, J.L., Hauser, S.L., 2003. HLA-DR2 dose effect on susceptibility to multiple sclerosis and influence on disease course. American journal of human genetics. 72**,** 710-6.

Barkhof, F., Filippi, M., Miller, D.H., Scheltens, P., Campi, A., Polman, C.H., Comi, G., Ader, H.J., Losseff, N., Valk, J., 1997. Comparison of MRI criteria at first presentation to predict conversion to clinically definite multiple sclerosis. Brain : a journal of neurology. 120 (Pt 11)**,** 2059-69.

Barkhof, F., van Walderveen, M., 1999. Characterization of tissue damage in multiple sclerosis by nuclear magnetic resonance. Philosophical transactions of the Royal Society of London. Series B, Biological sciences. 354**,** 1675-86.

Barkhof, F., 2002. The clinico-radiological paradox in multiple sclerosis revisited. Current opinion in neurology. 15**,** 239-45.

Barnett, M.H., Williams, D.B., Day, S., Macaskill, P., McLeod, J.G., 2003. Progressive increase in incidence and prevalence of multiple sclerosis in Newcastle, Australia: a 35-year study. Journal of the neurological sciences. 213**,** 1-6.

Barnett, M.H., Prineas, J.W., 2004. Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. Annals of neurology. 55**,** 458-68.

Barnett, M.H., Parratt, J.D., Cho, E.S., Prineas, J.W., 2009. Immunoglobulins and complement in postmortem multiple sclerosis tissue. Annals of neurology. 65**,** 32-46.

Beck, R.W., Cleary, P.A., 1993. Optic neuritis treatment trial. One-year follow-up results. Archives of ophthalmology. 111**,** 773-5.

Begolka, W.S., Miller, S.D., 1998. Cytokines as intrinsic and exogenous regulators of pathogenesis in experimental autoimmune encephalomyelitis. Research in immunology. 149**,** 771-81; discussion 843-4, 855-60.

Bejaoui, K., Rolak, L.A., 2010. What is the risk of permanent disability from a multiple sclerosis relapse? Neurology. 74**,** 900-2.

Bentzen, J., Flachs, E.M., Stenager, E., Bronnum-Hansen, H., Koch-Henriksen, N., 2010. Prevalence of multiple sclerosis in Denmark 1950--2005. Multiple sclerosis. 16**,** 520-5.

Bergamaschi, L., Leone, M.A., Fasano, M.E., Guerini, F.R., Ferrante, D., Bolognesi, E., Barizzone, N., Corrado, L., Naldi, P., Agliardi, C., Dametto, E., Salvetti, M., Visconti, A., Galimberti, D., Scarpini, E., Vercellino, M., Bergamaschi, R., Monaco, F., Caputo, D., Momigliano-Richiardi, P., D'Alfonso, S., 2010. HLA-class I markers and multiple sclerosis susceptibility in the Italian population. Genes and immunity. 11**,** 173-80.

Bergamaschi, R., Berzuini, C., Romani, A., Cosi, V., 2001. Predicting secondary progression in relapsing-remitting multiple sclerosis: a Bayesian analysis. Journal of the neurological sciences. 189**,** 13-21.

Berger, T., Rubner, P., Schautzer, F., Egg, R., Ulmer, H., Mayringer, I., Dilitz, E., Deisenhammer, F., Reindl, M., 2003. Antimyelin antibodies as a predictor

of clinically definite multiple sclerosis after a first demyelinating event. The New England journal of medicine. 349, 139-45.
Bermel, R.A., Weinstock-Guttman, B., Bourdette, D., Foulds, P., You, X., Rudick, R.A., 2010. Intramuscular interferon beta-1a therapy in patients with relapsing-remitting multiple sclerosis: a 15-year follow-up study. Multiple sclerosis. 16, 588-96.
Bermel, R.A., You, X., Foulds, P., Hyde, R., Simon, J.H., Fisher, E., Rudick, R.A., 2013. Predictors of long-term outcome in multiple sclerosis patients treated with interferon beta. Annals of neurology. 73, 95-103.
Bethoux, F., Miller, D.M., Kinkel, R.P., 2001. Recovery following acute exacerbations of multiple sclerosis: from impairment to quality of life. Multiple sclerosis. 7, 137-42.
Bhigjee, A.I., Moodley, K., Ramkissoon, K., 2007. Multiple sclerosis in KwaZulu Natal, South Africa: an epidemiological and clinical study. Multiple sclerosis. 13, 1095-9.
Bielekova, B., Sung, M.H., Kadom, N., Simon, R., McFarland, H., Martin, R., 2004. Expansion and functional relevance of high-avidity myelin-specific CD4+ T cells in multiple sclerosis. Journal of immunology. 172, 3893-904.
Bitsch, A., Schuchardt, J., Bunkowski, S., Kuhlmann, T., Bruck, W., 2000. Acute axonal injury in multiple sclerosis. Correlation with demyelination and inflammation. Brain : a journal of neurology. 123 (Pt 6), 1174-83.
Bitsch, A., Kuhlmann, T., Stadelmann, C., Lassmann, H., Lucchinetti, C., Bruck, W., 2001. A longitudinal MRI study of histopathologically defined hypointense multiple sclerosis lesions. Annals of neurology. 49, 793-6.
Bjartmar, C., Kidd, G., Mork, S., Rudick, R., Trapp, B.D., 2000. Neurological disability correlates with spinal cord axonal loss and reduced N-acetyl aspartate in chronic multiple sclerosis patients. Annals of neurology. 48, 893-901.
Bjartmar, C., Kinkel, R.P., Kidd, G., Rudick, R.A., Trapp, B.D., 2001. Axonal loss in normal-appearing white matter in a patient with acute MS. Neurology. 57, 1248-52.
Bjartmar, C., Trapp, B.D., 2001. Axonal and neuronal degeneration in multiple sclerosis: mechanisms and functional consequences. Current opinion in neurology. 14, 271-8.
Blanchette, F., Neuhaus, O., 2008. Glatiramer acetate: evidence for a dual mechanism of action. Journal of neurology. 255 Suppl 1, 26-36.
Bo, L., Vedeler, C.A., Nyland, H.I., Trapp, B.D., Mork, S.J., 2003. Subpial demyelination in the cerebral cortex of multiple sclerosis patients. Journal of neuropathology and experimental neurology. 62, 723-32.
Bo, L., Geurts, J.J., van der Valk, P., Polman, C., Barkhof, F., 2007. Lack of correlation between cortical demyelination and white matter pathologic changes in multiple sclerosis. Archives of neurology. 64, 76-80.
Bock, M., Brandt, A.U., Dorr, J., Kraft, H., Weinges-Evers, N., Gaede, G., Pfueller, C.F., Herges, K., Radbruch, H., Ohlraun, S., Bellmann-Strobl, J., Kuchenbecker, J., Zipp, F., Paul, F., 2010. Patterns of retinal nerve fiber layer loss in multiple sclerosis patients with or without optic neuritis and glaucoma patients. Clinical neurology and neurosurgery. 112, 647-52.
Bonduelle, M., 1967. [The mild forms of multiple sclerosis]. La Presse medicale. 75, 2023-6.

Bornstein, M.B., Miller, A., Slagle, S., Weitzman, M., Drexler, E., Keilson, M., Spada, V., Weiss, W., Appel, S., Rolak, L., et al., 1991. A placebo-controlled, double-blind, randomized, two-center, pilot trial of Cop 1 in chronic progressive multiple sclerosis. Neurology. 41, 533-9.

Bosca, I., Coret, F., Valero, C., Pascual, A.M., Magraner, M.J., Landete, L., Casanova, B., 2008. Effect of relapses over early progression of disability in multiple sclerosis patients treated with beta-interferon. Multiple sclerosis. 14, 636-9.

Bradburn, M.J., Clark, T.G., Love, S.B., Altman, D.G., 2003a. Survival analysis part II: multivariate data analysis--an introduction to concepts and methods. British journal of cancer. 89, 431-6.

Bradburn, M.J., Clark, T.G., Love, S.B., Altman, D.G., 2003b. Survival analysis Part III: multivariate data analysis -- choosing a model and assessing its adequacy and fit. British journal of cancer. 89, 605-11.

Brady, S.T., Witt, A.S., Kirkpatrick, L.L., de Waegh, S.M., Readhead, C., Tu, P.H., Lee, V.M., 1999. Formation of compact myelin is required for maturation of the axonal cytoskeleton. The Journal of neuroscience : the official journal of the Society for Neuroscience. 19, 7278-88.

Brain, W., 1930. Critical review: disseminated sclerosis QJ Med 23, 343-91.

Brain, W.R., 1935. Disseminated Sclerosis. British medical journal. 2, 1006.

Bramow, S., Frischer, J.M., Lassmann, H., Koch-Henriksen, N., Lucchinetti, C.F., Sorensen, P.S., Laursen, H., 2010. Demyelination versus remyelination in progressive multiple sclerosis. Brain : a journal of neurology. 133, 2983-98.

Brannon, P.M., Yetley, E.A., Bailey, R.L., Picciano, M.F., 2008. Overview of the conference "Vitamin D and Health in the 21st Century: an Update". The American journal of clinical nutrition. 88, 483S-490S.

Brex, P.A., Leary, S.M., O'Riordan, J.I., Miszkiel, K.A., Plant, G.T., Thompson, A.J., Miller, D.H., 2001. Measurement of spinal cord area in clinically isolated syndromes suggestive of multiple sclerosis. Journal of neurology, neurosurgery, and psychiatry. 70, 544-7.

Brex, P.A., Ciccarelli, O., O'Riordan, J.I., Sailer, M., Thompson, A.J., Miller, D.H., 2002. A longitudinal study of abnormalities on MRI and disability from multiple sclerosis. The New England journal of medicine. 346, 158-64.

Broman, T., Andersen, O., Bergmann, L., 1981. Clinical studies on multiple sclerosis. I. Presentation of an incidence material from Gothenburg. Acta neurologica Scandinavica. 63, 6-33.

Bronnum-Hansen, H., Koch-Henriksen, N., Hyllested, K., 1994. Survival of patients with multiple sclerosis in Denmark: a nationwide, long-term epidemiologic survey. Neurology. 44, 1901-7.

Bronnum-Hansen, H., Koch-Henriksen, N., Stenager, E., 2004. Trends in survival and cause of death in Danish patients with multiple sclerosis. Brain : a journal of neurology. 127, 844-50.

Bronnum-Hansen, H., Stenager, E., Nylev Stenager, E., Koch-Henriksen, N., 2005. Suicide among Danes with multiple sclerosis. Journal of neurology, neurosurgery, and psychiatry. 76, 1457-9.

Brownell, B., Hughes, J.T., 1962. The distribution of plaques in the cerebrum in multiple sclerosis. Journal of neurology, neurosurgery, and psychiatry. 25, 315-20.

Bruck, W., Porada, P., Poser, S., Rieckmann, P., Hanefeld, F., Kretzschmar, H.A., Lassmann, H., 1995. Monocyte/macrophage differentiation in early multiple sclerosis lesions. Annals of neurology. 38**,** 788-96.

Bruck, W., Bitsch, A., Kolenda, H., Bruck, Y., Stiefel, M., Lassmann, H., 1997. Inflammatory central nervous system demyelination: correlation of magnetic resonance imaging findings with lesion pathology. Annals of neurology. 42**,** 783-93.

Bruck, W., Wegner, C., 2011. Insight into the mechanism of laquinimod action. Journal of the neurological sciences. 306**,** 173-9.

Burns, J., Rosenzweig, A., Zweiman, B., Lisak, R.P., 1983. Isolation of myelin basic protein-reactive T-cell lines from normal human blood. Cellular immunology. 81**,** 435-40.

Burrell, A.M., Handel, A.E., Ramagopalan, S.V., Ebers, G.C., Morahan, J.M., 2011. Epigenetic mechanisms in multiple sclerosis and the major histocompatibility complex (MHC). Discovery medicine. 11**,** 187-96.

Burt, R.K., Cohen, B.A., Russell, E., Spero, K., Joshi, A., Oyama, Y., Karpus, W.J., Luo, K., Jovanovic, B., Traynor, A., Karlin, K., Stefoski, D., Burns, W.H., 2003. Hematopoietic stem cell transplantation for progressive multiple sclerosis: failure of a total body irradiation-based conditioning regimen to prevent disease progression in patients with high disability scores. Blood. 102**,** 2373-8.

Burt, R.K., Loh, Y., Cohen, B., Stefoski, D., Balabanov, R., Katsamakis, G., Oyama, Y., Russell, E.J., Stern, J., Muraro, P., Rose, J., Testori, A., Bucha, J., Jovanovic, B., Milanetti, F., Storek, J., Voltarelli, J.C., Burns, W.H., 2009a. Autologous non-myeloablative haemopoietic stem cell transplantation in relapsing-remitting multiple sclerosis: a phase I/II study. Lancet neurology. 8**,** 244-53.

Burt, R.K., Loh, Y., Cohen, B., Stefoski, D., Balabanov, R., Katsamakis, G., Oyama, Y., Russell, E.J., Stern, J., Muraro, P., Rose, J., Testori, A., Bucha, J., Jovanovic, B., Milanetti, F., Storek, J., Voltarelli, J.C., Burns, W.H., 2009b. Autologous non-myeloablative haemopoietic stem cell transplantation in relapsing-remitting multiple sclerosis: a phase I/II study. Lancet Neurol. 8**,** 244-53.

Cabarrocas, J., Bauer, J., Piaggio, E., Liblau, R., Lassmann, H., 2003. Effective and selective immune surveillance of the brain by MHC class I-restricted cytotoxic T lymphocytes. European journal of immunology. 33**,** 1174-82.

Cadavid, D., Wolansky, L.J., Skurnick, J., Lincoln, J., Cheriyan, J., Szczepanowski, K., Kamin, S.S., Pachner, A.R., Halper, J., Cook, S.D., 2009. Efficacy of treatment of MS with IFNbeta-1b or glatiramer acetate by monthly brain MRI in the BECOME study. Neurology. 72**,** 1976-83.

Caillier, S.J., Briggs, F., Cree, B.A., Baranzini, S.E., Fernandez-Vina, M., Ramsay, P.P., Khan, O., Royal, W., 3rd, Hauser, S.L., Barcellos, L.F., Oksenberg, J.R., 2008. Uncoupling the roles of HLA-DRB1 and HLA-DRB5 genes in multiple sclerosis. Journal of immunology. 181**,** 5473-80.

Calabrese, M., Atzori, M., Bernardi, V., Morra, A., Romualdi, C., Rinaldi, L., McAuliffe, M.J., Barachino, L., Perini, P., Fischl, B., Battistin, L., Gallo, P., 2007a. Cortical atrophy is relevant in multiple sclerosis at clinical onset. Journal of neurology. 254**,** 1212-20.

Calabrese, M., De Stefano, N., Atzori, M., Bernardi, V., Mattisi, I., Barachino, L., Morra, A., Rinaldi, L., Romualdi, C., Perini, P., Battistin, L., Gallo, P., 2007b.

Detection of cortical inflammatory lesions by double inversion recovery magnetic resonance imaging in patients with multiple sclerosis. Archives of neurology. 64**,** 1416-22.

Calabrese, M., Agosta, F., Rinaldi, F., Mattisi, I., Grossi, P., Favaretto, A., Atzori, M., Bernardi, V., Barachino, L., Rinaldi, L., Perini, P., Gallo, P., Filippi, M., 2009a. Cortical lesions and atrophy associated with cognitive impairment in relapsing-remitting multiple sclerosis. Archives of neurology. 66**,** 1144-50.

Calabrese, M., Filippi, M., Rovaris, M., Bernardi, V., Atzori, M., Mattisi, I., Favaretto, A., Grossi, P., Barachino, L., Rinaldi, L., Romualdi, C., Perini, P., Gallo, P., 2009b. Evidence for relative cortical sparing in benign multiple sclerosis: a longitudinal magnetic resonance imaging study. Multiple sclerosis. 15**,** 36-41.

Calabrese, M., Gallo, P., 2009. Magnetic resonance evidence of cortical onset of multiple sclerosis. Multiple sclerosis. 15**,** 933-41.

Calabrese, M., Rocca, M.A., Atzori, M., Mattisi, I., Favaretto, A., Perini, P., Gallo, P., Filippi, M., 2010. A 3-year magnetic resonance imaging study of cortical lesions in relapse-onset multiple sclerosis. Annals of neurology. 67**,** 376-83.

Calabrese, M., Poretto, V., Favaretto, A., Alessio, S., Bernardi, V., Romualdi, C., Rinaldi, F., Perini, P., Gallo, P., 2012. Cortical lesion load associates with progression of disability in multiple sclerosis. Brain : a journal of neurology. 135**,** 2952-61.

Calabrese, M., Romualdi, C., Poretto, V., Favaretto, A., Morra, A., Rinaldi, F., Perini, P., Gallo, P., 2013. The changing clinical course of multiple sclerosis: A matter of grey matter. Annals of neurology.

Cantorna, M.T., Hayes, C.E., DeLuca, H.F., 1996. 1,25-Dihydroxyvitamin D3 reversibly blocks the progression of relapsing encephalomyelitis, a model of multiple sclerosis. Proceedings of the National Academy of Sciences of the United States of America. 93**,** 7861-4.

Carter, S., Sciarra, D., Merritt, H.H., 1950. The course of multiple sclerosis as determined by autopsy proven cases. Research publications - Association for Research in Nervous and Mental Disease. 28**,** 471-511.

Celius, E.G., Vandvik, B., 2001. Multiple sclerosis in Oslo, Norway: prevalence on 1 January 1995 and incidence over a 25-year period. European journal of neurology : the official journal of the European Federation of Neurological Societies. 8**,** 463-9.

Celius, E.G., Smestad, C., 2009. Change in sex ratio, disease course and age at diagnosis in Oslo MS patients through seven decades. Acta neurologica Scandinavica. Supplementum. 27-9.

Chan, A., Weilbach, F.X., Toyka, K.V., Gold, R., 2005. Mitoxantrone induces cell death in peripheral blood leucocytes of multiple sclerosis patients. Clin Exp Immunol. 139**,** 152-8.

Chao, M.J., Ramagopalan, S.V., Herrera, B.M., Lincoln, M.R., Dyment, D.A., Sadovnick, A.D., Ebers, G.C., 2009. Epigenetics in multiple sclerosis susceptibility: difference in transgenerational risk localizes to the major histocompatibility complex. Human molecular genetics. 18**,** 261-6.

Charcot, J., 1880. Lecons sur les maladies du systeme nerveux faites a la Salpetriere. Bureau du Progres Medical.

Chard, D.T., Griffin, C.M., Parker, G.J., Kapoor, R., Thompson, A.J., Miller, D.H., 2002. Brain atrophy in clinically early relapsing-remitting multiple sclerosis. Brain : a journal of neurology. 125**,** 327-37.

Chaudhuri, A., Behan, P.O., 2004. Multiple sclerosis is not an autoimmune disease. Archives of neurology. 61**,** 1610-2.

Chen, S., Sims, G.P., Chen, X.X., Gu, Y.Y., Lipsky, P.E., 2007. Modulatory effects of 1,25-dihydroxyvitamin D3 on human B cell differentiation. Journal of immunology. 179**,** 1634-47.

Cheng, Q., Miao, L., Zhang, J., Ding, S.J., Liu, Z.G., Wang, X., Sun, X.J., Zhao, Z.X., Song, Y.J., Ding, X.Y., Guo, Z.L., Yang, Y., Chen, S.D., Jiang, G.X., Fredrikson, S., 2007. A population-based survey of multiple sclerosis in Shanghai, China. Neurology. 68**,** 1495-500.

Chiu, A.W., Richert, N., Ehrmantraut, M., Ohayon, J., Gupta, S., Bomboi, G., Gaindh, D., Cantor, F.K., Frank, J.A., McFarland, H.F., Bagnato, F., 2009. Heterogeneity in response to interferon beta in patients with multiple sclerosis: a 3-year monthly imaging study. Archives of neurology. 66**,** 39-43.

Choi, S.R., Howell, O.W., Carassiti, D., Magliozzi, R., Gveric, D., Muraro, P.A., Nicholas, R., Roncaroli, F., Reynolds, R., 2012. Meningeal inflammation plays a role in the pathology of primary progressive multiple sclerosis. Brain : a journal of neurology. 135**,** 2925-37.

Choi, S.S., Zawadzki, R.J., Keltner, J.L., Werner, J.S., 2008. Changes in cellular structures revealed by ultra-high resolution retinal imaging in optic neuropathies. Investigative ophthalmology & visual science. 49**,** 2103-19.

Chu, C.Q., Wittmer, S., Dalton, D.K., 2000. Failure to suppress the expansion of the activated CD4 T cell population in interferon gamma-deficient mice leads to exacerbation of experimental autoimmune encephalomyelitis. The Journal of experimental medicine. 192**,** 123-8.

Clark, T.G., Bradburn, M.J., Love, S.B., Altman, D.G., 2003. Survival analysis part I: basic concepts and first analyses. British journal of cancer. 89**,** 232-8.

Clark, V.A., Detels, R., Visscher, B.R., Valdiviezo, N.L., Malmgren, R.M., Dudley, J.P., 1982. Factors associated with a malignant or benign course of multiple sclerosis. JAMA : the journal of the American Medical Association. 248**,** 856-60.

Claussen, M.C., Korn, T., 2012. Immune mechanisms of new therapeutic strategies in MS: teriflunomide. Clinical immunology. 142**,** 49-56.

Cohen, B.A., Khan, O., Jeffery, D.R., Bashir, K., Rizvi, S.A., Fox, E.J., Agius, M., Bashir, R., Collins, T.E., Herndon, R., Kinkel, P., Mikol, D.D., Picone, M.A., Rivera, V., Tornatore, C., Zwibel, H., 2004. Identifying and treating patients with suboptimal responses. Neurology. 63**,** S33-40.

Cohen, J.A., Fischer, J.S., Bolibrush, D.M., Jak, A.J., Kniker, J.E., Mertz, L.A., Skaramagas, T.T., Cutter, G.R., 2000. Intrarater and interrater reliability of the MS functional composite outcome measure. Neurology. 54**,** 802-6.

Cohen, J.A., Cutter, G.R., Fischer, J.S., Goodman, A.D., Heidenreich, F.R., Jak, A.J., Kniker, J.E., Kooijmans, M.F., Lull, J.M., Sandrock, A.W., Simon, J.H., Simonian, N.A., Whitaker, J.N., 2001. Use of the multiple sclerosis functional composite as an outcome measure in a phase 3 clinical trial. Archives of neurology. 58**,** 961-7.

Cohen, J.A., Cutter, G.R., Fischer, J.S., Goodman, A.D., Heidenreich, F.R., Kooijmans, M.F., Sandrock, A.W., Rudick, R.A., Simon, J.H., Simonian, N.A., Tsao, E.C., Whitaker, J.N., 2002. Benefit of interferon beta-1a on MSFC progression in secondary progressive MS. Neurology. 59**,** 679-87.

Cohen, J.A., Barkhof, F., Comi, G., Hartung, H.P., Khatri, B.O., Montalban, X., Pelletier, J., Capra, R., Gallo, P., Izquierdo, G., Tiel-Wilck, K., de Vera, A., Jin, J., Stites, T., Wu, S., Aradhye, S., Kappos, L., 2010. Oral fingolimod or intramuscular interferon for relapsing multiple sclerosis. The New England journal of medicine. 362**,** 402-15.

Cohen, J.A., Coles, A.J., Arnold, D.L., Confavreux, C., Fox, E.J., Hartung, H.P., Havrdova, E., Selmaj, K.W., Weiner, H.L., Fisher, E., Brinar, V.V., Giovannoni, G., Stojanovic, M., Ertik, B.I., Lake, S.L., Margolin, D.H., Panzara, M.A., Compston, D.A., 2012a. Alemtuzumab versus interferon beta 1a as first-line treatment for patients with relapsing-remitting multiple sclerosis: a randomised controlled phase 3 trial. Lancet. 380**,** 1819-28.

Cohen, J.A., Reingold, S.C., Polman, C.H., Wolinsky, J.S., 2012b. Disability outcome measures in multiple sclerosis clinical trials: current status and future prospects. Lancet neurology. 11**,** 467-76.

Coles, A.J., Wing, M.G., Molyneux, P., Paolillo, A., Davie, C.M., Hale, G., Miller, D., Waldmann, H., Compston, A., 1999. Monoclonal antibody treatment exposes three mechanisms underlying the clinical course of multiple sclerosis. Annals of neurology. 46**,** 296-304.

Coles, A.J., Cox, A., Le Page, E., Jones, J., Trip, S.A., Deans, J., Seaman, S., Miller, D.H., Hale, G., Waldmann, H., Compston, D.A., 2006. The window of therapeutic opportunity in multiple sclerosis: evidence from monoclonal antibody therapy. Journal of neurology. 253**,** 98-108.

Coles, A.J., Compston, D.A., Selmaj, K.W., Lake, S.L., Moran, S., Margolin, D.H., Norris, K., Tandon, P.K., 2008. Alemtuzumab vs. interferon beta-1a in early multiple sclerosis. The New England journal of medicine. 359**,** 1786-801.

Coles, A.J., Fox, E., Vladic, A., Gazda, S.K., Brinar, V., Selmaj, K.W., Skoromets, A., Stolyarov, I., Bass, A., Sullivan, H., Margolin, D.H., Lake, S.L., Moran, S., Palmer, J., Smith, M.S., Compston, D.A., 2012a. Alemtuzumab more effective than interferon beta-1a at 5-year follow-up of CAMMS223 clinical trial. Neurology. 78**,** 1069-78.

Coles, A.J., Twyman, C.L., Arnold, D.L., Cohen, J.A., Confavreux, C., Fox, E.J., Hartung, H.P., Havrdova, E., Selmaj, K.W., Weiner, H.L., Miller, T., Fisher, E., Sandbrink, R., Lake, S.L., Margolin, D.H., Oyuela, P., Panzara, M.A., Compston, D.A., 2012b. Alemtuzumab for patients with relapsing multiple sclerosis after disease-modifying therapy: a randomised controlled phase 3 trial. Lancet. 380**,** 1829-39.

Comi, G., Filippi, M., Barkhof, F., Durelli, L., Edan, G., Fernandez, O., Hartung, H., Seeldrayers, P., Sorensen, P.S., Rovaris, M., Martinelli, V., Hommes, O.R., 2001a. Effect of early interferon treatment on conversion to definite multiple sclerosis: a randomised study. Lancet. 357**,** 1576-82.

Comi, G., Filippi, M., Wolinsky, J.S., 2001b. 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. Annals of neurology. 49, 290-7.
Comi, G., 2008. Induction vs. escalating therapy in multiple sclerosis: practical implications. Neurological sciences : official journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology. 29 Suppl 2, S253-5.
Comi, G., Martinelli, V., Rodegher, M., Moiola, L., Bajenaru, O., Carra, A., Elovaara, I., Fazekas, F., Hartung, H.P., Hillert, J., King, J., Komoly, S., Lubetzki, C., Montalban, X., Myhr, K.M., Ravnborg, M., Rieckmann, P., Wynn, D., Young, C., Filippi, M., 2009. 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. 374, 1503-11.
Comi, G., Jeffery, D., Kappos, L., Montalban, X., Boyko, A., Rocca, M.A., Filippi, M., 2012. Placebo-controlled trial of oral laquinimod for multiple sclerosis. The New England journal of medicine. 366, 1000-9.
Compston, A., Coles, A., 2002. Multiple sclerosis. Lancet. 359, 1221-31.
Compston, A., 2005. McAlpine's multiple sclerosis, Vol., Churchill Livingstone Elsevier, Philadelphia.
Compston, A., Coles, A., 2008. Multiple sclerosis. Lancet. 372, 1502-17.
Confavreux, C., Aimard, G., Devic, M., 1980. Course and prognosis of multiple sclerosis assessed by the computerized data processing of 349 patients. Brain : a journal of neurology. 103, 281-300.
Confavreux, C., Compston, D.A., Hommes, O.R., McDonald, W.I., Thompson, A.J., 1992a. EDMUS, a European database for multiple sclerosis. Journal of neurology, neurosurgery, and psychiatry. 55, 671-6.
Confavreux, C., Compston, D.A., Hommes, O.R., McDonald, W.I., Thompson, A.J., 1992b. EDMUS, a European database for multiple sclerosis. J Neurol Neurosurg Psychiatry. 55, 671-6.
Confavreux, C., Vukusic, S., Moreau, T., Adeleine, P., 2000. Relapses and progression of disability in multiple sclerosis. The New England journal of medicine. 343, 1430-8.
Confavreux, C., Vukusic, S., Adeleine, P., 2003. Early clinical predictors and progression of irreversible disability in multiple sclerosis: an amnesic process. Brain : a journal of neurology. 126, 770-82.
Confavreux, C., Vukusic, S., 2006a. Natural history of multiple sclerosis: a unifying concept. Brain : a journal of neurology. 129, 606-16.
Confavreux, C., Vukusic, S., 2006b. Age at disability milestones in multiple sclerosis. Brain : a journal of neurology. 129, 595-605.
Conway, D., Cohen, J.A., 2010. Emerging oral therapies in multiple sclerosis. Current neurology and neuroscience reports. 10, 381-8.
Coppola, G., Lanzillo, R., Florio, C., Orefice, G., Vivo, P., Ascione, S., Schiavone, V., Pagano, A., Vacca, G., De Michele, G., Morra, V.B., 2006. Long-term clinical experience with weekly interferon beta-1a in relapsing multiple sclerosis. European journal of neurology : the official journal of the European Federation of Neurological Societies. 13, 1014-21.
Costello, F., Coupland, S., Hodge, W., Lorello, G.R., Koroluk, J., Pan, Y.I., Freedman, M.S., Zackon, D.H., Kardon, R.H., 2006. Quantifying axonal loss after optic

neuritis with optical coherence tomography. Annals of neurology. 59, 963-9.

Cottrell, D.A., Kremenchutzky, M., Rice, G.P., Hader, W., Baskerville, J., Ebers, G.C., 1999a. The natural history of multiple sclerosis: a geographically based study. 6. Applications to planning and interpretation of clinical therapeutic trials in primary progressive multiple sclerosis. Brain : a journal of neurology. 122 (Pt 4), 641-7.

Cottrell, D.A., Kremenchutzky, M., Rice, G.P., Koopman, W.J., Hader, W., Baskerville, J., Ebers, G.C., 1999b. The natural history of multiple sclerosis: a geographically based study. 5. The clinical features and natural history of primary progressive multiple sclerosis. Brain : a journal of neurology. 122 (Pt 4), 625-39.

Cournu-Rebeix, I., Genin, E., Leray, E., Babron, M.C., Cohen, J., Gout, C., Alizadeh, M., Perdry, H., Semana, G., Brassat, D., Clerget-Darpoux, F., Yaouanq, J., Edan, G., Rosenheim, M., Fontaine, B., 2008. HLA-DRB1*15 allele influences the later course of relapsing remitting multiple sclerosis. Genes and immunity. 9, 570-4.

Craner, M.J., Fugger, L., 2011. Axonal injury in reverse. Nature medicine. 17, 423-6.

Cree, B.A., Rioux, J.D., McCauley, J.L., Gourraud, P.A., Goyette, P., McElroy, J., De Jager, P., Santaniello, A., Vyse, T.J., Gregersen, P.K., Mirel, D., Hafler, D.A., Haines, J.L., Pericak-Vance, M.A., Compston, A., Sawcer, S.J., Oksenberg, J.R., Hauser, S.L., 2010. A major histocompatibility Class I locus contributes to multiple sclerosis susceptibility independently from HLA-DRB1*15:01. PloS one. 5, e11296.

Cutter, G.R., Baier, M.L., Rudick, R.A., Cookfair, D.L., Fischer, J.S., Petkau, J., Syndulko, K., Weinshenker, B.G., Antel, J.P., Confavreux, C., Ellison, G.W., Lublin, F., Miller, A.E., Rao, S.M., Reingold, S., Thompson, A., Willoughby, E., 1999. Development of a multiple sclerosis functional composite as a clinical trial outcome measure. Brain : a journal of neurology. 122 (Pt 5), 871-82.

Dalton, C.M., Brex, P.A., Miszkiel, K.A., Hickman, S.J., MacManus, D.G., Plant, G.T., Thompson, A.J., Miller, D.H., 2002. Application of the new McDonald criteria to patients with clinically isolated syndromes suggestive of multiple sclerosis. Annals of neurology. 52, 47-53.

Daumer, M., Neuhaus, A., Morrissey, S., Hintzen, R., Ebers, G.C., 2009. MRI as an outcome in multiple sclerosis clinical trials. Neurology. 72, 705-11.

De Castro, S., Cartoni, D., Millefiorini, E., Funaro, S., Gasperini, C., Morino, S., Tallarico, D., Beni, S., 1995. Noninvasive assessment of mitoxantrone cardiotoxicity in relapsing remitting multiple sclerosis. J Clin Pharmacol. 35, 627-32.

De Jager, P.L., Simon, K.C., Munger, K.L., Rioux, J.D., Hafler, D.A., Ascherio, A., 2008. Integrating risk factors: HLA-DRB1*1501 and Epstein-Barr virus in multiple sclerosis. Neurology. 70, 1113-8.

De Stefano, N., Matthews, P.M., Antel, J.P., Preul, M., Francis, G., Arnold, D.L., 1995. Chemical pathology of acute demyelinating lesions and its correlation with disability. Annals of neurology. 38, 901-9.

De Stefano, N., Matthews, P.M., Filippi, M., Agosta, F., De Luca, M., Bartolozzi, M.L., Guidi, L., Ghezzi, A., Montanari, E., Cifelli, A., Federico, A., Smith, S.M., 2003.

Evidence of early cortical atrophy in MS: relevance to white matter changes and disability. Neurology. 60, 1157-62.
Dean, G., Kurtzke, J.F., 1970. A critical age for the acquisition of multiple sclerosis. Transactions of the American Neurological Association. 95, 232-3.
Debouverie, M., Pittion-Vouyovitch, S., Louis, S., Roederer, T., Guillemin, F., 2007. Increasing incidence of multiple sclerosis among women in Lorraine, Eastern France. Multiple sclerosis. 13, 962-7.
Debouverie, M., Pittion-Vouyovitch, S., Louis, S., Guillemin, F., 2008. Natural history of multiple sclerosis in a population-based cohort. European journal of neurology : the official journal of the European Federation of Neurological Societies. 15, 916-21.
Degenhardt, A., Ramagopalan, S.V., Scalfari, A., Ebers, G.C., 2009. Clinical prognostic factors in multiple sclerosis: a natural history review. Nature reviews. Neurology. 5, 672-82.
DeLorenze, G.N., Munger, K.L., Lennette, E.T., Orentreich, N., Vogelman, J.H., Ascherio, A., 2006. Epstein-Barr virus and multiple sclerosis: evidence of association from a prospective study with long-term follow-up. Archives of neurology. 63, 839-44.
DeLuca, G.C., Williams, K., Evangelou, N., Ebers, G.C., Esiri, M.M., 2006. The contribution of demyelination to axonal loss in multiple sclerosis. Brain : a journal of neurology. 129, 1507-16.
DeLuca, G.C., Ramagopalan, S.V., Herrera, B.M., Dyment, D.A., Lincoln, M.R., Montpetit, A., Pugliatti, M., Barnardo, M.C., Risch, N.J., Sadovnick, A.D., Chao, M., Sotgiu, S., Hudson, T.J., Ebers, G.C., 2007. An extremes of outcome strategy provides evidence that multiple sclerosis severity is determined by alleles at the HLA-DRB1 locus. Proceedings of the National Academy of Sciences of the United States of America. 104, 20896-901.
Deluca, G.C., Alterman, R., Martin, J.L., Mittal, A., Blundell, S., Bird, S., Beale, H., Hong, L.S., Esiri, M.M., 2013. Casting light on multiple sclerosis heterogeneity: the role of HLA-DRB1 on spinal cord pathology. Brain : a journal of neurology. 136, 1025-34.
Deluca, H.F., Cantorna, M.T., 2001. Vitamin D: its role and uses in immunology. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 15, 2579-85.
Derfuss, T., Kappos, L., 2012. Evaluating the potential benefit of interferon treatment in multiple sclerosis. JAMA : the journal of the American Medical Association. 308, 290-1.
Detels, R., Clark, V.A., Valdiviezo, N.L., Visscher, B.R., Malmgren, R.M., Dudley, J.P., 1982. Factors associated with a rapid course of multiple sclerosis. Archives of neurology. 39, 337-41.
Dimick, J.B., Livingston, E.H., 2010. Comparing treatments using observational study designs: what can we do about selection bias? Archives of surgery. 145, 927.
Disanto, G., Pakpoor, J., Morahan, J.M., Hall, C., Meier, U.C., Giovannoni, G., Ramagopalan, S.V., 2012. Epstein-Barr virus, latitude and multiple sclerosis. Multiple sclerosis.
Durelli, L., Verdun, E., Barbero, P., Bergui, M., Versino, E., Ghezzi, A., Montanari, E., Zaffaroni, M., 2002. Every-other-day interferon beta-1b versus once-weekly interferon beta-1a for multiple sclerosis: results of a 2-year

prospective randomised multicentre study (INCOMIN). Lancet. 359, 1453-60.
Dutta, R., McDonough, J., Yin, X., Peterson, J., Chang, A., Torres, T., Gudz, T., Macklin, W.B., Lewis, D.A., Fox, R.J., Rudick, R., Mirnics, K., Trapp, B.D., 2006. Mitochondrial dysfunction as a cause of axonal degeneration in multiple sclerosis patients. Annals of neurology. 59, 478-89.
Dutta, R., Trapp, B.D., 2007. Pathogenesis of axonal and neuronal damage in multiple sclerosis. Neurology. 68, S22-31; discussion S43-54.
Dutta, R., Trapp, B.D., 2011. Mechanisms of neuronal dysfunction and degeneration in multiple sclerosis. Progress in neurobiology. 93, 1-12.
Dyment, D.A., Ebers, G.C., Sadovnick, A.D., 2004. Genetics of multiple sclerosis. Lancet neurology. 3, 104-10.
Dyment, D.A., Herrera, B.M., Cader, M.Z., Willer, C.J., Lincoln, M.R., Sadovnick, A.D., Risch, N., Ebers, G.C., 2005. Complex interactions among MHC haplotypes in multiple sclerosis: susceptibility and resistance. Human molecular genetics. 14, 2019-26.
Ebers, G.C., Koopman, W.J., Hader, W., Sadovnick, A.D., Kremenchutzky, M., Mandalfino, P., Wingerchuk, D.M., Baskerville, J., Rice, G.P., 2000. The natural history of multiple sclerosis: a geographically based study: 8: familial multiple sclerosis. Brain : a journal of neurology. 123 Pt 3, 641-9.
Ebers, G.C., 2001. Natural history of multiple sclerosis. Journal of neurology, neurosurgery, and psychiatry. 71 Suppl 2, ii16-9.
Ebers, G.C., Sadovnick, A.D., Dyment, D.A., Yee, I.M., Willer, C.J., Risch, N., 2004. Parent-of-origin effect in multiple sclerosis: observations in half-siblings. Lancet. 363, 1773-4.
Ebers, G.C., 2008. Environmental factors and multiple sclerosis. Lancet neurology. 7, 268-77.
Ebers, G.C., Heigenhauser, L., Daumer, M., Lederer, C., Noseworthy, J.H., 2008. Disability as an outcome in MS clinical trials. Neurology. 71, 624-31.
Ebers, G.C., Reder, A.T., Traboulsee, A., Li, D., Langdon, D., Goodin, D.S., Wolf, C., Beckmann, K., Konieczny, A., 2009. Long-term follow-up of the original interferon-beta1b trial in multiple sclerosis: design and lessons from a 16-year observational study. Clinical therapeutics. 31, 1724-36.
Ebers, G.C., Traboulsee, A., Li, D., Langdon, D., Reder, A.T., Goodin, D.S., Bogumil, T., Beckmann, K., Wolf, C., Konieczny, A., 2010. Analysis of clinical outcomes according to original treatment groups 16 years after the pivotal IFNB-1b trial. Journal of neurology, neurosurgery, and psychiatry. 81, 907-12.
Ebers, G.C., Daumer, M., Scalfari, A., 2011. Surrogate endpoints for EDSS worsening in multiple sclerosis: a meta-analytic approach: measuring disability in relapsing-remitting MS. Neurology. 76, 1025; author reply 1025-6.
Ebers, G.C., Scalfari, A., Daumer, M., Lederer, C., 2012. Combined MRI lesions and relapses as a surrogate for disability in MS. Neurology. 78, 1367; author reply 1367.
Edan, G., 2012. There is no such thing as a mild MS relapse. The mild relapse is an Anglo-Saxon delusion - Yes. Multiple sclerosis. 18, 927-9.
Edan, G., Le Page, E., 2013. Induction Therapy for Patients with Multiple Sclerosis: Why? When? How? CNS drugs.

Edland, A., Nyland, H., Riise, T., Larsen, J.P., 1996. Epidemiology of multiple sclerosis in the county of Vestfold, eastern Norway: incidence and prevalence calculations. Acta neurologica Scandinavica. 93**,** 104-9.

Ekestern, E., Lebhart, G., 2004. Mortality from multiple sclerosis in Austria 1970-2001: dynamics, trends, and prospects. European journal of neurology : the official journal of the European Federation of Neurological Societies. 11**,** 511-20.

El-Salem, K., Al-Shimmery, E., Horany, K., Al-Refai, A., Al-Hayk, K., Khader, Y., 2006. Multiple sclerosis in Jordan: A clinical and epidemiological study. Journal of neurology. 253**,** 1210-6.

Emre, M., de Decker, C., 1992. Effects of cigarette smoking on motor functions in patients with multiple sclerosis. Archives of neurology. 49**,** 1243-7.

Eriksson, M., Andersen, O., Runmarker, B., 2003. Long-term follow up of patients with clinically isolated syndromes, relapsing-remitting and secondary progressive multiple sclerosis. Multiple sclerosis. 9**,** 260-74.

Evangelou, N., Esiri, M.M., Smith, S., Palace, J., Matthews, P.M., 2000. Quantitative pathological evidence for axonal loss in normal appearing white matter in multiple sclerosis. Annals of neurology. 47**,** 391-5.

Fagius, J., Lundgren, J., Oberg, G., 2009. Early highly aggressive MS successfully treated by hematopoietic stem cell transplantation. Mult Scler. 15**,** 229-37.

Farrell, R.A., Antony, D., Wall, G.R., Clark, D.A., Fisniku, L., Swanton, J., Khaleeli, Z., Schmierer, K., Miller, D.H., Giovannoni, G., 2009. Humoral immune response to EBV in multiple sclerosis is associated with disease activity on MRI. Neurology. 73**,** 32-8.

Fazekas, F., Offenbacher, H., Fuchs, S., Schmidt, R., Niederkorn, K., Horner, S., Lechner, H., 1988. Criteria for an increased specificity of MRI interpretation in elderly subjects with suspected multiple sclerosis. Neurology. 38**,** 1822-5.

Fazekas, F., Barkhof, F., Filippi, M., Grossman, R.I., Li, D.K., McDonald, W.I., McFarland, H.F., Paty, D.W., Simon, J.H., Wolinsky, J.S., Miller, D.H., 1999. The contribution of magnetic resonance imaging to the diagnosis of multiple sclerosis. Neurology. 53**,** 448-56.

Ferber, I.A., Brocke, S., Taylor-Edwards, C., Ridgway, W., Dinisco, C., Steinman, L., Dalton, D., Fathman, C.G., 1996. Mice with a disrupted IFN-gamma gene are susceptible to the induction of experimental autoimmune encephalomyelitis (EAE). Journal of immunology. 156**,** 5-7.

Ferguson, B., Matyszak, M.K., Esiri, M.M., Perry, V.H., 1997. Axonal damage in acute multiple sclerosis lesions. Brain : a journal of neurology. 120 (Pt 3)**,** 393-9.

Filippi, M., Horsfield, M.A., Morrissey, S.P., MacManus, D.G., Rudge, P., McDonald, W.I., Miller, D.H., 1994. Quantitative brain MRI lesion load predicts the course of clinically isolated syndromes suggestive of multiple sclerosis. Neurology. 44**,** 635-41.

Filippi, M., 2000. Enhanced magnetic resonance imaging in multiple sclerosis. Multiple sclerosis. 6**,** 320-6.

Filippi, M., Bozzali, M., Rovaris, M., Gonen, O., Kesavadas, C., Ghezzi, A., Martinelli, V., Grossman, R.I., Scotti, G., Comi, G., Falini, A., 2003. Evidence for

widespread axonal damage at the earliest clinical stage of multiple sclerosis. Brain : a journal of neurology. 126**,** 433-7.

Filippi, M., Rocca, M.A., Barkhof, F., Bruck, W., Chen, J.T., Comi, G., DeLuca, G., De Stefano, N., Erickson, B.J., Evangelou, N., Fazekas, F., Geurts, J.J., Lucchinetti, C., Miller, D.H., Pelletier, D., Popescu, B.F., Lassmann, H., 2012. Association between pathological and MRI findings in multiple sclerosis. Lancet neurology. 11**,** 349-60.

Filippi, M., Rocca, M.A., Pagani, E., De Stefano, N., Jeffery, D., Kappos, L., Montalban, X., Boyko, A.N., Comi, G., 2013. Placebo-controlled trial of oral laquinimod in multiple sclerosis: MRI evidence of an effect on brain tissue damage. Journal of neurology, neurosurgery, and psychiatry.

Fisher, J.B., Jacobs, D.A., Markowitz, C.E., Galetta, S.L., Volpe, N.J., Nano-Schiavi, M.L., Baier, M.L., Frohman, E.M., Winslow, H., Frohman, T.C., Calabresi, P.A., Maguire, M.G., Cutter, G.R., Balcer, L.J., 2006. Relation of visual function to retinal nerve fiber layer thickness in multiple sclerosis. Ophthalmology. 113**,** 324-32.

Fisniku, L.K., Brex, P.A., Altmann, D.R., Miszkiel, K.A., Benton, C.E., Lanyon, R., Thompson, A.J., Miller, D.H., 2008a. Disability and T2 MRI lesions: a 20-year follow-up of patients with relapse onset of multiple sclerosis. Brain : a journal of neurology. 131**,** 808-17.

Fisniku, L.K., Chard, D.T., Jackson, J.S., Anderson, V.M., Altmann, D.R., Miszkiel, K.A., Thompson, A.J., Miller, D.H., 2008b. Gray matter atrophy is related to long-term disability in multiple sclerosis. Annals of neurology. 64**,** 247-54.

Flynn, J.M., Byrd, J.C., 2000. Campath-1H monoclonal antibody therapy. Current opinion in oncology. 12**,** 574-81.

Fog, T., Linnemann, F., 1970. The course of multiple sclerosis in 73 cases with computer-designed curves. Acta neurologica Scandinavica. Supplementum. 47**,** 3-175.

Fois, A.F., Wotton, C.J., Yeates, D., Turner, M.R., Goldacre, M.J., 2010. Cancer in patients with motor neuron disease, multiple sclerosis and Parkinson's disease: record linkage studies. Journal of neurology, neurosurgery, and psychiatry. 81**,** 215-21.

Forbes, R.B., Wilson, S.V., Swingler, R.J., 1999. The prevalence of multiple sclerosis in Tayside, Scotland: do latitudinal gradients really exist? Journal of neurology. 246**,** 1033-40.

Ford, C., Goodman, A.D., Johnson, K., Kachuck, N., Lindsey, J.W., Lisak, R., Luzzio, C., Myers, L., Panitch, H., Preiningerova, J., Pruitt, A., Rose, J., Rus, H., Wolinsky, J., 2010. Continuous long-term immunomodulatory therapy in relapsing multiple sclerosis: results from the 15-year analysis of the US prospective open-label study of glatiramer acetate. Multiple sclerosis. 16**,** 342-50.

Fox, R.I., Herrmann, M.L., Frangou, C.G., Wahl, G.M., Morris, R.E., Strand, V., Kirschbaum, B.J., 1999. Mechanism of action for leflunomide in rheumatoid arthritis. Clinical immunology. 93**,** 198-208.

Fox, R.J., Miller, D.H., Phillips, J.T., Hutchinson, M., Havrdova, E., Kita, M., Yang, M., Raghupathi, K., Novas, M., Sweetser, M.T., Viglietta, V., Dawson, K.T., 2012. Placebo-controlled phase 3 study of oral BG-12 or glatiramer in multiple sclerosis. The New England journal of medicine. 367**,** 1087-97.

Frank, J.A., Stone, L.A., Smith, M.E., Albert, P.S., Maloni, H., McFarland, H.F., 1994. Serial contrast-enhanced magnetic resonance imaging in patients with early relapsing-remitting multiple sclerosis: implications for treatment trials. Annals of neurology. 36 Suppl, S86-90.

Franklin, R.J., 2002. Why does remyelination fail in multiple sclerosis? Nature reviews. Neuroscience. 3, 705-14.

Franklin, R.J., Kotter, M.R., 2008. The biology of CNS remyelination: the key to therapeutic advances. Journal of neurology. 255 Suppl 1, 19-25.

Fredrikson, S., Cheng, Q., Jiang, G.X., Wasserman, D., 2003. Elevated suicide risk among patients with multiple sclerosis in Sweden. Neuroepidemiology. 22, 146-52.

Freedman, D.M., Dosemeci, M., Alavanja, M.C., 2000. Mortality from multiple sclerosis and exposure to residential and occupational solar radiation: a case-control study based on death certificates. Occupational and environmental medicine. 57, 418-21.

Freedman, M.S., Patry, D.G., Grand'Maison, F., Myles, M.L., Paty, D.W., Selchen, D.H., 2004. Treatment optimization in multiple sclerosis. The Canadian journal of neurological sciences. Le journal canadien des sciences neurologiques. 31, 157-68.

Freedman, M.S., Thompson, E.J., Deisenhammer, F., Giovannoni, G., Grimsley, G., Keir, G., Ohman, S., Racke, M.K., Sharief, M., Sindic, C.J., Sellebjerg, F., Tourtellotte, W.W., 2005. Recommended standard of cerebrospinal fluid analysis in the diagnosis of multiple sclerosis: a consensus statement. Archives of neurology. 62, 865-70.

Freedman, M.S., 2011. Long-term follow-up of clinical trials of multiple sclerosis therapies. Neurology. 76, S26-34.

Freedman, M.S., Wolinsky, J.S., Wamil, B., Confavreux, C., Comi, G., Kappos, L., Olsson, T.P., Miller, A., Benzerdjeb, H., Li, H., Simonson, C., O'Connor, P.W., 2012. Teriflunomide added to interferon-beta in relapsing multiple sclerosis: a randomized phase II trial. Neurology. 78, 1877-85.

Friese, M.A., Fugger, L., 2005. Autoreactive CD8+ T cells in multiple sclerosis: a new target for therapy? Brain : a journal of neurology. 128, 1747-63.

Frischer, J.M., Bramow, S., Dal-Bianco, A., Lucchinetti, C.F., Rauschka, H., Schmidbauer, M., Laursen, H., Sorensen, P.S., Lassmann, H., 2009. The relation between inflammation and neurodegeneration in multiple sclerosis brains. Brain : a journal of neurology. 132, 1175-89.

Frohman, E.M., Havrdova, E., Lublin, F., Barkhof, F., Achiron, A., Sharief, M.K., Stuve, O., Racke, M.K., Steinman, L., Weiner, H., Olek, M., Zivadinov, R., Corboy, J., Raine, C., Cutter, G., Richert, J., Filippi, M., 2006a. Most patients with multiple sclerosis or a clinically isolated demyelinating syndrome should be treated at the time of diagnosis. Archives of neurology. 63, 614-9.

Frohman, E.M., Racke, M.K., Raine, C.S., 2006b. Multiple sclerosis--the plaque and its pathogenesis. The New England journal of medicine. 354, 942-55.

Gale, C.R., Martyn, C.N., 1995. Migrant studies in multiple sclerosis. Progress in neurobiology. 47, 425-48.

Gay, F.W., Drye, T.J., Dick, G.W., Esiri, M.M., 1997. The application of multifactorial cluster analysis in the staging of plaques in early multiple sclerosis.

Identification and characterization of the primary demyelinating lesion. Brain : a journal of neurology. 120 (Pt 8), 1461-83.
Geurts, J.J., Roosendaal, S.D., Calabrese, M., Ciccarelli, O., Agosta, F., Chard, D.T., Gass, A., Huerga, E., Moraal, B., Pareto, D., Rocca, M.A., Wattjes, M.P., Yousry, T.A., Uitdehaag, B.M., Barkhof, F., 2011. Consensus recommendations for MS cortical lesion scoring using double inversion recovery MRI. Neurology. 76, 418-24.
Geurts, J.J., Calabrese, M., Fisher, E., Rudick, R.A., 2012. Measurement and clinical effect of grey matter pathology in multiple sclerosis. Lancet neurology. 11, 1082-92.
Giacomini, P.S., Arnold, D.L., Bar-Or, A., Antel, J.P., 2009. Defining multiple sclerosis treatment response with magnetic resonance imaging: how much activity is too much? Archives of neurology. 66, 19-20.
Gilmore, C.P., Donaldson, I., Bo, L., Owens, T., Lowe, J., Evangelou, N., 2009. Regional variations in the extent and pattern of grey matter demyelination in multiple sclerosis: a comparison between the cerebral cortex, cerebellar cortex, deep grey matter nuclei and the spinal cord. Journal of neurology, neurosurgery, and psychiatry. 80, 182-7.
Giorgio, A., Stromillo, M.L., Rossi, F., Battaglini, M., Hakiki, B., Portaccio, E., Federico, A., Amato, M.P., De Stefano, N., 2011. Cortical lesions in radiologically isolated syndrome. Neurology. 77, 1896-9.
Giovannoni, G., Southam, E., Waubant, E., 2012. Systematic review of disease-modifying therapies to assess unmet needs in multiple sclerosis: tolerability and adherence. Multiple sclerosis. 18, 932-46.
Gold, R., Kappos, L., Arnold, D.L., Bar-Or, A., Giovannoni, G., Selmaj, K., Tornatore, C., Sweetser, M.T., Yang, M., Sheikh, S.I., Dawson, K.T., 2012. Placebo-controlled phase 3 study of oral BG-12 for relapsing multiple sclerosis. The New England journal of medicine. 367, 1098-107.
Gonzalez-Navajas, J.M., Lee, J., David, M., Raz, E., 2012. Immunomodulatory functions of type I interferons. Nature reviews. Immunology. 12, 125-35.
Goodin, D.S., Arnason, B.G., Coyle, P.K., Frohman, E.M., Paty, D.W., 2003. The use of mitoxantrone (Novantrone) for the treatment of multiple sclerosis: report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. Neurology. 61, 1332-8.
Goodin, D.S., 2004. Disease-modifying therapy in MS: a critical review of the literature. Part I: Analysis of clinical trial errors. Journal of neurology. 251 Suppl 5, v3-v11.
Goodin, D.S., 2009. The causal cascade to multiple sclerosis: a model for MS pathogenesis. PloS one. 4, e4565.
Goodin, D.S., Reder, A.T., Ebers, G.C., Cutter, G., Kremenchutzky, M., Oger, J., Langdon, D., Rametta, M., Beckmann, K., DeSimone, T.M., Knappertz, V., 2012. Survival in MS: a randomized cohort study 21 years after the start of the pivotal IFNbeta-1b trial. Neurology. 78, 1315-22.
Goodkin, D.E., Hertsgaard, D., Seminary, J., 1988. Upper extremity function in multiple sclerosis: improving assessment sensitivity with box-and-block and nine-hole peg tests. Archives of physical medicine and rehabilitation. 69, 850-4.
Goodkin, D.E., Hertsgaard, D., 1989. Seasonal variation of multiple sclerosis exacerbations in North Dakota. Archives of neurology. 46, 1015-8.

Goodkin, D.E., Hertsgaard, D., Rudick, R.A., 1989. Exacerbation rates and adherence to disease type in a prospectively followed-up population with multiple sclerosis. Implications for clinical trials. Archives of neurology. 46**,** 1107-12.

Goodkin, D.E., Cookfair, D., Wende, K., Bourdette, D., Pullicino, P., Scherokman, B., Whitham, R., 1992. Inter- and intrarater scoring agreement using grades 1.0 to 3.5 of the Kurtzke Expanded Disability Status Scale (EDSS). Multiple Sclerosis Collaborative Research Group. Neurology. 42**,** 859-63.

Gordon-Lipkin, E., Chodkowski, B., Reich, D.S., Smith, S.A., Pulicken, M., Balcer, L.J., Frohman, E.M., Cutter, G., Calabresi, P.A., 2007. Retinal nerve fiber layer is associated with brain atrophy in multiple sclerosis. Neurology. 69**,** 1603-9.

Gosselink, R., Kovacs, L., Decramer, M., 1999. Respiratory muscle involvement in multiple sclerosis. The European respiratory journal : official journal of the European Society for Clinical Respiratory Physiology. 13**,** 449-54.

Gosselink, R., Kovacs, L., Ketelaer, P., Carton, H., Decramer, M., 2000. Respiratory muscle weakness and respiratory muscle training in severely disabled multiple sclerosis patients. Archives of physical medicine and rehabilitation. 81**,** 747-51.

Gout, O., 2008. Confounders in natural history of interferon-beta-treated relapsing multiple sclerosis. Annals of neurology. 63**,** 126; author reply 126-7.

Goverman, J., Woods, A., Larson, L., Weiner, L.P., Hood, L., Zaller, D.M., 1993. Transgenic mice that express a myelin basic protein-specific T cell receptor develop spontaneous autoimmunity. Cell. 72**,** 551-60.

Granberg, T., Martola, J., Kristoffersen-Wiberg, M., Aspelin, P., Fredrikson, S., 2013. Radiologically isolated syndrome - incidental magnetic resonance imaging findings suggestive of multiple sclerosis, a systematic review. Multiple sclerosis. 19**,** 271-80.

Granieri, E., Casetta, I., Govoni, V., Tola, M.R., Marchi, D., Murgia, S.B., Ticca, A., Pugliatti, M., Murgia, B., Rosati, G., 2000. The increasing incidence and prevalence of MS in a Sardinian province. Neurology. 55**,** 842-8.

Gray, O.M., McDonnell, G.V., Hawkins, S.A., 2008. Factors in the rising prevalence of multiple sclerosis in the north-east of Ireland. Multiple sclerosis. 14**,** 880-6.

Grazioli, E., Zivadinov, R., Weinstock-Guttman, B., Lincoff, N., Baier, M., Wong, J.R., Hussein, S., Cox, J.L., Hojnacki, D., Ramanathan, M., 2008. Retinal nerve fiber layer thickness is associated with brain MRI outcomes in multiple sclerosis. Journal of the neurological sciences. 268**,** 12-7.

Green, M.S., Symons, M.J., 1983. A comparison of the logistic risk function and the proportional hazards model in prospective epidemiologic studies. Journal of chronic diseases. 36**,** 715-23.

Gresle, M.M., Butzkueven, H., Shaw, G., 2011. Neurofilament proteins as body fluid biomarkers of neurodegeneration in multiple sclerosis. Multiple sclerosis international. 2011**,** 315406.

Grimaldi, L.M., Palmeri, B., Salemi, G., Giglia, G., D'Amelio, M., Grimaldi, R., Vitello, G., Ragonese, P., Savettieri, G., 2007. High prevalence and fast rising incidence of multiple sclerosis in Caltanissetta, Sicily, southern Italy. Neuroepidemiology. 28**,** 28-32.

Gronwall, D.M., 1977. Paced auditory serial-addition task: a measure of recovery from concussion. Perceptual and motor skills. 44, 367-73.
Gross, K., Kokk, A., Kaasik, A.E., 1993. Prevalence of MS in south Estonia. Evidence of a new border of the Fennoscandian focus. Acta neurologica Scandinavica. 88, 241-6.
Grytten, N., Glad, S.B., Aarseth, J.H., Nyland, H., Midgard, R., Myhr, K.M., 2006. A 50-year follow-up of the incidence of multiple sclerosis in Hordaland County, Norway. Neurology. 66, 182-6.
Grytten Torkildsen, N., Lie, S.A., Aarseth, J.H., Nyland, H., Myhr, K.M., 2008. Survival and cause of death in multiple sclerosis: results from a 50-year follow-up in Western Norway. Multiple sclerosis. 14, 1191-8.
Gunnarsson, M., Malmestrom, C., Axelsson, M., Sundstrom, P., Dahle, C., Vrethem, M., Olsson, T., Piehl, F., Norgren, N., Rosengren, L., Svenningsson, A., Lycke, J., 2011. Axonal damage in relapsing multiple sclerosis is markedly reduced by natalizumab. Annals of neurology. 69, 83-9.
Hader, W.J., 1982. Prevalence of multiple sclerosis in Saskatoon. Canadian Medical Association journal. 127, 295-7.
Hader, W.J., Elliot, M., Ebers, G.C., 1988. Epidemiology of multiple sclerosis in London and Middlesex County, Ontario, Canada. Neurology. 38, 617-21.
Hader, W.J., Yee, I.M., 2007. Incidence and prevalence of multiple sclerosis in Saskatoon, Saskatchewan. Neurology. 69, 1224-9.
Hader, W.J., 2010. Disability and survival of multiple sclerosis in Saskatoon, Saskatchewan. The Canadian journal of neurological sciences. Le journal canadien des sciences neurologiques. 37, 28-35.
Haghighi, S., Andersen, O., Oden, A., Rosengren, L., 2004. Cerebrospinal fluid markers in MS patients and their healthy siblings. Acta neurologica Scandinavica. 109, 97-9.
Haines, J.L., Terwedow, H.A., Burgess, K., Pericak-Vance, M.A., Rimmler, J.B., Martin, E.R., Oksenberg, J.R., Lincoln, R., Zhang, D.Y., Banatao, D.R., Gatto, N., Goodkin, D.E., Hauser, S.L., 1998. Linkage of the MHC to familial multiple sclerosis suggests genetic heterogeneity. The Multiple Sclerosis Genetics Group. Human molecular genetics. 7, 1229-34.
Hallgren, B., Sourander, P., 1958. The effect of age on the non-haemin iron in the human brain. Journal of neurochemistry. 3, 41-51.
Hammond, S.R., McLeod, J.G., Millingen, K.S., Stewart-Wynne, E.G., English, D., Holland, J.T., McCall, M.G., 1988. The epidemiology of multiple sclerosis in three Australian cities: Perth, Newcastle and Hobart. Brain : a journal of neurology. 111 (Pt 1), 1-25.
Hammond, S.R., English, D.R., McLeod, J.G., 2000. The age-range of risk of developing multiple sclerosis: evidence from a migrant population in Australia. Brain : a journal of neurology. 123 (Pt 5), 968-74.
Handel, A.E., Ebers, G.C., Ramagopalan, S.V., 2010a. Epigenetics: molecular mechanisms and implications for disease. Trends in molecular medicine. 16, 7-16.
Handel, A.E., Giovannoni, G., Ebers, G.C., Ramagopalan, S.V., 2010b. Environmental factors and their timing in adult-onset multiple sclerosis. Nature reviews. Neurology. 6, 156-66.

Handel, A.E., Ramagopalan, S.V., 2010. Multiple sclerosis and risk of cancer: a meta-analysis. Journal of neurology, neurosurgery, and psychiatry. 81, 1413-4.

Handel, A.E., Williamson, A.J., Disanto, G., Handunnetthi, L., Giovannoni, G., Ramagopalan, S.V., 2010c. An updated meta-analysis of risk of multiple sclerosis following infectious mononucleosis. PloS one. 5.

Hanisch, U.K., 2002. Microglia as a source and target of cytokines. Glia. 40, 140-55.

Hansen, T., Skytthe, A., Stenager, E., Petersen, H.C., Bronnum-Hansen, H., Kyvik, K.O., 2005. Concordance for multiple sclerosis in Danish twins: an update of a nationwide study. Multiple sclerosis. 11, 504-10.

Hartline, D.K., Colman, D.R., 2007. Rapid conduction and the evolution of giant axons and myelinated fibers. Current biology : CB. 17, R29-35.

Hartung, H.P., Gonsette, R., Konig, N., Kwiecinski, H., Guseo, A., Morrissey, S.P., Krapf, H., Zwingers, T., 2002. Mitoxantrone in progressive multiple sclerosis: a placebo-controlled, double-blind, randomised, multicentre trial. Lancet. 360, 2018-25.

Harty, J.T., Tvinnereim, A.R., White, D.W., 2000. CD8+ T cell effector mechanisms in resistance to infection. Annual review of immunology. 18, 275-308.

Harty, J.T., Badovinac, V.P., 2008. Shaping and reshaping CD8+ T-cell memory. Nature reviews. Immunology. 8, 107-19.

Hauser, S.L., Weiner, H.L., Che, M., Shapiro, M.E., Gilles, F., Letvin, N.L., 1984. Prevention of experimental allergic encephalomyelitis (EAE) in the SJL/J mouse by whole body ultraviolet irradiation. Journal of immunology. 132, 1276-81.

Hauser, S.L., Waubant, E., Arnold, D.L., Vollmer, T., Antel, J., Fox, R.J., Bar-Or, A., Panzara, M., Sarkar, N., Agarwal, S., Langer-Gould, A., Smith, C.H., 2008. B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. The New England journal of medicine. 358, 676-88.

Hawkins, S.A., McDonnell, G.V., 1999. Benign multiple sclerosis? Clinical course, long term follow up, and assessment of prognostic factors. J Neurol Neurosurg Psychiatry. 67, 148-52.

Hayes, C.E., Donald Acheson, E., 2008. A unifying multiple sclerosis etiology linking virus infection, sunlight, and vitamin D, through viral interleukin-10. Medical hypotheses. 71, 85-90.

Healy, B.C., Ali, E.N., Guttmann, C.R., Chitnis, T., Glanz, B.I., Buckle, G., Houtchens, M., Stazzone, L., Moodie, J., Berger, A.M., Duan, Y., Bakshi, R., Khoury, S., Weiner, H., Ascherio, A., 2009. Smoking and disease progression in multiple sclerosis. Archives of neurology. 66, 858-64.

Hedstrom, A.K., Baarnhielm, M., Olsson, T., Alfredsson, L., 2009. Tobacco smoking, but not Swedish snuff use, increases the risk of multiple sclerosis. Neurology. 73, 696-701.

Helmick, C.G., Wrigley, J.M., Zack, M.M., Bigler, W.J., Lehman, J.L., Janssen, R.S., Hartwig, E.C., Witte, J.J., 1989. Multiple sclerosis in Key West, Florida. American journal of epidemiology. 130, 935-49.

Henderson, A.P., Trip, S.A., Schlottmann, P.G., Altmann, D.R., Garway-Heath, D.F., Plant, G.T., Miller, D.H., 2008. An investigation of the retinal nerve fibre layer in progressive multiple sclerosis using optical coherence tomography. Brain : a journal of neurology. 131, 277-87.

Henderson, A.P., Barnett, M.H., Parratt, J.D., Prineas, J.W., 2009. Multiple sclerosis: distribution of inflammatory cells in newly forming lesions. Annals of neurology. 66, 739-53.
Hernan, M.A., Olek, M.J., Ascherio, A., 2001. Cigarette smoking and incidence of multiple sclerosis. American journal of epidemiology. 154, 69-74.
Hernan, M.A., Jick, S.S., Logroscino, G., Olek, M.J., Ascherio, A., Jick, H., 2005. Cigarette smoking and the progression of multiple sclerosis. Brain : a journal of neurology. 128, 1461-5.
Herrera, B.M., Ramagopalan, S.V., Lincoln, M.R., Orton, S.M., Chao, M.J., Sadovnick, A.D., Ebers, G.C., 2008. Parent-of-origin effects in MS: observations from avuncular pairs. Neurology. 71, 799-803.
Hirst, C., Ingram, G., Pearson, O., Pickersgill, T., Scolding, N., Robertson, N., 2008a. Contribution of relapses to disability in multiple sclerosis. Journal of neurology. 255, 280-7.
Hirst, C., Swingler, R., Compston, D.A., Ben-Shlomo, Y., Robertson, N.P., 2008b. Survival and cause of death in multiple sclerosis: a prospective population-based study. Journal of neurology, neurosurgery, and psychiatry. 79, 1016-21.
Hirst, C., Ingram, G., Pickersgill, T., Swingler, R., Compston, D.A., Robertson, N.P., 2009. Increasing prevalence and incidence of multiple sclerosis in South East Wales. Journal of neurology, neurosurgery, and psychiatry. 80, 386-91.
Hochmeister, S., Grundtner, R., Bauer, J., Engelhardt, B., Lyck, R., Gordon, G., Korosec, T., Kutzelnigg, A., Berger, J.J., Bradl, M., Bittner, R.E., Lassmann, H., 2006. Dysferlin is a new marker for leaky brain blood vessels in multiple sclerosis. Journal of neuropathology and experimental neurology. 65, 855-65.
Hofman, F.M., Hinton, D.R., Johnson, K., Merrill, J.E., 1989. Tumor necrosis factor identified in multiple sclerosis brain. The Journal of experimental medicine. 170, 607-12.
Hohnoki, K., Inoue, A., Koh, C.S., 1998. Elevated serum levels of IFN-gamma, IL-4 and TNF-alpha/unelevated serum levels of IL-10 in patients with demyelinating diseases during the acute stage. Journal of neuroimmunology. 87, 27-32.
Holick, M.F., 2003. Vitamin D: A millenium perspective. Journal of cellular biochemistry. 88, 296-307.
Holick, M.F., 2004. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. The American journal of clinical nutrition. 80, 1678S-88S.
Holmoy, T., 2008. Vitamin D status modulates the immune response to Epstein Barr virus: Synergistic effect of risk factors in multiple sclerosis. Medical hypotheses. 70, 66-9.
Hong, J., Li, N., Zhang, X., Zheng, B., Zhang, J.Z., 2005. Induction of CD4+CD25+ regulatory T cells by copolymer-I through activation of transcription factor Foxp3. Proceedings of the National Academy of Sciences of the United States of America. 102, 6449-54.
Hoogervorst, E.L., Kalkers, N.F., Uitdehaag, B.M., Polman, C.H., 2002. A study validating changes in the multiple sclerosis functional composite. Archives of neurology. 59, 113-6.

Hoppenbrouwers, I.A., Hintzen, R.Q., 2011. Genetics of multiple sclerosis. Biochimica et biophysica acta. 1812**,** 194-201.
Hori, S., Haury, M., Coutinho, A., Demengeot, J., 2002. Specificity requirements for selection and effector functions of CD25+4+ regulatory T cells in anti-myelin basic protein T cell receptor transgenic mice. Proceedings of the National Academy of Sciences of the United States of America. 99**,** 8213-8.
Houzen, H., Niino, M., Kikuchi, S., Fukazawa, T., Nogoshi, S., Matsumoto, H., Tashiro, K., 2003. The prevalence and clinical characteristics of MS in northern Japan. Journal of the neurological sciences. 211**,** 49-53.
Houzen, H., Niino, M., Hata, D., Nakano, F., Kikuchi, S., Fukazawa, T., Sasaki, H., 2008. Increasing prevalence and incidence of multiple sclerosis in northern Japan. Multiple sclerosis. 14**,** 887-92.
Howell, O.W., Reeves, C.A., Nicholas, R., Carassiti, D., Radotra, B., Gentleman, S.M., Serafini, B., Aloisi, F., Roncaroli, F., Magliozzi, R., Reynolds, R., 2011. Meningeal inflammation is widespread and linked to cortical pathology in multiple sclerosis. Brain : a journal of neurology. 134**,** 2755-71.
Huang, D., Swanson, E.A., Lin, C.P., Schuman, J.S., Stinson, W.G., Chang, W., Hee, M.R., Flotte, T., Gregory, K., Puliafito, C.A., et al., 1991. Optical coherence tomography. Science. 254**,** 1178-81.
Hulet, S.W., Powers, S., Connor, J.R., 1999. Distribution of transferrin and ferritin binding in normal and multiple sclerotic human brains. Journal of the neurological sciences. 165**,** 48-55.
Hunter, S.F., Hafler, D.A., 2000. Ubiquitous pathogens: links between infection and autoimmunity in MS? Neurology. 55**,** 164-5.
Hutchinson, M., Kappos, L., Calabresi, P.A., Confavreux, C., Giovannoni, G., Galetta, S.L., Havrdova, E., Lublin, F.D., Miller, D.H., O'Connor, P.W., Phillips, J.T., Polman, C.H., Radue, E.W., Rudick, R.A., Stuart, W.H., Wajgt, A., Weinstock-Guttman, B., Wynn, D.R., Lynn, F., Panzara, M.A., 2009. The efficacy of natalizumab in patients with relapsing multiple sclerosis: subgroup analyses of AFFIRM and SENTINEL. Journal of neurology. 256**,** 405-15.
Hutchinson, M., 2011. Relapses do not matter in relation to long-term disability: commentary. Multiple sclerosis. 17**,** 1417.
Inusah, S., Sormani, M.P., Cofield, S.S., Aban, I.B., Musani, S.K., Srinivasasainagendra, V., Cutter, G.R., 2010. Assessing changes in relapse rates in multiple sclerosis. Multiple sclerosis. 16**,** 1414-21.
Irvine, K.A., Blakemore, W.F., 2008. Remyelination protects axons from demyelination-associated axon degeneration. Brain : a journal of neurology. 131**,** 1464-77.
Islam, T., Gauderman, W.J., Cozen, W., Hamilton, A.S., Burnett, M.E., Mack, T.M., 2006. Differential twin concordance for multiple sclerosis by latitude of birthplace. Annals of neurology. 60**,** 56-64.
Islam, T., Gauderman, W.J., Cozen, W., Mack, T.M., 2007. Childhood sun exposure influences risk of multiple sclerosis in monozygotic twins. Neurology. 69**,** 381-8.
Jacobs, L.D., Cookfair, D.L., Rudick, R.A., Herndon, R.M., Richert, J.R., Salazar, A.M., Fischer, J.S., Goodkin, D.E., Granger, C.V., Simon, J.H., Alam, J.J., Bartoszak, D.M., Bourdette, D.N., Braiman, J., Brownscheidle, C.M., Coats, M.E., Cohan, S.L., Dougherty, D.S., Kinkel, R.P., Mass, M.K., Munschauer, F.E., 3rd, Priore, R.L., Pullicino, P.M., Scherokman, B.J., Whitham, R.H., et al., 1996a.

Intramuscular interferon beta-1a for disease progression in relapsing multiple sclerosis. The Multiple Sclerosis Collaborative Research Group (MSCRG). Annals of neurology. 39**,** 285-94.

Jacobs, L.D., Cookfair, D.L., Rudick, R.A., Herndon, R.M., Richert, J.R., Salazar, A.M., Fischer, J.S., Goodkin, D.E., Granger, C.V., Simon, J.H., Alam, J.J., Bartoszak, D.M., Bourdette, D.N., Braiman, J., Brownscheidle, C.M., Coats, M.E., Cohan, S.L., Dougherty, D.S., Kinkel, R.P., Mass, M.K., Munschauer, F.E., 3rd, Priore, R.L., Pullicino, P.M., Scherokman, B.J., Whitham, R.H., et al., 1996b. Intramuscular interferon beta-1a for disease progression in relapsing multiple sclerosis. The Multiple Sclerosis Collaborative Research Group (MSCRG). Ann Neurol. 39**,** 285-94.

Jacobs, L.D., Beck, R.W., Simon, J.H., Kinkel, R.P., Brownscheidle, C.M., Murray, T.J., Simonian, N.A., Slasor, P.J., Sandrock, A.W., 2000. Intramuscular interferon beta-1a therapy initiated during a first demyelinating event in multiple sclerosis. CHAMPS Study Group. The New England journal of medicine. 343**,** 898-904.

Jersild, C., Fog, T., Hansen, G.S., Thomsen, M., Svejgaard, A., Dupont, B., 1973. Histocompatibility determinants in multiple sclerosis, with special reference to clinical course. Lancet. 2**,** 1221-5.

Jiang, H., Zhang, S.I., Pernis, B., 1992. Role of CD8+ T cells in murine experimental allergic encephalomyelitis. Science. 256**,** 1213-5.

Jiang, H., Curran, S., Ruiz-Vazquez, E., Liang, B., Winchester, R., Chess, L., 2003. Regulatory CD8+ T cells fine-tune the myelin basic protein-reactive T cell receptor V beta repertoire during experimental autoimmune encephalomyelitis. Proceedings of the National Academy of Sciences of the United States of America. 100**,** 8378-83.

Johnson, K.P., Brooks, B.R., Cohen, J.A., Ford, C.C., Goldstein, J., Lisak, R.P., Myers, L.W., Panitch, H.S., Rose, J.W., Schiffer, R.B., 1995. Copolymer 1 reduces relapse rate and improves disability in relapsing-remitting multiple sclerosis: results of a phase III multicenter, double-blind placebo-controlled trial. The Copolymer 1 Multiple Sclerosis Study Group. Neurology. 45**,** 1268-76.

Johnson, K.P., Brooks, B.R., Cohen, J.A., Ford, C.C., Goldstein, J., Lisak, R.P., Myers, L.W., Panitch, H.S., Rose, J.W., Schiffer, R.B., Vollmer, T., Weiner, L.P., Wolinsky, J.S., 1998. Extended use of glatiramer acetate (Copaxone) is well tolerated and maintains its clinical effect on multiple sclerosis relapse rate and degree of disability. Copolymer 1 Multiple Sclerosis Study Group. Neurology. 50**,** 701-8.

Jousilahti, P., Vartiainen, E., Tuomilehto, J., Puska, P., 1999. Sex, age, cardiovascular risk factors, and coronary heart disease: a prospective follow-up study of 14 786 middle-aged men and women in Finland. Circulation. 99**,** 1165-72.

Junker, A., Ivanidze, J., Malotka, J., Eiglmeier, I., Lassmann, H., Wekerle, H., Meinl, E., Hohlfeld, R., Dornmair, K., 2007. Multiple sclerosis: T-cell receptor expression in distinct brain regions. Brain : a journal of neurology. 130**,** 2789-99.

Kabat, E.A., Glusman, M., Knaub, V., 1948. Quantitative estimation of the albumin and gamma globulin in normal and pathologic cerebrospinal fluid by immunochemical methods. The American journal of medicine. 4**,** 653-62.

Kalkers, N.F., de Groot, V., Lazeron, R.H., Killestein, J., Ader, H.J., Barkhof, F., Lankhorst, G.J., Polman, C.H., 2000. MS functional composite: relation to disease phenotype and disability strata. Neurology. 54**,** 1233-9.

Kalkers, N.F., Bergers, L., de Groot, V., Lazeron, R.H., van Walderveen, M.A., Uitdehaag, B.M., Polman, C.H., Barkhof, F., 2001. Concurrent validity of the MS Functional Composite using MRI as a biological disease marker. Neurology. 56**,** 215-9.

Kampman, M.T., Wilsgaard, T., Mellgren, S.I., 2007. Outdoor activities and diet in childhood and adolescence relate to MS risk above the Arctic Circle. Journal of neurology. 254**,** 471-7.

Kampman, M.T., Aarseth, J.H., Grytten, N., Benjaminsen, E., Celius, E.G., Dahl, O.P., Holmoy, T., Loken-Amsrud, K., Midgard, R., Myhr, K.M., Risberg, G., Vatne, A., Torkildsen, O., 2013. Sex ratio of multiple sclerosis in persons born from 1930 to 1979 and its relation to latitude in Norway. Journal of neurology.

Kantarci, O., Siva, A., Eraksoy, M., Karabudak, R., Sutlas, N., Agaoglu, J., Turan, F., Ozmenoglu, M., Togrul, E., Demirkiran, M., 1998. Survival and predictors of disability in Turkish MS patients. Turkish Multiple Sclerosis Study Group (TUMSSG). Neurology. 51**,** 765-72.

Kappos, L., Moeri, D., Radue, E.W., Schoetzau, A., Schweikert, K., Barkhof, F., Miller, D., Guttmann, C.R., Weiner, H.L., Gasperini, C., Filippi, M., 1999. Predictive value of gadolinium-enhanced magnetic resonance imaging for relapse rate and changes in disability or impairment in multiple sclerosis: a meta-analysis. Gadolinium MRI Meta-analysis Group. Lancet. 353**,** 964-9.

Kappos, L., Weinshenker, B., Pozzilli, C., Thompson, A.J., Dahlke, F., Beckmann, K., Polman, C., McFarland, H., 2004. Interferon beta-1b in secondary progressive MS: a combined analysis of the two trials. Neurology. 63**,** 1779-87.

Kappos, L., Antel, J., Comi, G., Montalban, X., O'Connor, P., Polman, C.H., Haas, T., Korn, A.A., Karlsson, G., Radue, E.W., 2006a. Oral fingolimod (FTY720) for relapsing multiple sclerosis. The New England journal of medicine. 355**,** 1124-40.

Kappos, L., Polman, C.H., Freedman, M.S., Edan, G., Hartung, H.P., Miller, D.H., Montalban, X., Barkhof, F., Bauer, L., Jakobs, P., Pohl, C., Sandbrink, R., 2006b. Treatment with interferon beta-1b delays conversion to clinically definite and McDonald MS in patients with clinically isolated syndromes. Neurology. 67**,** 1242-9.

Kappos, L., Traboulsee, A., Constantinescu, C., Eralinna, J.P., Forrestal, F., Jongen, P., Pollard, J., Sandberg-Wollheim, M., Sindic, C., Stubinski, B., Uitdehaag, B., Li, D., 2006c. Long-term subcutaneous interferon beta-1a therapy in patients with relapsing-remitting MS. Neurology. 67**,** 944-53.

Kappos, L., Gold, R., Miller, D.H., Macmanus, D.G., Havrdova, E., Limmroth, V., Polman, C.H., Schmierer, K., Yousry, T.A., Yang, M., Eraksoy, M., Meluzinova, E., Rektor, I., Dawson, K.T., Sandrock, A.W., O'Neill, G.N., 2008. Efficacy and safety of oral fumarate in patients with relapsing-remitting multiple sclerosis: a multicentre, randomised, double-blind, placebo-controlled phase IIb study. Lancet. 372**,** 1463-72.

Kappos, L., Freedman, M.S., Polman, C.H., Edan, G., Hartung, H.P., Miller, D.H., Montalban, X., Barkhof, F., Radu, E.W., Metzig, C., Bauer, L., Lanius, V.,

Sandbrink, R., Pohl, C., 2009. Long-term effect of early treatment with interferon beta-1b after a first clinical event suggestive of multiple sclerosis: 5-year active treatment extension of the phase 3 BENEFIT trial. Lancet neurology. 8**,** 987-97.

Kappos, L., Radue, E.W., O'Connor, P., Polman, C., Hohlfeld, R., Calabresi, P., Selmaj, K., Agoropoulou, C., Leyk, M., Zhang-Auberson, L., Burtin, P., 2010. A placebo-controlled trial of oral fingolimod in relapsing multiple sclerosis. The New England journal of medicine. 362**,** 387-401.

Kappos, L., Bates, D., Edan, G., Eraksoy, M., Garcia-Merino, A., Grigoriadis, N., Hartung, H.P., Havrdova, E., Hillert, J., Hohlfeld, R., Kremenchutzky, M., Lyon-Caen, O., Miller, A., Pozzilli, C., Ravnborg, M., Saida, T., Sindic, C., Vass, K., Clifford, D.B., Hauser, S., Major, E.O., O'Connor, P.W., Weiner, H.L., Clanet, M., Gold, R., Hirsch, H.H., Radu, E.W., Sorensen, P.S., King, J., 2011. Natalizumab treatment for multiple sclerosis: updated recommendations for patient selection and monitoring. Lancet neurology. 10**,** 745-58.

Kappos, L., Gold, R., Miller, D.H., MacManus, D.G., Havrdova, E., Limmroth, V., Polman, C.H., Schmierer, K., Yousry, T.A., Eraksoy, M., Meluzinova, E., Dufek, M., Yang, M., Dawson, K., O'Neill, G.N., 2012. Effect of BG-12 on contrast-enhanced lesions in patients with relapsing--remitting multiple sclerosis: subgroup analyses from the phase 2b study. Multiple sclerosis. 18**,** 314-21.

Karni, A., Bakimer-Kleiner, R., Abramsky, O., Ben-Nun, A., 1999. Elevated levels of antibody to myelin oligodendrocyte glycoprotein is not specific for patients with multiple sclerosis. Archives of neurology. 56**,** 311-5.

Katz, R., 2004. Biomarkers and surrogate markers: an FDA perspective. NeuroRx : the journal of the American Society for Experimental NeuroTherapeutics. 1**,** 189-95.

Kaufman, M., Moyer, D., Norton, J., 2000. The significant change for the Timed 25-foot Walk in the multiple sclerosis functional composite. Multiple sclerosis. 6**,** 286-90.

Kebir, H., Kreymborg, K., Ifergan, I., Dodelet-Devillers, A., Cayrol, R., Bernard, M., Giuliani, F., Arbour, N., Becher, B., Prat, A., 2007. Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. Nature medicine. 13**,** 1173-5.

Kennedy, M.K., Tan, L.J., Dal Canto, M.C., Tuohy, V.K., Lu, Z.J., Trotter, J.L., Miller, S.D., 1990. Inhibition of murine relapsing experimental autoimmune encephalomyelitis by immune tolerance to proteolipid protein and its encephalitogenic peptides. Journal of immunology. 144**,** 909-15.

Khademi, M., Bornsen, L., Rafatnia, F., Andersson, M., Brundin, L., Piehl, F., Sellebjerg, F., Olsson, T., 2009. The effects of natalizumab on inflammatory mediators in multiple sclerosis: prospects for treatment-sensitive biomarkers. European journal of neurology : the official journal of the European Federation of Neurological Societies. 16**,** 528-36.

Khatri, B., Barkhof, F., Comi, G., Hartung, H.P., Kappos, L., Montalban, X., Pelletier, J., Stites, T., Wu, S., Holdbrook, F., Zhang-Auberson, L., Francis, G., Cohen, J.A., 2011. Comparison of fingolimod with interferon beta-1a in relapsing-remitting multiple sclerosis: a randomised extension of the TRANSFORMS study. Lancet neurology. 10**,** 520-9.

Khoury, S.J., Guttmann, C.R., Orav, E.J., Hohol, M.J., Ahn, S.S., Hsu, L., Kikinis, R., Mackin, G.A., Jolesz, F.A., Weiner, H.L., 1994. Longitudinal MRI in multiple sclerosis: correlation between disability and lesion burden. Neurology. 44**,** 2120-4.

Kidd, D., Barkhof, F., McConnell, R., Algra, P.R., Allen, I.V., Revesz, T., 1999. Cortical lesions in multiple sclerosis. Brain : a journal of neurology. 122 (Pt 1)**,** 17-26.

Kieseier, B.C., 2011. The mechanism of action of interferon-beta in relapsing multiple sclerosis. CNS drugs. 25**,** 491-502.

Kimiskidis, V., Sakellari, I., Tsimourtou, V., Kapina, V., Papagiannopoulos, S., Kazis, D., Vlaikidis, N., Anagnostopoulos, A., Fassas, A., 2008. Autologous stem-cell transplantation in malignant multiple sclerosis: a case with a favorable long-term outcome. Mult Scler. 14**,** 278-83.

Kingwell, E., van der Kop, M., Zhao, Y., Shirani, A., Zhu, F., Oger, J., Tremlett, H., 2012. Relative mortality and survival in multiple sclerosis: findings from British Columbia, Canada. Journal of neurology, neurosurgery, and psychiatry. 83**,** 61-6.

Kinkel, R.P., Dontchev, M., Kollman, C., Skaramagas, T.T., O'Connor, P.W., Simon, J.H., 2012. Association between immediate initiation of intramuscular interferon beta-1a at the time of a clinically isolated syndrome and long-term outcomes: a 10-year follow-up of the Controlled High-Risk Avonex Multiple Sclerosis Prevention Study in Ongoing Neurological Surveillance. Archives of neurology. 69**,** 183-90.

Kinnunen, E., 1984. Multiple sclerosis in Finland: evidence of increasing frequency and uneven geographic distribution. Neurology. 34**,** 457-61.

Kirk, J., Plumb, J., Mirakhur, M., McQuaid, S., 2003. Tight junctional abnormality in multiple sclerosis white matter affects all calibres of vessel and is associated with blood-brain barrier leakage and active demyelination. The Journal of pathology. 201**,** 319-27.

Kivisakk, P., Imitola, J., Rasmussen, S., Elyaman, W., Zhu, B., Ransohoff, R.M., Khoury, S.J., 2009. Localizing central nervous system immune surveillance: meningeal antigen-presenting cells activate T cells during experimental autoimmune encephalomyelitis. Annals of neurology. 65**,** 457-69.

Kleinschmidt-DeMasters, B.K., Tyler, K.L., 2005. Progressive multifocal leukoencephalopathy complicating treatment with natalizumab and interferon beta-1a for multiple sclerosis. The New England journal of medicine. 353**,** 369-74.

Klistorner, A., Arvind, H., Nguyen, T., Garrick, R., Paine, M., Graham, S., O'Day, J., Grigg, J., Billson, F., Yiannikas, C., 2008. Axonal loss and myelin in early ON loss in postacute optic neuritis. Annals of neurology. 64**,** 325-31.

Koch, M., Mostert, J., Heersema, D., De Keyser, J., 2007. Progression in multiple sclerosis: further evidence of an age dependent process. Journal of the neurological sciences. 255**,** 35-41.

Koch, M., Mostert, J., De Keyser, J., Tremlett, H., Filippini, G., 2008. Interferon-beta treatment and the natural history of relapsing-remitting multiple sclerosis. Annals of neurology. 63**,** 125-6; author reply 126-7.

Koch, M., Kingwell, E., Rieckmann, P., Tremlett, H., 2009. The natural history of primary progressive multiple sclerosis. Neurology. 73**,** 1996-2002.

Koch, M., Kingwell, E., Rieckmann, P., Tremlett, H., 2010. The natural history of secondary progressive multiple sclerosis. Journal of neurology, neurosurgery, and psychiatry. 81**,** 1039-43.

Koch-Henriksen, N., Bronnum-Hansen, H., Stenager, E., 1998. Underlying cause of death in Danish patients with multiple sclerosis: results from the Danish Multiple Sclerosis Registry. Journal of neurology, neurosurgery, and psychiatry. 65**,** 56-9.

Koch-Henriksen, N., Sorensen, P.S., 2010. The changing demographic pattern of multiple sclerosis epidemiology. Lancet neurology. 9**,** 520-32.

Kooi, E.J., Geurts, J.J., van Horssen, J., Bo, L., van der Valk, P., 2009. Meningeal inflammation is not associated with cortical demyelination in chronic multiple sclerosis. Journal of neuropathology and experimental neurology. 68**,** 1021-8.

Korn, T., Magnus, T., Toyka, K., Jung, S., 2004. Modulation of effector cell functions in experimental autoimmune encephalomyelitis by leflunomide--mechanisms independent of pyrimidine depletion. Journal of leukocyte biology. 76**,** 950-60.

Korn, T., Reddy, J., Gao, W., Bettelli, E., Awasthi, A., Petersen, T.R., Backstrom, B.T., Sobel, R.A., Wucherpfennig, K.W., Strom, T.B., Oukka, M., Kuchroo, V.K., 2007. Myelin-specific regulatory T cells accumulate in the CNS but fail to control autoimmune inflammation. Nature medicine. 13**,** 423-31.

Korn, T., Bettelli, E., Oukka, M., Kuchroo, V.K., 2009. IL-17 and Th17 Cells. Annual review of immunology. 27**,** 485-517.

Kornek, B., Lassmann, H., 1999. Axonal pathology in multiple sclerosis. A historical note. Brain pathology. 9**,** 651-6.

Kornek, B., Storch, M.K., Weissert, R., Wallstroem, E., Stefferl, A., Olsson, T., Linington, C., Schmidbauer, M., Lassmann, H., 2000. Multiple sclerosis and chronic autoimmune encephalomyelitis: a comparative quantitative study of axonal injury in active, inactive, and remyelinated lesions. The American journal of pathology. 157**,** 267-76.

Kovacs, G.G., Hoftberger, R., Majtenyi, K., Horvath, R., Barsi, P., Komoly, S., Lassmann, H., Budka, H., Jakab, G., 2005. Neuropathology of white matter disease in Leber's hereditary optic neuropathy. Brain : a journal of neurology. 128**,** 35-41.

Kremenchutzky, M., Cottrell, D., Rice, G., Hader, W., Baskerville, J., Koopman, W., Ebers, G.C., 1999a. The natural history of multiple sclerosis: a geographically based study. 7. Progressive-relapsing and relapsing-progressive multiple sclerosis: a re-evaluation. Brain : a journal of neurology. 122 (Pt 10)**,** 1941-50.

Kremenchutzky, M., Cottrell, D., Rice, G., Hader, W., Baskerville, J., Koopman, W., Ebers, G.C., 1999b. The natural history of multiple sclerosis: a geographically based study. 7. Progressive-relapsing and relapsing-progressive multiple sclerosis: a re-evaluation. Brain. 122 (Pt 10)**,** 1941-50.

Kremenchutzky, M., Rice, G.P., Baskerville, J., Wingerchuk, D.M., Ebers, G.C., 2006a. The natural history of multiple sclerosis: a geographically based study 9: observations on the progressive phase of the disease. Brain : a journal of neurology. 129**,** 584-94.

Kremenchutzky, M., Rice, G.P., Baskerville, J., Wingerchuk, D.M., Ebers, G.C., 2006b. The natural history of multiple sclerosis: a geographically based study 9: observations on the progressive phase of the disease. Brain. 129, 584-94.

Kroenke, M.A., Carlson, T.J., Andjelkovic, A.V., Segal, B.M., 2008. IL-12- and IL-23-modulated T cells induce distinct types of EAE based on histology, CNS chemokine profile, and response to cytokine inhibition. The Journal of experimental medicine. 205, 1535-41.

Kuenz, B., Lutterotti, A., Ehling, R., Gneiss, C., Haemmerle, M., Rainer, C., Deisenhammer, F., Schocke, M., Berger, T., Reindl, M., 2008. Cerebrospinal fluid B cells correlate with early brain inflammation in multiple sclerosis. PloS one. 3, e2559.

Kuhle, J., Leppert, D., Petzold, A., Regeniter, A., Schindler, C., Mehling, M., Anthony, D.C., Kappos, L., Lindberg, R.L., 2011. Neurofilament heavy chain in CSF correlates with relapses and disability in multiple sclerosis. Neurology. 76, 1206-13.

Kuhlmann, T., Lingfeld, G., Bitsch, A., Schuchardt, J., Bruck, W., 2002. Acute axonal damage in multiple sclerosis is most extensive in early disease stages and decreases over time. Brain : a journal of neurology. 125, 2202-12.

Kuhlmann, T., Miron, V., Cui, Q., Wegner, C., Antel, J., Bruck, W., 2008. Differentiation block of oligodendroglial progenitor cells as a cause for remyelination failure in chronic multiple sclerosis. Brain : a journal of neurology. 131, 1749-58.

Kurtzke, J.F., 1955. A new scale for evaluating disability in multiple sclerosis. Neurology. 5, 580-3.

Kurtzke, J.F., Dean, G., Botha, D.P., 1970. A method for estimating the age at immigration of white immigrants to South Africa, with an example of its importance. South African medical journal = Suid-Afrikaanse tydskrif vir geneeskunde. 44, 663-9.

Kurtzke, J.F., Hyllested, K., 1975. Multiple sclerosis: an epidemic disease in the Faeroes. Transactions of the American Neurological Association. 100, 213-5.

Kurtzke, J.F., Beebe, G.W., Nagler, B., Kurland, L.T., Auth, T.L., 1977. Studies on the natural history of multiple sclerosis--8. Early prognostic features of the later course of the illness. Journal of chronic diseases. 30, 819-30.

Kurtzke, J.F., Beebe, G.W., Norman, J.E., Jr., 1979. Epidemiology of multiple sclerosis in U.S. veterans: 1. Race, sex, and geographic distribution. Neurology. 29, 1228-35.

Kurtzke, J.F., Gudmundsson, K.R., Bergmann, S., 1982. Multiple sclerosis in Iceland: 1. Evidence of a postwar epidemic. Neurology. 32, 143-50.

Kurtzke, J.F., 1983. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology. 33, 1444-52.

Kurtzke, J.F., Beebe, G.W., Norman, J.E., Jr., 1985. Epidemiology of multiple sclerosis in US veterans: III. Migration and the risk of MS. Neurology. 35, 672-8.

Kurtzke, J.F., Hyllested, K., Heltberg, A., Olsen, A., 1993. Multiple sclerosis in the Faroe Islands. 5. The occurrence of the fourth epidemic as validation of transmission. Acta neurologica Scandinavica. 88, 161-73.

Kurtzke, J.F., 2000. Multiple sclerosis in time and space--geographic clues to cause. Journal of neurovirology. 6 Suppl 2, S134-40.
Kurtzke, J.F., Heltberg, A., 2001. Multiple sclerosis in the Faroe Islands: an epitome. Journal of clinical epidemiology. 54, 1-22.
Kutok, J.L., Wang, F., 2006. Spectrum of Epstein-Barr virus-associated diseases. Annual review of pathology. 1, 375-404.
Kutzelnigg, A., Lucchinetti, C.F., Stadelmann, C., Bruck, W., Rauschka, H., Bergmann, M., Schmidbauer, M., Parisi, J.E., Lassmann, H., 2005. Cortical demyelination and diffuse white matter injury in multiple sclerosis. Brain : a journal of neurology. 128, 2705-12.
La Mantia, L., Vacchi, L., Rovaris, M., Di Pietrantonj, C., Ebers, G., Fredrikson, S., Filippini, G., 2012. Interferon beta for secondary progressive multiple sclerosis: a systematic review. Journal of neurology, neurosurgery, and psychiatry.
Langer-Gould, A., Atlas, S.W., Green, A.J., Bollen, A.W., Pelletier, D., 2005. Progressive multifocal leukoencephalopathy in a patient treated with natalizumab. The New England journal of medicine. 353, 375-81.
Larsen, J.P., Aarli, J.A., Nyland, H., Riise, T., 1984a. Western Norway, a high-risk area for multiple sclerosis: a prevalence/incidence study in the county of Hordaland. Neurology. 34, 1202-7.
Larsen, J.P., Kvaale, G., Riise, T., Nyland, H., Aarli, J.A., 1984b. An increase in the incidence of multiple sclerosis in western Norway. Acta neurologica Scandinavica. 70, 96-103.
Lassmann, H., Bruck, W., Lucchinetti, C., 2001. Heterogeneity of multiple sclerosis pathogenesis: implications for diagnosis and therapy. Trends in molecular medicine. 7, 115-21.
Lassmann, H., Ransohoff, R.M., 2004. The CD4-Th1 model for multiple sclerosis: a critical [correction of crucial] re-appraisal. Trends in immunology. 25, 132-7.
Lassmann, H., 2007. Multiple sclerosis: is there neurodegeneration independent from inflammation? Journal of the neurological sciences. 259, 3-6.
Lassmann, H., Bruck, W., Lucchinetti, C.F., 2007. The immunopathology of multiple sclerosis: an overview. Brain pathology. 17, 210-8.
Lassmann, H., 2010. What drives disease in multiple sclerosis: inflammation or neurodegeneration ? Clinical and experimental Neuroimmunology. 1, 2-11.
Lassmann, H., Niedobitek, G., Aloisi, F., Middeldorp, J.M., 2011. Epstein-Barr virus in the multiple sclerosis brain: a controversial issue--report on a focused workshop held in the Centre for Brain Research of the Medical University of Vienna, Austria. Brain : a journal of neurology. 134, 2772-86.
Lassmann, H., 2012. Cortical lesions in multiple sclerosis: inflammation versus neurodegeneration. Brain : a journal of neurology. 135, 2904-5.
Lassmann, H., van Horssen, J., Mahad, D., 2012. Progressive multiple sclerosis: pathology and pathogenesis. Nature reviews. Neurology. 8, 647-56.
Lau, K.K., Wong, L.K., Li, L.S., Chan, Y.W., Li, H.L., Wong, V., 2002. Epidemiological study of multiple sclerosis in Hong Kong Chinese: questionnaire survey. Hong Kong medical journal = Xianggang yi xue za zhi / Hong Kong Academy of Medicine. 8, 77-80.

Le Page, E., Leray, E., Taurin, G., Coustans, M., Chaperon, J., Morrissey, S.P., Edan, G., 2008. Mitoxantrone as induction treatment in aggressive relapsing remitting multiple sclerosis: treatment response factors in a 5 year follow-up observational study of 100 consecutive patients. J Neurol Neurosurg Psychiatry. 79, 52-6.

Lee, D.H., Linker, R.A., Gold, R., 2008. Spotlight on fumarates. International MS journal / MS Forum. 15, 12-8.

Leibowitz, U., Alter, M., Halpern, L., 1964. Clinical Studies of Multiple Sclerosis in Israel. 3. Clinical Course and Prognosis Related to Age at Onset. Neurology. 14, 926-32.

Leibowitz, U., Sharon, D., Alter, M., 1967. Geographical considerations in multiple sclerosis. Brain : a journal of neurology. 90, 871-86.

Leibowitz, U., Kahana, E., Alter, M., 1969. Survival and death in multiple sclerosis. Brain : a journal of neurology. 92, 115-30.

Lemire, J.M., Archer, D.C., 1991. 1,25-dihydroxyvitamin D3 prevents the in vivo induction of murine experimental autoimmune encephalomyelitis. The Journal of clinical investigation. 87, 1103-7.

Leray, E., Morrissey, S., Yaouanq, J., Coustans, M., Le Page, E., Chaperon, J., Edan, G., 2007. Long-term survival of patients with multiple sclerosis in West France. Multiple sclerosis. 13, 865-74.

Leray, E., Yaouanq, J., Le Page, E., Coustans, M., Laplaud, D., Oger, J., Edan, G., 2010. Evidence for a two-stage disability progression in multiple sclerosis. Brain : a journal of neurology. 133, 1900-13.

Levin, L.I., Munger, K.L., Rubertone, M.V., Peck, C.A., Lennette, E.T., Spiegelman, D., Ascherio, A., 2005. Temporal relationship between elevation of epstein-barr virus antibody titers and initial onset of neurological symptoms in multiple sclerosis. JAMA : the journal of the American Medical Association. 293, 2496-500.

Levin, L.I., Munger, K.L., O'Reilly, E.J., Falk, K.I., Ascherio, A., 2010. Primary infection with the Epstein-Barr virus and risk of multiple sclerosis. Annals of neurology. 67, 824-30.

Liguori, M., Marrosu, M.G., Pugliatti, M., Giuliani, F., De Robertis, F., Cocco, E., Zimatore, G.B., Livrea, P., Trojano, M., 2000. Age at onset in multiple sclerosis. Neurological sciences : official journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology. 21, S825-9.

Lin, W., Kemper, A., Dupree, J.L., Harding, H.P., Ron, D., Popko, B., 2006. Interferon-gamma inhibits central nervous system remyelination through a process modulated by endoplasmic reticulum stress. Brain : a journal of neurology. 129, 1306-18.

Lincoln, M.R., Montpetit, A., Cader, M.Z., Saarela, J., Dyment, D.A., Tiislar, M., Ferretti, V., Tienari, P.J., Sadovnick, A.D., Peltonen, L., Ebers, G.C., Hudson, T.J., 2005. A predominant role for the HLA class II region in the association of the MHC region with multiple sclerosis. Nature genetics. 37, 1108-12.

Link, J., Lorentzen, A.R., Kockum, I., Duvefelt, K., Lie, B.A., Celius, E.G., Harbo, H.F., Hillert, J., Brynedal, B., 2010. Two HLA class I genes independently associated with multiple sclerosis. Journal of neuroimmunology. 226, 172-6.

Liu, C., Blumhardt, L.D., 2000. Disability outcome measures in therapeutic trials of relapsing-remitting multiple sclerosis: effects of heterogeneity of disease course in placebo cohorts. Journal of neurology, neurosurgery, and psychiatry. 68**,** 450-7.

Lo, A.C., Saab, C.Y., Black, J.A., Waxman, S.G., 2003. Phenytoin protects spinal cord axons and preserves axonal conduction and neurological function in a model of neuroinflammation in vivo. Journal of neurophysiology. 90**,** 3566-71.

Lock, C., Hermans, G., Pedotti, R., Brendolan, A., Schadt, E., Garren, H., Langer-Gould, A., Strober, S., Cannella, B., Allard, J., Klonowski, P., Austin, A., Lad, N., Kaminski, N., Galli, S.J., Oksenberg, J.R., Raine, C.S., Heller, R., Steinman, L., 2002. Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. Nature medicine. 8**,** 500-8.

Locksley, R.M., Killeen, N., Lenardo, M.J., 2001. The TNF and TNF receptor superfamilies: integrating mammalian biology. Cell. 104**,** 487-501.

Looker, A.C., Pfeiffer, C.M., Lacher, D.A., Schleicher, R.L., Picciano, M.F., Yetley, E.A., 2008. Serum 25-hydroxyvitamin D status of the US population: 1988-1994 compared with 2000-2004. The American journal of clinical nutrition. 88**,** 1519-27.

Lou, Y.H., Park, K.K., Agersborg, S., Alard, P., Tung, K.S., 2000. Retargeting T cell-mediated inflammation: a new perspective on autoantibody action. Journal of immunology. 164**,** 5251-7.

Lovas, G., Szilagyi, N., Majtenyi, K., Palkovits, M., Komoly, S., 2000. Axonal changes in chronic demyelinated cervical spinal cord plaques. Brain : a journal of neurology. 123 (Pt 2)**,** 308-17.

Lublin, F.D., Lavasa, M., Viti, C., Knobler, R.L., 1987. Suppression of acute and relapsing experimental allergic encephalomyelitis with mitoxantrone. Clin Immunol Immunopathol. 45**,** 122-8.

Lublin, F.D., Reingold, S.C., 1996. 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. 46**,** 907-11.

Lublin, F.D., Baier, M., Cutter, G., 2003. Effect of relapses on development of residual deficit in multiple sclerosis. Neurology. 61**,** 1528-32.

Lublin, F.D., 2011. Relapses do not matter in relation to long-term disability: no (they do). Multiple sclerosis. 17**,** 1415-6.

Lucas, R.M., Hughes, A.M., Lay, M.L., Ponsonby, A.L., Dwyer, D.E., Taylor, B.V., Pender, M.P., 2011. Epstein-Barr virus and multiple sclerosis. Journal of neurology, neurosurgery, and psychiatry. 82**,** 1142-8.

Lucchinetti, C., Bruck, W., Parisi, J., Scheithauer, B., Rodriguez, M., Lassmann, H., 2000. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. Annals of neurology. 47**,** 707-17.

Lucchinetti, C.F., Popescu, B.F., Bunyan, R.F., Moll, N.M., Roemer, S.F., Lassmann, H., Bruck, W., Parisi, J.E., Scheithauer, B.W., Giannini, C., Weigand, S.D., Mandrekar, J., Ransohoff, R.M., 2011. Inflammatory cortical demyelination in early multiple sclerosis. The New England journal of medicine. 365**,** 2188-97.

Luks, T.L., Goodkin, D.E., Nelson, S.J., Majumdar, S., Bacchetti, P., Portnoy, D., Sloan, R., 2000. A longitudinal study of ventricular volume in early relapsing-remitting multiple sclerosis. Multiple sclerosis. 6, 332-7.

Lycke, J.N., Karlsson, J.E., Andersen, O., Rosengren, L.E., 1998. Neurofilament protein in cerebrospinal fluid: a potential marker of activity in multiple sclerosis. Journal of neurology, neurosurgery, and psychiatry. 64, 402-4.

Mackay, R.P., 1950. The familial occurrence of multiple sclerosis and its implications. Annals of internal medicine. 33, 298-320.

Maghzi, A.H., Ghazavi, H., Ahsan, M., Etemadifar, M., Mousavi, S., Khorvash, F., Minagar, A., 2010. Increasing female preponderance of multiple sclerosis in Isfahan, Iran: a population-based study. Multiple sclerosis. 16, 359-61.

Magliozzi, R., Howell, O., Vora, A., Serafini, B., Nicholas, R., Puopolo, M., Reynolds, R., Aloisi, F., 2007. Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. Brain : a journal of neurology. 130, 1089-104.

Magliozzi, R., Howell, O.W., Reeves, C., Roncaroli, F., Nicholas, R., Serafini, B., Aloisi, F., Reynolds, R., 2010. A Gradient of neuronal loss and meningeal inflammation in multiple sclerosis. Annals of neurology. 68, 477-93.

Maimone, D., Gregory, S., Arnason, B.G., Reder, A.T., 1991. Cytokine levels in the cerebrospinal fluid and serum of patients with multiple sclerosis. Journal of neuroimmunology. 32, 67-74.

Major, E.O., Amemiya, K., Tornatore, C.S., Houff, S.A., Berger, J.R., 1992. Pathogenesis and molecular biology of progressive multifocal leukoencephalopathy, the JC virus-induced demyelinating disease of the human brain. Clinical microbiology reviews. 5, 49-73.

Malmestrom, C., Haghighi, S., Rosengren, L., Andersen, O., Lycke, J., 2003. Neurofilament light protein and glial fibrillary acidic protein as biological markers in MS. Neurology. 61, 1720-5.

Mancardi, G., Hart, B.A., Capello, E., Brok, H.P., Ben-Nun, A., Roccatagliata, L., Giunti, D., Gazzola, P., Dono, M., Kerlero de Rosbo, N., Colombo, M., Uccelli, A., 2000. Restricted immune responses lead to CNS demyelination and axonal damage. Journal of neuroimmunology. 107, 178-83.

Mancardi, G., Saccardi, R., 2008. Autologous haematopoietic stem-cell transplantation in multiple sclerosis. Lancet Neurol. 7, 626-36.

Mancardi, G.L., Saccardi, R., Filippi, M., Gualandi, F., Murialdo, A., Inglese, M., Marrosu, M.G., Meucci, G., Massacesi, L., Lugaresi, A., Pagliai, F., Sormani, M.P., Sardanelli, F., Marmont, A., 2001. Autologous hematopoietic stem cell transplantation suppresses Gd-enhanced MRI activity in MS. Neurology. 57, 62-8.

Marik, C., Felts, P.A., Bauer, J., Lassmann, H., Smith, K.J., 2007. Lesion genesis in a subset of patients with multiple sclerosis: a role for innate immunity? Brain : a journal of neurology. 130, 2800-15.

Marrie, R.A., 2004. Environmental risk factors in multiple sclerosis aetiology. Lancet neurology. 3, 709-18.

Marriott, J.J., Miyasaki, J.M., Gronseth, G., O'Connor, P.W., 2010. Evidence Report: The efficacy and safety of mitoxantrone (Novantrone) in the treatment of multiple sclerosis: Report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. Neurology. 74, 1463-70.

Marrosu, M.G., Murru, R., Murru, M.R., Costa, G., Zavattari, P., Whalen, M., Cocco, E., Mancosu, C., Schirru, L., Solla, E., Fadda, E., Melis, C., Porru, I., Rolesu, M., Cucca, F., 2001. Dissection of the HLA association with multiple sclerosis in the founder isolated population of Sardinia. Human molecular genetics. 10**,** 2907-16.

Martinez-Yelamos, S., Martinez-Yelamos, A., Martin Ozaeta, G., Casado, V., Carmona, O., Arbizu, T., 2006. Regression to the mean in multiple sclerosis. Multiple sclerosis. 12**,** 826-9.

Masterman, T., Ligers, A., Olsson, T., Andersson, M., Olerup, O., Hillert, J., 2000. HLA-DR15 is associated with lower age at onset in multiple sclerosis. Annals of neurology. 48**,** 211-9.

Matsuoka, T., Matsushita, T., Osoegawa, M., Kawano, Y., Minohara, M., Mihara, F., Nishimura, Y., Ohyagi, Y., Kira, J., 2008. Association of the HLA-DRB1 alleles with characteristic MRI features of Asian multiple sclerosis. Multiple sclerosis. 14**,** 1181-90.

Matusevicius, D., Kivisakk, P., He, B., Kostulas, N., Ozenci, V., Fredrikson, S., Link, H., 1999. Interleukin-17 mRNA expression in blood and CSF mononuclear cells is augmented in multiple sclerosis. Multiple sclerosis. 5**,** 101-4.

Mayr, W.T., Pittock, S.J., McClelland, R.L., Jorgensen, N.W., Noseworthy, J.H., Rodriguez, M., 2003. Incidence and prevalence of multiple sclerosis in Olmsted County, Minnesota, 1985-2000. Neurology. 61**,** 1373-7.

Mc, A.D., Compston, N., 1952. Some aspects of the natural history of disseminated sclerosis. The Quarterly journal of medicine. 21**,** 135-67.

McAlpine, D., 1961. The benign form of multiple sclerosis. A study based on 241 cases seen within three years of onset and followed up until the tenth year or more of the disease. Brain : a journal of neurology. 84**,** 186-203.

McDonald, W.I., Miller, D.H., Thompson, A.J., 1994. Are magnetic resonance findings predictive of clinical outcome in therapeutic trials in multiple sclerosis? The dilemma of interferon-beta. Annals of neurology. 36**,** 14-8.

McDonald, W.I., Compston, A., Edan, G., Goodkin, D., Hartung, H.P., Lublin, F.D., McFarland, H.F., Paty, D.W., Polman, C.H., Reingold, S.C., Sandberg-Wollheim, M., Sibley, W., Thompson, A., van den Noort, S., Weinshenker, B.Y., Wolinsky, J.S., 2001. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. Annals of neurology. 50**,** 121-7.

McFarland, H.F., Martin, R., 2007. Multiple sclerosis: a complicated picture of autoimmunity. Nature immunology. 8**,** 913-9.

McGeachy, M.J., Stephens, L.A., Anderton, S.M., 2005. Natural recovery and protection from autoimmune encephalomyelitis: contribution of CD4+CD25+ regulatory cells within the central nervous system. Journal of immunology. 175**,** 3025-32.

McGraw, C.A., Lublin, F.D., 2012. Interferon Beta and Glatiramer Acetate Therapy. Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics.

McLeod, J.G., Hammond, S.R., Hallpike, J.F., 1994. Epidemiology of multiple sclerosis in Australia. With NSW and SA survey results. The Medical journal of Australia. 160**,** 117-22.

Mehling, M., Lindberg, R., Raulf, F., Kuhle, J., Hess, C., Kappos, L., Brinkmann, V., 2010. Th17 central memory T cells are reduced by FTY720 in patients with multiple sclerosis. Neurology. 75**,** 403-10.

Meinl, E., Krumbholz, M., Derfuss, T., Junker, A., Hohlfeld, R., 2008. Compartmentalization of inflammation in the CNS: a major mechanism driving progressive multiple sclerosis. Journal of the neurological sciences. 274**,** 42-4.

Melcon, M.O., Gold, L., Carra, A., Caceres, F., Correale, J., Cristiano, E., Fernandez Liguori, N., Garcea, O., Luetic, G., Kremenchutzky, M., 2008. Argentine Patagonia: prevalence and clinical features of multiple sclerosis. Multiple sclerosis. 14**,** 656-62.

Mendel, I., Kerlero de Rosbo, N., Ben-Nun, A., 1995. A myelin oligodendrocyte glycoprotein peptide induces typical chronic experimental autoimmune encephalomyelitis in H-2b mice: fine specificity and T cell receptor V beta expression of encephalitogenic T cells. European journal of immunology. 25**,** 1951-9.

Mews, I., Bergmann, M., Bunkowski, S., Gullotta, F., Bruck, W., 1998. Oligodendrocyte and axon pathology in clinically silent multiple sclerosis lesions. Multiple sclerosis. 4**,** 55-62.

Micu, I., Jiang, Q., Coderre, E., Ridsdale, A., Zhang, L., Woulfe, J., Yin, X., Trapp, B.D., McRory, J.E., Rehak, R., Zamponi, G.W., Wang, W., Stys, P.K., 2006. NMDA receptors mediate calcium accumulation in myelin during chemical ischaemia. Nature. 439**,** 988-92.

Midgard, R., Riise, T., Nyland, H., 1991. Epidemiologic trends in multiple sclerosis in More and Romsdal, Norway: a prevalence/incidence study in a stable population. Neurology. 41**,** 887-92.

Midgard, R., Albrektsen, G., Riise, T., Kvale, G., Nyland, H., 1995. Prognostic factors for survival in multiple sclerosis: a longitudinal, population based study in More and Romsdal, Norway. Journal of neurology, neurosurgery, and psychiatry. 58**,** 417-21.

Midgard, R., Riise, T., Svanes, C., Kvale, G., Nyland, H., 1996. Incidence of multiple sclerosis in More and Romsdal, Norway from 1950 to 1991. An age-period-cohort analysis. Brain : a journal of neurology. 119 (Pt 1)**,** 203-11.

Mikol, D.D., Barkhof, F., Chang, P., Coyle, P.K., Jeffery, D.R., Schwid, S.R., Stubinski, B., Uitdehaag, B., 2008. Comparison of subcutaneous interferon beta-1a with glatiramer acetate in patients with relapsing multiple sclerosis (the REbif vs Glatiramer Acetate in Relapsing MS Disease [REGARD] study): a multicentre, randomised, parallel, open-label trial. Lancet neurology. 7**,** 903-14.

Milanese, C., La Mantia, L., Palumbo, R., Martinelli, V., Murialdo, A., Zaffaroni, M., Caputo, D., Capra, R., Bergamaschi, R., 2003. A post-marketing study on interferon beta 1b and 1a treatment in relapsing-remitting multiple sclerosis: different response in drop-outs and treated patients. Journal of neurology, neurosurgery, and psychiatry. 74**,** 1689-92.

Milanov, I., Georgiev, D., Kmetska, K., Jordanova, L., Topalov, N., 1997. Prevalence of multiple sclerosis in Bulgaria. Neuroepidemiology. 16**,** 304-7.

Miller, D.H., Hornabrook, R.W., Purdie, G., 1992. The natural history of multiple sclerosis: a regional study with some longitudinal data. Journal of neurology, neurosurgery, and psychiatry. 55**,** 341-6.

Miller, D.H., Albert, P.S., Barkhof, F., Francis, G., Frank, J.A., Hodgkinson, S., Lublin, F.D., Paty, D.W., Reingold, S.C., Simon, J., 1996. Guidelines for the use of magnetic resonance techniques in monitoring the treatment of multiple sclerosis. US National MS Society Task Force. Annals of neurology. 39**,** 6-16.

Miller, D.H., Weinshenker, B.G., Filippi, M., Banwell, B.L., Cohen, J.A., Freedman, M.S., Galetta, S.L., Hutchinson, M., Johnson, R.T., Kappos, L., Kira, J., Lublin, F.D., McFarland, H.F., Montalban, X., Panitch, H., Richert, J.R., Reingold, S.C., Polman, C.H., 2008. Differential diagnosis of suspected multiple sclerosis: a consensus approach. Multiple sclerosis. 14**,** 1157-74.

Miller, D.H., Chard, D.T., Ciccarelli, O., 2012. Clinically isolated syndromes. Lancet neurology. 11**,** 157-69.

Minderhoud, J.M., Prange, A.J., Luyckx, G.J., 1988a. A long-term double-blind controlled study on the effect of azathioprine in the treatment of multiple sclerosis. Clinical neurology and neurosurgery. 90**,** 25-8.

Minderhoud, J.M., van der Hoeven, J.H., Prange, A.J., 1988b. Course and prognosis of chronic progressive multiple sclerosis. Results of an epidemiological study. Acta neurologica Scandinavica. 78**,** 10-5.

Minneboo, A., Barkhof, F., Polman, C.H., Uitdehaag, B.M., Knol, D.L., Castelijns, J.A., 2004. Infratentorial lesions predict long-term disability in patients with initial findings suggestive of multiple sclerosis. Archives of neurology. 61**,** 217-21.

Mladenovic, V., Domljan, Z., Rozman, B., Jajic, I., Mihajlovic, D., Dordevic, J., Popovic, M., Dimitrijevic, M., Zivkovic, M., Campion, G., et al., 1995. Safety and effectiveness of leflunomide in the treatment of patients with active rheumatoid arthritis. Results of a randomized, placebo-controlled, phase II study. Arthritis and rheumatism. 38**,** 1595-603.

Montalban, X., Tintore, M., Swanton, J., Barkhof, F., Fazekas, F., Filippi, M., Frederiksen, J., Kappos, L., Palace, J., Polman, C., Rovaris, M., de Stefano, N., Thompson, A., Yousry, T., Rovira, A., Miller, D.H., 2010. MRI criteria for MS in patients with clinically isolated syndromes. Neurology. 74**,** 427-34.

Montgomery, S.M., Bahmanyar, S., Hillert, J., Ekbom, A., Olsson, T., 2008. Maternal smoking during pregnancy and multiple sclerosis amongst offspring. European journal of neurology : the official journal of the European Federation of Neurological Societies. 15**,** 1395-9.

Mowry, E.M., Deen, S., Malikova, I., Pelletier, J., Bacchetti, P., Waubant, E., 2009a. The onset location of multiple sclerosis predicts the location of subsequent relapses. Journal of neurology, neurosurgery, and psychiatry. 80**,** 400-3.

Mowry, E.M., Pesic, M., Grimes, B., Deen, S., Bacchetti, P., Waubant, E., 2009b. Demyelinating events in early multiple sclerosis have inherent severity and recovery. Neurology. 72**,** 602-8.

Mowry, E.M., Pesic, M., Grimes, B., Deen, S.R., Bacchetti, P., Waubant, E., 2009c. Clinical predictors of early second event in patients with clinically isolated syndrome. Journal of neurology. 256**,** 1061-6.

Mowry, E.M., Carey, R.F., Blasco, M.R., Pelletier, J., Duquette, P., Villoslada, P., Malikova, I., Roger, E., Kinkel, R.P., McDonald, J., Bacchetti, P., Waubant, E., 2013a. Association of multiple sclerosis susceptibility variants and early attack location in the CNS. PloS one. 8**,** e75565.

Mowry, E.M., Carey, R.F., Blasco, M.R., Pelletier, J., Duquette, P., Villoslada, P., Malikova, I., Roger, E., Kinkel, R.P., McDonald, J., Bacchetti, P., Waubant, E., 2013b. Multiple sclerosis susceptibility genes: associations with relapse severity and recovery. PloS one. 8**,** e75416.

Mrowietz, U., Christophers, E., Altmeyer, P., 1999. Treatment of severe psoriasis with fumaric acid esters: scientific background and guidelines for therapeutic use. The German Fumaric Acid Ester Consensus Conference. The British journal of dermatology. 141**,** 424-9.

Muller, R., 1949. Studies on disseminated sclerosis with special reference to symptomatology, course and prognosis. Acta medica Scandinavica. 222**,** 1-214.

Muller, R., 1951. The clinical aspects of disseminated sclerosis. Acta psychiatrica et neurologica Scandinavica. Supplementum. 74**,** 32-47.

Munger, K.L., Zhang, S.M., O'Reilly, E., Hernan, M.A., Olek, M.J., Willett, W.C., Ascherio, A., 2004. Vitamin D intake and incidence of multiple sclerosis. Neurology. 62**,** 60-5.

Munger, K.L., Levin, L.I., Hollis, B.W., Howard, N.S., Ascherio, A., 2006. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. JAMA : the journal of the American Medical Association. 296**,** 2832-8.

Muraro, P.A., Cassiani Ingoni, R., Martin, R., 2003. Hematopoietic stem cell transplantation for multiple sclerosis: current status and future challenges. Curr Opin Neurol. 16**,** 299-305.

Mutluay, F.K., Gurses, H.N., Saip, S., 2005. Effects of multiple sclerosis on respiratory functions. Clinical rehabilitation. 19**,** 426-32.

Myhr, K.M., Riise, T., Vedeler, C., Nortvedt, M.W., Gronning, R., Midgard, R., Nyland, H.I., 2001. Disability and prognosis in multiple sclerosis: demographic and clinical variables important for the ability to walk and awarding of disability pension. Multiple sclerosis. 7**,** 59-65.

Narayana, P.A., 2005. Magnetic resonance spectroscopy in the monitoring of multiple sclerosis. Journal of neuroimaging : official journal of the American Society of Neuroimaging. 15**,** 46S-57S.

Nataf, S., Garcion, E., Darcy, F., Chabannes, D., Muller, J.Y., Brachet, P., 1996. 1,25 Dihydroxyvitamin D3 exerts regional effects in the central nervous system during experimental allergic encephalomyelitis. Journal of neuropathology and experimental neurology. 55**,** 904-14.

Newhook, L.A., Sloka, S., Grant, M., Randell, E., Kovacs, C.S., Twells, L.K., 2009. Vitamin D insufficiency common in newborns, children and pregnant women living in Newfoundland and Labrador, Canada. Maternal & child nutrition. 5**,** 186-91.

Nguyen, L.T., Ramanathan, M., Munschauer, F., Brownscheidle, C., Krantz, S., Umhauer, M., Miller, C., DeNardin, E., Jacobs, L.D., 1999. Flow cytometric analysis of in vitro proinflammatory cytokine secretion in peripheral blood from multiple sclerosis patients. Journal of clinical immunology. 19**,** 179-85.

Nicholas, R., Straube, S., Schmidli, H., Schneider, S., Friede, T., 2011. Trends in annualized relapse rates in relapsing-remitting multiple sclerosis and consequences for clinical trial design. Multiple sclerosis. 17**,** 1211-7.

Nicoletti, A., Patti, F., Lo Fermo, S., Sorbello, V., Reggio, E., Maimone, D., Zappia, M., Reggio, A., 2005. Possible increasing risk of multiple sclerosis in Catania, Sicily. Neurology. 65**,** 1259-63.

Nielsen, N.M., Rostgaard, K., Rasmussen, S., Koch-Henriksen, N., Storm, H.H., Melbye, M., Hjalgrim, H., 2006. Cancer risk among patients with multiple sclerosis: a population-based register study. International journal of cancer. Journal international du cancer. 118**,** 979-84.

Nielsen, T.R., Rostgaard, K., Nielsen, N.M., Koch-Henriksen, N., Haahr, S., Sorensen, P.S., Hjalgrim, H., 2007. Multiple sclerosis after infectious mononucleosis. Archives of neurology. 64**,** 72-5.

Nielsen, T.R., Rostgaard, K., Askling, J., Steffensen, R., Oturai, A., Jersild, C., Koch-Henriksen, N., Sorensen, P.S., Hjalgrim, H., 2009. Effects of infectious mononucleosis and HLA-DRB1*15 in multiple sclerosis. Multiple sclerosis. 15**,** 431-6.

Noonan, C.W., Kathman, S.J., White, M.C., 2002. Prevalence estimates for MS in the United States and evidence of an increasing trend for women. Neurology. 58**,** 136-8.

Norgren, N., Sundstrom, P., Svenningsson, A., Rosengren, L., Stigbrand, T., Gunnarsson, M., 2004. Neurofilament and glial fibrillary acidic protein in multiple sclerosis. Neurology. 63**,** 1586-90.

Noseworthy, J.H., Vandervoort, M.K., Hopkins, M., Ebers, G.C., 1989. A referendum on clinical trial research in multiple sclerosis: the opinion of the participants at the Jekyll Island workshop. Neurology. 39**,** 977-81.

Noseworthy, J.H., Vandervoort, M.K., Wong, C.J., Ebers, G.C., 1990. Interrater variability with the Expanded Disability Status Scale (EDSS) and Functional Systems (FS) in a multiple sclerosis clinical trial. The Canadian Cooperation MS Study Group. Neurology. 40**,** 971-5.

O'Connor, P., Filippi, M., Arnason, B., Comi, G., Cook, S., Goodin, D., Hartung, H.P., Jeffery, D., Kappos, L., Boateng, F., Filippov, V., Groth, M., Knappertz, V., Kraus, C., Sandbrink, R., Pohl, C., Bogumil, T., 2009. 250 microg or 500 microg interferon beta-1b versus 20 mg glatiramer acetate in relapsing-remitting multiple sclerosis: a prospective, randomised, multicentre study. Lancet neurology. 8**,** 889-97.

O'Connor, P., Wolinsky, J.S., Confavreux, C., Comi, G., Kappos, L., Olsson, T.P., Benzerdjeb, H., Truffinet, P., Wang, L., Miller, A., Freedman, M.S., 2011. Randomized trial of oral teriflunomide for relapsing multiple sclerosis. The New England journal of medicine. 365**,** 1293-303.

O'Connor, P.W., Li, D., Freedman, M.S., Bar-Or, A., Rice, G.P., Confavreux, C., Paty, D.W., Stewart, J.A., Scheyer, R., 2006. A Phase II study of the safety and efficacy of teriflunomide in multiple sclerosis with relapses. Neurology. 66**,** 894-900.

Oksenberg, J.R., Panzara, M.A., Begovich, A.B., Mitchell, D., Erlich, H.A., Murray, R.S., Shimonkevitz, R., Sherritt, M., Rothbard, J., Bernard, C.C., et al., 1993. Selection for T-cell receptor V beta-D beta-J beta gene rearrangements with specificity for a myelin basic protein peptide in brain lesions of multiple sclerosis. Nature. 362**,** 68-70.

Okuda, D.T., Mowry, E.M., Beheshtian, A., Waubant, E., Baranzini, S.E., Goodin, D.S., Hauser, S.L., Pelletier, D., 2009. Incidental MRI anomalies suggestive

of multiple sclerosis: the radiologically isolated syndrome. Neurology. 72**,** 800-5.

Olsson, T., Zhi, W.W., Hojeberg, B., Kostulas, V., Jiang, Y.P., Anderson, G., Ekre, H.P., Link, H., 1990. Autoreactive T lymphocytes in multiple sclerosis determined by antigen-induced secretion of interferon-gamma. The Journal of clinical investigation. 86**,** 981-5.

Ormerod, I.E., Miller, D.H., McDonald, W.I., du Boulay, E.P., Rudge, P., Kendall, B.E., Moseley, I.F., Johnson, G., Tofts, P.S., Halliday, A.M., et al., 1987. The role of NMR imaging in the assessment of multiple sclerosis and isolated neurological lesions. A quantitative study. Brain : a journal of neurology. 110 (Pt 6)**,** 1579-616.

Orton, S.M., Herrera, B.M., Yee, I.M., Valdar, W., Ramagopalan, S.V., Sadovnick, A.D., Ebers, G.C., 2006. Sex ratio of multiple sclerosis in Canada: a longitudinal study. Lancet neurology. 5**,** 932-6.

Orton, S.M., Ramagopalan, S.V., Brocklebank, D., Herrera, B.M., Dyment, D.A., Yee, I.M., Sadovnick, A.D., Ebers, G.C., 2010. Effect of immigration on multiple sclerosis sex ratio in Canada: the Canadian Collaborative Study. Journal of neurology, neurosurgery, and psychiatry. 81**,** 31-6.

Osoegawa, M., Kira, J., Fukazawa, T., Fujihara, K., Kikuchi, S., Matsui, M., Kohriyama, T., Sobue, G., Yamamura, T., Itoyama, Y., Saida, T., Sakata, K., Ochi, H., Matsuoka, T., 2009. Temporal changes and geographical differences in multiple sclerosis phenotypes in Japanese: nationwide survey results over 30 years. Multiple sclerosis. 15**,** 159-73.

Pachner, A.R., 2011. Experimental models of multiple sclerosis. Current opinion in neurology. 24**,** 291-9.

Pakpoor, J., Disanto, G., Gerber, J.E., Dobson, R., Meier, U.C., Giovannoni, G., Ramagopalan, S.V., 2012. The risk of developing multiple sclerosis in individuals seronegative for Epstein-Barr virus: a meta-analysis. Multiple sclerosis.

Palacios, N., Alonso, A., Bronnum-Hansen, H., Ascherio, A., 2011. Smoking and increased risk of multiple sclerosis: parallel trends in the sex ratio reinforce the evidence. Annals of epidemiology. 21**,** 536-42.

Panelius, M., 1969. Studies on epidemiological, clinical and etiological aspects of multiple sclerosis. Acta neurologica Scandinavica. Suppl 39:1-82.

Panitch, H., Goodin, D.S., Francis, G., Chang, P., Coyle, P.K., O'Connor, P., Monaghan, E., Li, D., Weinshenker, B., 2002. Randomized, comparative study of interferon beta-1a treatment regimens in MS: The EVIDENCE Trial. Neurology. 59**,** 1496-506.

Panitch, H., Miller, A., Paty, D., Weinshenker, B., 2004. Interferon beta-1b in secondary progressive MS: results from a 3-year controlled study. Neurology. 63**,** 1788-95.

Panitch, H.S., Hirsch, R.L., Schindler, J., Johnson, K.P., 1987. Treatment of multiple sclerosis with gamma interferon: exacerbations associated with activation of the immune system. Neurology. 37**,** 1097-102.

Paolillo, A., Coles, A.J., Molyneux, P.D., Gawne-Cain, M., MacManus, D., Barker, G.J., Compston, D.A., Miller, D.H., 1999. Quantitative MRI in patients with secondary progressive MS treated with monoclonal antibody Campath 1H. Neurology. 53**,** 751-7.

Parry, A., Clare, S., Jenkinson, M., Smith, S., Palace, J., Matthews, P.M., 2002. White matter and lesion T1 relaxation times increase in parallel and correlate with disability in multiple sclerosis. Journal of neurology. 249**,** 1279-86.

Patrikios, P., Stadelmann, C., Kutzelnigg, A., Rauschka, H., Schmidbauer, M., Laursen, H., Sorensen, P.S., Bruck, W., Lucchinetti, C., Lassmann, H., 2006. Remyelination is extensive in a subset of multiple sclerosis patients. Brain : a journal of neurology. 129**,** 3165-72.

Paty, D.W., Oger, J.J., Kastrukoff, L.F., Hashimoto, S.A., Hooge, J.P., Eisen, A.A., Eisen, K.A., Purves, S.J., Low, M.D., Brandejs, V., et al., 1988. MRI in the diagnosis of MS: a prospective study with comparison of clinical evaluation, evoked potentials, oligoclonal banding, and CT. Neurology. 38**,** 180-5.

Paty, D.W., Li, D.K., 1993. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. II. MRI analysis results of a multicenter, randomized, double-blind, placebo-controlled trial. UBC MS/MRI Study Group and the IFNB Multiple Sclerosis Study Group. Neurology. 43**,** 662-7.

Patzold, U., Pocklington, P.R., 1982. Course of multiple sclerosis. First results of a prospective study carried out of 102 MS patients from 1976-1980. Acta neurologica Scandinavica. 65**,** 248-66.

Peferoen, L.A., Lamers, F., Lodder, L.N., Gerritsen, W.H., Huitinga, I., Melief, J., Giovannoni, G., Meier, U., Hintzen, R.Q., Verjans, G.M., van Nierop, G.P., Vos, W., Peferoen-Baert, R.M., Middeldorp, J.M., van der Valk, P., Amor, S., 2010. Epstein Barr virus is not a characteristic feature in the central nervous system in established multiple sclerosis. Brain : a journal of neurology. 133**,** e137.

Pekmezovic, T., Jarebinski, M., Drulovic, J., Stojsavljevic, N., Levic, Z., 2001. Prevalence of multiple sclerosis in Belgrade, Yugoslavia. Acta neurologica Scandinavica. 104**,** 353-7.

Pelletier, D., Hafler, D.A., 2012. Fingolimod for multiple sclerosis. The New England journal of medicine. 366**,** 339-47.

Pender, M.P., 2011. The essential role of Epstein-Barr virus in the pathogenesis of multiple sclerosis. The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry. 17**,** 351-67.

Perkin, G.D., Bowden, P., Rose, F.C., 1975. Smoking and optic neuritis. Postgraduate medical journal. 51**,** 382-5.

Peterson, J.W., Bo, L., Mork, S., Chang, A., Trapp, B.D., 2001. Transected neurites, apoptotic neurons, and reduced inflammation in cortical multiple sclerosis lesions. Annals of neurology. 50**,** 389-400.

Peto, R., Pike, M.C., Armitage, P., Breslow, N.E., Cox, D.R., Howard, S.V., Mantel, N., McPherson, K., Peto, J., Smith, P.G., 1977. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. analysis and examples. British journal of cancer. 35**,** 1-39.

Pettinelli, C.B., McFarlin, D.E., 1981. Adoptive transfer of experimental allergic encephalomyelitis in SJL/J mice after in vitro activation of lymph node cells by myelin basic protein: requirement for Lyt 1+ 2- T lymphocytes. Journal of immunology. 127**,** 1420-3.

Petzold, A., Eikelenboom, M.J., Keir, G., Grant, D., Lazeron, R.H., Polman, C.H., Uitdehaag, B.M., Thompson, E.J., Giovannoni, G., 2005. Axonal damage accumulates in the progressive phase of multiple sclerosis: three year

follow up study. Journal of neurology, neurosurgery, and psychiatry. 76, 206-11.

Petzold, A., de Boer, J.F., Schippling, S., Vermersch, P., Kardon, R., Green, A., Calabresi, P.A., Polman, C., 2010. Optical coherence tomography in multiple sclerosis: a systematic review and meta-analysis. Lancet neurology. 9, 921-32.

Phadke, J.G., 1987. Survival pattern and cause of death in patients with multiple sclerosis: results from an epidemiological survey in north east Scotland. Journal of neurology, neurosurgery, and psychiatry. 50, 523-31.

Phadke, J.G., 1990. Clinical aspects of multiple sclerosis in north-east Scotland with particular reference to its course and prognosis. Brain : a journal of neurology. 113 (Pt 6), 1597-628.

Pina, M.A., Ara, J.R., Lasierra, P., Modrego, P.J., Larrad, L., 1999. Study of HLA as a predisposing factor and its possible influence on the outcome of multiple sclerosis in the sanitary district of Calatayud, northern Spain. Neuroepidemiology. 18, 203-9.

Pirko, I., Lucchinetti, C.F., Sriram, S., Bakshi, R., 2007. Gray matter involvement in multiple sclerosis. Neurology. 68, 634-42.

Pittock, S.J., Weinshenker, B.G., Noseworthy, J.H., Lucchinetti, C.F., Keegan, M., Wingerchuk, D.M., Carter, J., Shuster, E., Rodriguez, M., 2006. Not every patient with multiple sclerosis should be treated at time of diagnosis. Archives of neurology. 63, 611-4.

Pittock, S.J., 2007. Interferon beta in multiple sclerosis: how much BENEFIT? Lancet. 370, 363-4.

Pittock, S.J., 2009. Uncertain BENEFIT of early interferon beta-1b treatment. Lancet neurology. 8, 970-1.

Polman, C., Barkhof, F., Sandberg-Wollheim, M., Linde, A., Nordle, O., Nederman, T., 2005a. Treatment with laquinimod reduces development of active MRI lesions in relapsing MS. Neurology. 64, 987-91.

Polman, C.H., Reingold, S.C., Edan, G., Filippi, M., Hartung, H.P., Kappos, L., Lublin, F.D., Metz, L.M., McFarland, H.F., O'Connor, P.W., Sandberg-Wollheim, M., Thompson, A.J., Weinshenker, B.G., Wolinsky, J.S., 2005b. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". Annals of neurology. 58, 840-6.

Polman, C.H., O'Connor, P.W., Havrdova, E., Hutchinson, M., Kappos, L., Miller, D.H., Phillips, J.T., Lublin, F.D., Giovannoni, G., Wajgt, A., Toal, M., Lynn, F., Panzara, M.A., Sandrock, A.W., 2006. A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. The New England journal of medicine. 354, 899-910.

Polman, C.H., Rudick, R.A., 2010. The multiple sclerosis functional composite: a clinically meaningful measure of disability. Neurology. 74 Suppl 3, S8-15.

Polman, C.H., Reingold, S.C., Banwell, B., Clanet, M., Cohen, J.A., Filippi, M., Fujihara, K., Havrdova, E., Hutchinson, M., Kappos, L., Lublin, F.D., Montalban, X., O'Connor, P., Sandberg-Wollheim, M., Thompson, A.J., Waubant, E., Weinshenker, B., Wolinsky, J.S., 2011. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Annals of neurology. 69, 292-302.

Poppe, A.Y., Wolfson, C., Zhu, B., 2008. Prevalence of multiple sclerosis in Canada: a systematic review. The Canadian journal of neurological sciences. Le journal canadien des sciences neurologiques. 35, 593-601.

Portaccio, E., Amato, M.P., Siracusa, G., Pagliai, F., Sorbi, S., Guidi, S., Bosi, A., Saccardi, R., 2007. Autologous hematopoietic stem cell transplantation for very active relapsing-remitting multiple sclerosis: report of two cases. Mult Scler. In press.

Poser, C.M., Paty, D.W., Scheinberg, L., McDonald, W.I., Davis, F.A., Ebers, G.C., Johnson, K.P., Sibley, W.A., Silberberg, D.H., Tourtellotte, W.W., 1983. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Annals of neurology. 13, 227-31.

Poser, C.M., Hibberd, P.L., Benedikz, J., Gudmundsson, G., 1988. Analysis of the 'epidemic' of multiple sclerosis in the Faroe Islands. I. Clinical and epidemiological aspects. Neuroepidemiology. 7, 168-80.

Poser, C.M., Brinar, V.V., 2004. Diagnostic criteria for multiple sclerosis: an historical review. Clinical neurology and neurosurgery. 106, 147-58.

Poser, S., Bauer, H.J., Poser, W., 1982a. Prognosis of multiple sclerosis. Results from an epidemiological area in Germany. Acta neurologica Scandinavica. 65, 347-54.

Poser, S., Raun, N.E., Poser, W., 1982b. Age at onset, initial symptomatology and the course of multiple sclerosis. Acta neurologica Scandinavica. 66, 355-62.

Poser, S., Stickel, B., Krtsch, U., Burckhardt, D., Nordman, B., 1989. Increasing incidence of multiple sclerosis in South Lower Saxony, Germany. Neuroepidemiology. 8, 207-13.

Pozzilli, C., Prosperini, L., Sbardella, E., De Giglio, L., Onesti, E., Tomassini, V., 2005. Post-marketing survey on clinical response to interferon beta in relapsing multiple sclerosis: the Roman experience. Neurological sciences : official journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology. 26 Suppl 4, S174-8.

Prat, A., Biernacki, K., Antel, J.P., 2005. Th1 and Th2 lymphocyte migration across the human BBB is specifically regulated by interferon beta and copolymer-1. Journal of autoimmunity. 24, 119-24.

Prencipe, M., Ferretti, C., Casini, A.R., Santini, M., Giubilei, F., Culasso, F., 1997. Stroke, disability, and dementia: results of a population survey. Stroke; a journal of cerebral circulation. 28, 531-6.

Prineas, J.W., Barnard, R.O., Kwon, E.E., Sharer, L.R., Cho, E.S., 1993. Multiple sclerosis: remyelination of nascent lesions. Annals of neurology. 33, 137-51.

Prineas, J.W., Kwon, E.E., Cho, E.S., Sharer, L.R., Barnett, M.H., Oleszak, E.L., Hoffman, B., Morgan, B.P., 2001. Immunopathology of secondary-progressive multiple sclerosis. Annals of neurology. 50, 646-57.

Pueyo, V., Martin, J., Fernandez, J., Almarcegui, C., Ara, J., Egea, C., Pablo, L., Honrubia, F., 2008. Axonal loss in the retinal nerve fiber layer in patients with multiple sclerosis. Multiple sclerosis. 14, 609-14.

Pugliatti, M., Sotgiu, S., Rosati, G., 2002. The worldwide prevalence of multiple sclerosis. Clinical neurology and neurosurgery. 104, 182-91.

Pugliatti, M., Riise, T., Sotgiu, M.A., Sotgiu, S., Satta, W.M., Mannu, L., Sanna, G., Rosati, G., 2005. Increasing incidence of multiple sclerosis in the province of Sassari, northern Sardinia. Neuroepidemiology. 25, 129-34.

Radhakrishnan, K., Ashok, P.P., Sridharan, R., Mousa, M.E., 1985. Prevalence and pattern of multiple sclerosis in Benghazi, north-eastern Libya. Journal of the neurological sciences. 70, 39-46.

Ragonese, P., Aridon, P., Salemi, G., D'Amelio, M., Savettieri, G., 2008. Mortality in multiple sclerosis: a review. European journal of neurology : the official journal of the European Federation of Neurological Societies. 15, 123-7.

Ragonese, P., Aridon, P., Mazzola, M.A., Callari, G., Palmeri, B., Famoso, G., Terruso, V., Salemi, G., D'Amelio, M., Savettieri, G., 2010. Multiple sclerosis survival: a population-based study in Sicily. European journal of neurology : the official journal of the European Federation of Neurological Societies. 17, 391-7.

Ramagopalan, S.V., Morris, A.P., Dyment, D.A., Herrera, B.M., DeLuca, G.C., Lincoln, M.R., Orton, S.M., Chao, M.J., Sadovnick, A.D., Ebers, G.C., 2007. The inheritance of resistance alleles in multiple sclerosis. PLoS genetics. 3, 1607-13.

Ramagopalan, S.V., Deluca, G.C., Degenhardt, A., Ebers, G.C., 2008. The genetics of clinical outcome in multiple sclerosis. Journal of neuroimmunology. 201-202, 183-99.

Ramagopalan, S.V., Knight, J.C., Ebers, G.C., 2009a. Multiple sclerosis and the major histocompatibility complex. Current opinion in neurology. 22, 219-25.

Ramagopalan, S.V., McMahon, R., Dyment, D.A., Sadovnick, A.D., Ebers, G.C., Wittkowski, K.M., 2009b. An extension to a statistical approach for family based association studies provides insights into genetic risk factors for multiple sclerosis in the HLA-DRB1 gene. BMC medical genetics. 10, 10.

Ramagopalan, S.V., Valdar, W., Dyment, D.A., DeLuca, G.C., Yee, I.M., Giovannoni, G., Ebers, G.C., Sadovnick, A.D., 2009c. Association of infectious mononucleosis with multiple sclerosis. A population-based study. Neuroepidemiology. 32, 257-62.

Ramagopalan, S.V., Byrnes, J.K., Orton, S.M., Dyment, D.A., Guimond, C., Yee, I.M., Ebers, G.C., Sadovnick, A.D., 2010. Sex ratio of multiple sclerosis and clinical phenotype. European journal of neurology : the official journal of the European Federation of Neurological Societies. 17, 634-7.

Ramagopalan, S.V., Handel, A.E., Giovannoni, G., Rutherford Siegel, S., Ebers, G.C., Chaplin, G., 2011. Relationship of UV exposure to prevalence of multiple sclerosis in England. Neurology. 76, 1410-4.

Ramakrishna, C., Stohlman, S.A., Atkinson, R.A., Hinton, D.R., Bergmann, C.C., 2004. Differential regulation of primary and secondary CD8+ T cells in the central nervous system. Journal of immunology. 173, 6265-73.

Ramsaransing, G.S., De Keyser, J., 2006. Benign course in multiple sclerosis: a review. Acta Neurol Scand. 113, 359-69.

Redelings, M.D., McCoy, L., Sorvillo, F., 2006. Multiple sclerosis mortality and patterns of comorbidity in the United States from 1990 to 2001. Neuroepidemiology. 26, 102-7.

Redford, E.J., Kapoor, R., Smith, K.J., 1997. Nitric oxide donors reversibly block axonal conduction: demyelinated axons are especially susceptible. Brain : a journal of neurology. 120 (Pt 12), 2149-57.

Renoux, C., Vukusic, S., Mikaeloff, Y., Edan, G., Clanet, M., Dubois, B., Debouverie, M., Brochet, B., Lebrun-Frenay, C., Pelletier, J., Moreau, T., Lubetzki, C., Vermersch, P., Roullet, E., Magy, L., Tardieu, M., Suissa, S., Confavreux, C., 2007. Natural history of multiple sclerosis with childhood onset. The New England journal of medicine. 356, 2603-13.

Renoux, C., Suissa, S., 2008. Immortal time bias in the study of effectiveness of interferon-beta in multiple sclerosis. Annals of neurology. 64, 109-10; author reply 110.

Revesz, T., Kidd, D., Thompson, A.J., Barnard, R.O., McDonald, W.I., 1994. A comparison of the pathology of primary and secondary progressive multiple sclerosis. Brain : a journal of neurology. 117 (Pt 4), 759-65.

Rice, C.M., Cottrell, D., Wilkins, A., Scolding, N.J., 2013. Primary progressive multiple sclerosis: progress and challenges. Journal of neurology, neurosurgery, and psychiatry.

Rice, G.P., Hartung, H.P., Calabresi, P.A., 2005. Anti-alpha4 integrin therapy for multiple sclerosis: mechanisms and rationale. Neurology. 64, 1336-42.

Rieckmann, P., 2008. Clinical trials in multiple sclerosis: current and future requirements - potential pitfalls. Journal of neurology. 255 Suppl 6, 66-8.

Riise, T., Gronning, M., Aarli, J.A., Nyland, H., Larsen, J.P., Edland, A., 1988. Prognostic factors for life expectancy in multiple sclerosis analysed by Cox-models. Journal of clinical epidemiology. 41, 1031-6.

Riise, T., Gronning, M., Fernandez, O., Lauer, K., Midgard, R., Minderhoud, J.M., Nyland, H., Palffy, G., Poser, S., Aarli, J.A., 1992. Early prognostic factors for disability in multiple sclerosis, a European multicenter study. Acta neurologica Scandinavica. 85, 212-8.

Riise, T., Nortvedt, M.W., Ascherio, A., 2003. Smoking is a risk factor for multiple sclerosis. Neurology. 61, 1122-4.

Rio, J., Nos, C., Tintore, M., Tellez, N., Galan, I., Pelayo, R., Comabella, M., Montalban, X., 2006. Defining the response to interferon-beta in relapsing-remitting multiple sclerosis patients. Annals of neurology. 59, 344-52.

Rio, J., Rovira, A., Tintore, M., Huerga, E., Nos, C., Tellez, N., Tur, C., Comabella, M., Montalban, X., 2008. Relationship between MRI lesion activity and response to IFN-beta in relapsing-remitting multiple sclerosis patients. Multiple sclerosis. 14, 479-84.

Rio, J., Castillo, J., Rovira, A., Tintore, M., Sastre-Garriga, J., Horga, A., Nos, C., Comabella, M., Aymerich, X., Montalban, X., 2009a. Measures in the first year of therapy predict the response to interferon beta in MS. Multiple sclerosis. 15, 848-53.

Rio, J., Comabella, M., Montalban, X., 2009b. Predicting responders to therapies for multiple sclerosis. Nature reviews. Neurology. 5, 553-60.

Rio, J., Comabella, M., Montalban, X., 2011. Multiple sclerosis: current treatment algorithms. Current opinion in neurology. 24, 230-7.

Rist, J.M., Franklin, R.J., 2008. Taking ageing into account in remyelination-based therapies for multiple sclerosis. Journal of the neurological sciences. 274, 64-7.

Robertson, N.P., Fraser, M., Deans, J., Clayton, D., Walker, N., Compston, D.A., 1996. Age-adjusted recurrence risks for relatives of patients with multiple sclerosis. Brain : a journal of neurology. 119 (Pt 2), 449-55.

Rocca, M.A., Anzalone, N., Falini, A., Filippi, M., 2013. Contribution of magnetic resonance imaging to the diagnosis and monitoring of multiple sclerosis. La Radiologia medica. 118, 251-64.

Rohn, T.A., Jennings, G.T., Hernandez, M., Grest, P., Beck, M., Zou, Y., Kopf, M., Bachmann, M.F., 2006. Vaccination against IL-17 suppresses autoimmune arthritis and encephalomyelitis. European journal of immunology. 36, 2857-67.

Romero-Pinel, L., Pujal, J.M., Martinez-Yelamos, S., Gubieras, L., Matas, E., Bau, L., Torrabadella, M., Azqueta, C., Arbizu, T., 2011. HLA-DRB1: genetic susceptibility and disability progression in a Spanish multiple sclerosis population. European journal of neurology : the official journal of the European Federation of Neurological Societies. 18, 337-42.

Roosendaal, S.D., Moraal, B., Pouwels, P.J., Vrenken, H., Castelijns, J.A., Barkhof, F., Geurts, J.J., 2009. Accumulation of cortical lesions in MS: relation with cognitive impairment. Multiple sclerosis. 15, 708-14.

Roosendaal, S.D., Bendfeldt, K., Vrenken, H., Polman, C.H., Borgwardt, S., Radue, E.W., Kappos, L., Pelletier, D., Hauser, S.L., Matthews, P.M., Barkhof, F., Geurts, J.J., 2011. Grey matter volume in a large cohort of MS patients: relation to MRI parameters and disability. Multiple sclerosis. 17, 1098-106.

Roquer, J., Campello, A.R., Gomis, M., 2003. Sex differences in first-ever acute stroke. Stroke; a journal of cerebral circulation. 34, 1581-5.

Rosati, G., Aiello, I., Pirastru, M.I., Mannu, L., Sanna, G., Sau, G.F., Sotgiu, S., 1996. Epidemiology of multiple sclerosis in Northwestern Sardinia: further evidence for higher frequency in Sardinians compared to other Italians. Neuroepidemiology. 15, 10-9.

Rosati, G., 2001. The prevalence of multiple sclerosis in the world: an update. Neurological sciences : official journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology. 22, 117-39.

Rothwell, P.M., Charlton, D., 1998. High incidence and prevalence of multiple sclerosis in south east Scotland: evidence of a genetic predisposition. Journal of neurology, neurosurgery, and psychiatry. 64, 730-5.

Rovaris, M., Bozzali, M., Santuccio, G., Iannucci, G., Sormani, M.P., Colombo, B., Comi, G., Filippi, M., 2000. Relative contributions of brain and cervical cord pathology to multiple sclerosis disability: a study with magnetisation transfer ratio histogram analysis. Journal of neurology, neurosurgery, and psychiatry. 69, 723-7.

Ruckh, J.M., Zhao, J.W., Shadrach, J.L., van Wijngaarden, P., Rao, T.N., Wagers, A.J., Franklin, R.J., 2012. Rejuvenation of regeneration in the aging central nervous system. Cell stem cell. 10, 96-103.

Rudick, R., Antel, J., Confavreux, C., Cutter, G., Ellison, G., Fischer, J., Lublin, F., Miller, A., Petkau, J., Rao, S., Reingold, S., Syndulko, K., Thompson, A., Wallenberg, J., Weinshenker, B., Willoughby, E., 1997. Recommendations from the National Multiple Sclerosis Society Clinical Outcomes Assessment Task Force. Annals of neurology. 42, 379-82.

Rudick, R.A., Fisher, E., Lee, J.C., Simon, J., Jacobs, L., 1999. Use of the brain parenchymal fraction to measure whole brain atrophy in relapsing-remitting MS. Multiple Sclerosis Collaborative Research Group. Neurology. 53, 1698-704.

Rudick, R.A., Lee, J.C., Simon, J., Ransohoff, R.M., Fisher, E., 2004. Defining interferon beta response status in multiple sclerosis patients. Annals of neurology. 56, 548-55.

Rudick, R.A., Lee, J.C., Simon, J., Fisher, E., 2006a. Significance of T2 lesions in multiple sclerosis: A 13-year longitudinal study. Annals of neurology. 60, 236-42.

Rudick, R.A., Stuart, W.H., Calabresi, P.A., Confavreux, C., Galetta, S.L., Radue, E.W., Lublin, F.D., Weinstock-Guttman, B., Wynn, D.R., Lynn, F., Panzara, M.A., Sandrock, A.W., 2006b. Natalizumab plus interferon beta-1a for relapsing multiple sclerosis. The New England journal of medicine. 354, 911-23.

Rudick, R.A., Lee, J.C., Nakamura, K., Fisher, E., 2009. Gray matter atrophy correlates with MS disability progression measured with MSFC but not EDSS. Journal of the neurological sciences. 282, 106-11.

Runia, T.F., Hop, W.C., de Rijke, Y.B., Buljevac, D., Hintzen, R.Q., 2012. Lower serum vitamin D levels are associated with a higher relapse risk in multiple sclerosis. Neurology. 79, 261-6.

Runmarker, B., Andersen, O., 1993. Prognostic factors in a multiple sclerosis incidence cohort with twenty-five years of follow-up. Brain : a journal of neurology. 116 (Pt 1), 117-34.

Saccardi, R., Mancardi, G.L., Solari, A., Bosi, A., Bruzzi, P., Di Bartolomeo, P., Donelli, A., Filippi, M., Guerrasio, A., Gualandi, F., La Nasa, G., Murialdo, A., Pagliai, F., Papineschi, F., Scappini, B., Marmont, A.M., 2005. Autologous HSCT for severe progressive multiple sclerosis in a multicenter trial: impact on disease activity and quality of life. Blood. 105, 2601-7.

Saccardi, R., Kozak, T., Bocelli-Tyndall, C., Fassas, A., Kazis, A., Havrdova, E., Carreras, E., Saiz, A., Lowenberg, B., te Boekhorst, P.A., Gualandio, F., Openshaw, H., Longo, G., Pagliai, F., Massacesi, L., Deconink, E., Ouyang, J., Nagore, F.J., Besalduch, J., Lisukov, I.A., Bonini, A., Merelli, E., Slavino, S., Gratwohl, A., Passweg, J., Tyndall, A., Steck, A.J., Andolina, M., Capobianco, M., Martin, J.L., Lugaresi, A., Meucci, G., Saez, R.A., Clark, R.E., Fernandez, M.N., Fouillard, L., Herstenstein, B., Koza, V., Cocco, E., Baurmann, H., Mancardi, G.L., 2006. Autologous stem cell transplantation for progressive multiple sclerosis: update of the European Group for Blood and Marrow Transplantation autoimmune diseases working party database. Mult Scler. 12, 814-23.

Sadovnick, A.D., Bulman, D., Ebers, G.C., 1991a. Parent-child concordance in multiple sclerosis. Annals of neurology. 29, 252-5.

Sadovnick, A.D., Eisen, K., Ebers, G.C., Paty, D.W., 1991b. Cause of death in patients attending multiple sclerosis clinics. Neurology. 41, 1193-6.

Sadovnick, A.D., 2009. European Charcot Foundation Lecture: the natural history of multiple sclerosis and gender. Journal of the neurological sciences. 286, 1-5.

Sahraian, M.A., Khorramnia, S., Ebrahim, M.M., Moinfar, Z., Lotfi, J., Pakdaman, H., 2010. Multiple sclerosis in Iran: a demographic study of 8,000 patients and changes over time. European neurology. 64, 331-6.

Sailer, M., O'Riordan, J.I., Thompson, A.J., Kingsley, D.P., MacManus, D.G., McDonald, W.I., Miller, D.H., 1999. Quantitative MRI in patients with clinically isolated syndromes suggestive of demyelination. Neurology. 52**,** 599-606.

Saiz, A., Carreras, E., Berenguer, J., Yague, J., Martinez, C., Marin, P., Rovira, M., Pujol, T., Arbizu, T., Graus, F., 2001. MRI and CSF oligoclonal bands after autologous hematopoietic stem cell transplantation in MS. Neurology. 56**,** 1084-9.

Salzer, J., Svenningsson, A., Sundstrom, P., 2010. Neurofilament light as a prognostic marker in multiple sclerosis. Multiple sclerosis. 16**,** 287-92.

Santiago, O., Gutierrez, J., Sorlozano, A., de Dios Luna, J., Villegas, E., Fernandez, O., 2010. Relation between Epstein-Barr virus and multiple sclerosis: analytic study of scientific production. European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology. 29**,** 857-66.

Sargsyan, S.A., Shearer, A.J., Ritchie, A.M., Burgoon, M.P., Anderson, S., Hemmer, B., Stadelmann, C., Gattenlohner, S., Owens, G.P., Gilden, D., Bennett, J.L., 2010. Absence of Epstein-Barr virus in the brain and CSF of patients with multiple sclerosis. Neurology. 74**,** 1127-35.

Sawcer, S., Hellenthal, G., Pirinen, M., Spencer, C.C., Patsopoulos, N.A., Moutsianas, L., Dilthey, A., Su, Z., Freeman, C., Hunt, S.E., Edkins, S., Gray, E., Booth, D.R., Potter, S.C., Goris, A., Band, G., Oturai, A.B., Strange, A., Saarela, J., Bellenguez, C., Fontaine, B., Gillman, M., Hemmer, B., Gwilliam, R., Zipp, F., Jayakumar, A., Martin, R., Leslie, S., Hawkins, S., Giannoulatou, E., D'Alfonso, S., Blackburn, H., Martinelli Boneschi, F., Liddle, J., Harbo, H.F., Perez, M.L., Spurkland, A., Waller, M.J., Mycko, M.P., Ricketts, M., Comabella, M., Hammond, N., Kockum, I., McCann, O.T., Ban, M., Whittaker, P., Kemppinen, A., Weston, P., Hawkins, C., Widaa, S., Zajicek, J., Dronov, S., Robertson, N., Bumpstead, S.J., Barcellos, L.F., Ravindrarajah, R., Abraham, R., Alfredsson, L., Ardlie, K., Aubin, C., Baker, A., Baker, K., Baranzini, S.E., Bergamaschi, L., Bergamaschi, R., Bernstein, A., Berthele, A., Boggild, M., Bradfield, J.P., Brassat, D., Broadley, S.A., Buck, D., Butzkueven, H., Capra, R., Carroll, W.M., Cavalla, P., Celius, E.G., Cepok, S., Chiavacci, R., Clerget-Darpoux, F., Clysters, K., Comi, G., Cossburn, M., Cournu-Rebeix, I., Cox, M.B., Cozen, W., Cree, B.A., Cross, A.H., Cusi, D., Daly, M.J., Davis, E., de Bakker, P.I., Debouverie, M., D'Hooghe M, B., Dixon, K., Dobosi, R., Dubois, B., Ellinghaus, D., Elovaara, I., Esposito, F., Fontenille, C., Foote, S., Franke, A., Galimberti, D., Ghezzi, A., Glessner, J., Gomez, R., Gout, O., Graham, C., Grant, S.F., Guerini, F.R., Hakonarson, H., Hall, P., Hamsten, A., Hartung, H.P., Heard, R.N., Heath, S., Hobart, J., Hoshi, M., Infante-Duarte, C., Ingram, G., Ingram, W., Islam, T., Jagodic, M., Kabesch, M., Kermode, A.G., Kilpatrick, T.J., Kim, C., Klopp, N., Koivisto, K., Larsson, M., Lathrop, M., Lechner-Scott, J.S., Leone, M.A., Leppa, V., Liljedahl, U., Bomfim, I.L., Lincoln, R.R., Link, J., Liu, J., Lorentzen, A.R., Lupoli, S., Macciardi, F., Mack, T., Marriott, M., Martinelli, V., Mason, D., McCauley, J.L., Mentch, F., Mero, I.L., Mihalova, T., Montalban, X., Mottershead, J., Myhr, K.M., Naldi, P., Ollier, W., Page, A., Palotie, A., Pelletier, J., Piccio, L., Pickersgill, T., Piehl, F., Pobywajlo, S., Quach, H.L., Ramsay, P.P., Reunanen, M., Reynolds, R., Rioux, J.D., Rodegher, M., Roesner, S., Rubio, J.P., Ruckert, I.M., Salvetti, M., Salvi,

E., Santaniello, A., Schaefer, C.A., Schreiber, S., Schulze, C., Scott, R.J., Sellebjerg, F., Selmaj, K.W., Sexton, D., Shen, L., Simms-Acuna, B., Skidmore, S., Sleiman, P.M., Smestad, C., Sorensen, P.S., Sondergaard, H.B., Stankovich, J., Strange, R.C., Sulonen, A.M., Sundqvist, E., Syvanen, A.C., Taddeo, F., Taylor, B., Blackwell, J.M., Tienari, P., Bramon, E., Tourbah, A., Brown, M.A., Tronczynska, E., Casas, J.P., Tubridy, N., Corvin, A., Vickery, J., Jankowski, J., Villoslada, P., Markus, H.S., Wang, K., Mathew, C.G., Wason, J., Palmer, C.N., Wichmann, H.E., Plomin, R., Willoughby, E., Rautanen, A., Winkelmann, J., Wittig, M., Trembath, R.C., Yaouanq, J., Viswanathan, A.C., Zhang, H., Wood, N.W., Zuvich, R., Deloukas, P., Langford, C., Duncanson, A., Oksenberg, J.R., Pericak-Vance, M.A., Haines, J.L., Olsson, T., Hillert, J., Ivinson, A.J., De Jager, P.L., Peltonen, L., Stewart, G.J., Hafler, D.A., Hauser, S.L., McVean, G., Donnelly, P., Compston, A., 2011. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. Nature. 476**,** 214-9.

Saxena, A., Bauer, J., Scheikl, T., Zappulla, J., Audebert, M., Desbois, S., Waisman, A., Lassmann, H., Liblau, R.S., Mars, L.T., 2008. Cutting edge: Multiple sclerosis-like lesions induced by effector CD8 T cells recognizing a sequestered antigen on oligodendrocytes. Journal of immunology. 181**,** 1617-21.

Sayao, A.L., Devonshire, V., Tremlett, H., 2007. Longitudinal follow-up of "benign" multiple sclerosis at 20 years. Neurology. 68**,** 496-500.

Scalfari, A., Neuhaus, A., Degenhardt, A., Rice, G.P., Muraro, P.A., Daumer, M., Ebers, G.C., 2010. The natural history of multiple sclerosis: a geographically based study 10: relapses and long-term disability. Brain : a journal of neurology. 133**,** 1914-29.

Schapira, K., Poskanzer, D.C., Miller, H., 1963. Familial and conjugal multiple sclerosis. Brain : a journal of neurology. 86**,** 315-32.

Schattenkirchner, M., 2000. The use of leflunomide in the treatment of rheumatoid arthritis: an experimental and clinical review. Immunopharmacology. 47**,** 291-8.

Schmierer, K., Tozer, D.J., Scaravilli, F., Altmann, D.R., Barker, G.J., Tofts, P.S., Miller, D.H., 2007. Quantitative magnetization transfer imaging in postmortem multiple sclerosis brain. Journal of magnetic resonance imaging : JMRI. 26**,** 41-51.

Schumacher, G.A., Beebe, G., Kibler, R.F., Kurland, L.T., Kurtzke, J.F., McDowell, F., Nagler, B., Sibley, W.A., Tourtellotte, W.W., Willmon, T.L., 1965. Problems of Experimental Trials of Therapy in Multiple Sclerosis: Report by the Panel on the Evaluation of Experimental Trials of Therapy in Multiple Sclerosis. Annals of the New York Academy of Sciences. 122**,** 552-68.

Schutzer, S.E., Angel, T.E., Liu, T., Schepmoes, A.A., Xie, F., Bergquist, J., Vecsei, L., Zadori, D., Camp, D.G., 2nd, Holland, B.K., Smith, R.D., Coyle, P.K., 2013. Gray matter is targeted in first-attack multiple sclerosis. PloS one. 8**,** e66117.

Seamons, A., Perchellet, A., Goverman, J., 2003. Immune tolerance to myelin proteins. Immunologic research. 28**,** 201-21.

Seder, R.A., Ahmed, R., 2003. Similarities and differences in CD4+ and CD8+ effector and memory T cell generation. Nature immunology. 4**,** 835-42.

Seewann, A., Vrenken, H., van der Valk, P., Blezer, E.L., Knol, D.L., Castelijns, J.A., Polman, C.H., Pouwels, P.J., Barkhof, F., Geurts, J.J., 2009. Diffusely abnormal white matter in chronic multiple sclerosis: imaging and histopathologic analysis. Archives of neurology. 66**,** 601-9.

Seewann, A., Vrenken, H., Kooi, E.J., van der Valk, P., Knol, D.L., Polman, C.H., Pouwels, P.J., Barkhof, F., Geurts, J.J., 2011. Imaging the tip of the iceberg: visualization of cortical lesions in multiple sclerosis. Multiple sclerosis. 17**,** 1202-10.

Sellner, J., Kraus, J., Awad, A., Milo, R., Hemmer, B., Stuve, O., 2011. The increasing incidence and prevalence of female multiple sclerosis--a critical analysis of potential environmental factors. Autoimmunity reviews. 10**,** 495-502.

Selmaj, K., Raine, C.S., Cannella, B., Brosnan, C.F., 1991a. Identification of lymphotoxin and tumor necrosis factor in multiple sclerosis lesions. The Journal of clinical investigation. 87**,** 949-54.

Selmaj, K., Raine, C.S., Farooq, M., Norton, W.T., Brosnan, C.F., 1991b. Cytokine cytotoxicity against oligodendrocytes. Apoptosis induced by lymphotoxin. Journal of immunology. 147**,** 1522-9.

Selmaj, K.W., Farooq, M., Norton, W.T., Raine, C.S., Brosnan, C.F., 1990. Proliferation of astrocytes in vitro in response to cytokines. A primary role for tumor necrosis factor. Journal of immunology. 144**,** 129-35.

Semra, Y.K., Seidi, O.A., Sharief, M.K., 2002. Heightened intrathecal release of axonal cytoskeletal proteins in multiple sclerosis is associated with progressive disease and clinical disability. Journal of neuroimmunology. 122**,** 132-9.

Sepulcre, J., Murie-Fernandez, M., Salinas-Alaman, A., Garcia-Layana, A., Bejarano, B., Villoslada, P., 2007. Diagnostic accuracy of retinal abnormalities in predicting disease activity in MS. Neurology. 68**,** 1488-94.

Serafini, B., Rosicarelli, B., Magliozzi, R., Stigliano, E., Aloisi, F., 2004. Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis. Brain pathology. 14**,** 164-74.

Serafini, B., Rosicarelli, B., Franciotta, D., Magliozzi, R., Reynolds, R., Cinque, P., Andreoni, L., Trivedi, P., Salvetti, M., Faggioni, A., Aloisi, F., 2007. Dysregulated Epstein-Barr virus infection in the multiple sclerosis brain. The Journal of experimental medicine. 204**,** 2899-912.

Sharief, M.K., Hentges, R., 1991. Association between tumor necrosis factor-alpha and disease progression in patients with multiple sclerosis. The New England journal of medicine. 325**,** 467-72.

Shen, S., Sandoval, J., Swiss, V.A., Li, J., Dupree, J., Franklin, R.J., Casaccia-Bonnefil, P., 2008. Age-dependent epigenetic control of differentiation inhibitors is critical for remyelination efficiency. Nature neuroscience. 11**,** 1024-34.

Shenkenberg, T.D., Von Hoff, D.D., 1986. Mitoxantrone: a new anticancer drug with significant clinical activity. Ann Intern Med. 105**,** 67-81.

Shepherd, D.I., Downie, A.W., 1978. Prevalence of multiple sclerosis in north-east Scotland. British medical journal. 2**,** 1024.

Shirani, A., Zhao, Y., Karim, M.E., Evans, C., Kingwell, E., van der Kop, M.L., Oger, J., Gustafson, P., Petkau, J., Tremlett, H., 2012. Association between use of interferon beta and progression of disability in patients with relapsing-

remitting multiple sclerosis. JAMA : the journal of the American Medical Association. 308**,** 247-56.

Siger, M., Dziegielewski, K., Jasek, L., Bieniek, M., Nicpan, A., Nawrocki, J., Selmaj, K., 2008. Optical coherence tomography in multiple sclerosis: thickness of the retinal nerve fiber layer as a potential measure of axonal loss and brain atrophy. Journal of neurology. 255**,** 1555-60.

Sim, F.J., Zhao, C., Penderis, J., Franklin, R.J., 2002. The age-related decrease in CNS remyelination efficiency is attributable to an impairment of both oligodendrocyte progenitor recruitment and differentiation. The Journal of neuroscience : the official journal of the Society for Neuroscience. 22**,** 2451-9.

Simon, J.H., Jacobs, L.D., Campion, M.K., Rudick, R.A., Cookfair, D.L., Herndon, R.M., Richert, J.R., Salazar, A.M., Fischer, J.S., Goodkin, D.E., Simonian, N., Lajaunie, M., Miller, D.E., Wende, K., Martens-Davidson, A., Kinkel, R.P., Munschauer, F.E., 3rd, Brownscheidle, C.M., 1999. A longitudinal study of brain atrophy in relapsing multiple sclerosis. The Multiple Sclerosis Collaborative Research Group (MSCRG). Neurology. 53**,** 139-48.

Simon, K.C., van der Mei, I.A., Munger, K.L., Ponsonby, A., Dickinson, J., Dwyer, T., Sundstrom, P., Ascherio, A., 2010. Combined effects of smoking, anti-EBNA antibodies, and HLA-DRB1*1501 on multiple sclerosis risk. Neurology. 74**,** 1365-71.

Simpson, S., Jr., Taylor, B., Blizzard, L., Ponsonby, A.L., Pittas, F., Tremlett, H., Dwyer, T., Gies, P., van der Mei, I., 2010. Higher 25-hydroxyvitamin D is associated with lower relapse risk in multiple sclerosis. Annals of neurology. 68**,** 193-203.

Simpson, S., Jr., Blizzard, L., Otahal, P., Van der Mei, I., Taylor, B., 2011. Latitude is significantly associated with the prevalence of multiple sclerosis: a meta-analysis. Journal of neurology, neurosurgery, and psychiatry. 82**,** 1132-41.

Singh, R., Mukhopadhyay, K., 2011. Survival analysis in clinical trials: Basics and must know areas. Perspectives in clinical research. 2**,** 145-8.

Skegg, D.C., Corwin, P.A., Craven, R.S., Malloch, J.A., Pollock, M., 1987. Occurrence of multiple sclerosis in the north and south of New Zealand. Journal of neurology, neurosurgery, and psychiatry. 50**,** 134-9.

Skoog, B., Runmarker, B., Winblad, S., Ekholm, S., Andersen, O., 2012. A representative cohort of patients with non-progressive multiple sclerosis at the age of normal life expectancy. Brain : a journal of neurology. 135**,** 900-11.

Skulina, C., Schmidt, S., Dornmair, K., Babbe, H., Roers, A., Rajewsky, K., Wekerle, H., Hohlfeld, R., Goebels, N., 2004. Multiple sclerosis: brain-infiltrating CD8+ T cells persist as clonal expansions in the cerebrospinal fluid and blood. Proceedings of the National Academy of Sciences of the United States of America. 101**,** 2428-33.

Smestad, C., Sandvik, L., Celius, E.G., 2009. Excess mortality and cause of death in a cohort of Norwegian multiple sclerosis patients. Multiple sclerosis. 15**,** 1263-70.

Smith, A.D., Duckett, S., Waters, A.H., 1963. Neuropathological Changes in Chronic Cyanide Intoxication. Nature. 200**,** 179-81.

Smith, K.J., 1994. Conduction properties of central demyelinated and remyelinated axons, and their relation to symptom production in demyelinating disorders. Eye. 8 (Pt 2), 224-37.
Smith, K.J., Kapoor, R., Hall, S.M., Davies, M., 2001. Electrically active axons degenerate when exposed to nitric oxide. Annals of neurology. 49, 470-6.
Solari, A., Radice, D., Manneschi, L., Motti, L., Montanari, E., 2005. The multiple sclerosis functional composite: different practice effects in the three test components. Journal of the neurological sciences. 228, 71-4.
Sopori, M.L., Kozak, W., 1998. Immunomodulatory effects of cigarette smoke. Journal of neuroimmunology. 83, 148-56.
Sormani, M., Rio, J., Tintore, M., Signori, A., Li, D., Cornelisse, P., Stubinski, B., Stromillo, M., Montalban, X., De Stefano, N., 2012. Scoring treatment response in patients with relapsing multiple sclerosis. Multiple sclerosis.
Sormani, M.P., Bruzzi, P., Beckmann, K., Kappos, L., Miller, D.H., Polman, C., Pozzilli, C., Thompson, A.J., Wagner, K., Filippi, M., 2005. The distribution of magnetic resonance imaging response to interferonbeta-1b in multiple sclerosis. Journal of neurology. 252, 1455-8.
Sormani, M.P., Rovaris, M., Comi, G., Filippi, M., 2007. A composite score to predict short-term disease activity in patients with relapsing-remitting MS. Neurology. 69, 1230-5.
Sormani, M.P., Li, D.K., Bruzzi, P., Stubinski, B., Cornelisse, P., Rocak, S., De Stefano, N., 2011. Combined MRI lesions and relapses as a surrogate for disability in multiple sclerosis. Neurology. 77, 1684-90.
Sospedra, M., Martin, R., 2005. Immunology of multiple sclerosis. Annual review of immunology. 23, 683-747.
Spach, K.M., Nashold, F.E., Dittel, B.N., Hayes, C.E., 2006. IL-10 signaling is essential for 1,25-dihydroxyvitamin D3-mediated inhibition of experimental autoimmune encephalomyelitis. Journal of immunology. 177, 6030-7.
Stadelmann, C., Bruck, W., 2008. Interplay between mechanisms of damage and repair in multiple sclerosis. Journal of neurology. 255 Suppl 1, 12-8.
Stadelmann, C., 2011. Multiple sclerosis as a neurodegenerative disease: pathology, mechanisms and therapeutic implications. Current opinion in neurology. 24, 224-9.
Stankoff, B., Mrejen, S., Tourbah, A., Fontaine, B., Lyon-Caen, O., Lubetzki, C., Rosenheim, M., 2007. Age at onset determines the occurrence of the progressive phase of multiple sclerosis. Neurology. 68, 779-81.
Stankovich, J., Butzkueven, H., Marriott, M., Chapman, C., Tubridy, N., Tait, B.D., Varney, M.D., Taylor, B.V., Foote, S.J., Kilpatrick, T.J., Rubio, J.P., 2009. HLA-DRB1 associations with disease susceptibility and clinical course in Australians with multiple sclerosis. Tissue antigens. 74, 17-21.
Steinman, L., 2001. Myelin-specific CD8 T cells in the pathogenesis of experimental allergic encephalitis and multiple sclerosis. The Journal of experimental medicine. 194, F27-30.
Steinvorth, S.M., Rover, C., Schneider, S., Nicholas, R., Straube, S., Friede, T., 2013. Explaining temporal trends in annualised relapse rates in placebo groups of randomised controlled trials in relapsing multiple sclerosis: systematic review and meta-regression. Multiple sclerosis.

Sterman, A.B., Coyle, P.K., Panasci, D.J., Grimson, R., 1985. Disseminated abnormalities of cardiovascular autonomic functions in multiple sclerosis. Neurology. 35**,** 1665-8.

Storch, M.K., Piddlesden, S., Haltia, M., Iivanainen, M., Morgan, P., Lassmann, H., 1998. Multiple sclerosis: in situ evidence for antibody- and complement-mediated demyelination. Annals of neurology. 43**,** 465-71.

Stromillo, M.L., Giorgio, A., Rossi, F., Battaglini, M., Hakiki, B., Malentacchi, G., Santangelo, M., Gasperini, C., Bartolozzi, M.L., Portaccio, E., Amato, M.P., De Stefano, N., 2013. Brain metabolic changes suggestive of axonal damage in radiologically isolated syndrome. Neurology.

Stromnes, I.M., Cerretti, L.M., Liggitt, D., Harris, R.A., Goverman, J.M., 2008. Differential regulation of central nervous system autoimmunity by T(H)1 and T(H)17 cells. Nature medicine. 14**,** 337-42.

Strotmann, J.M., Spindler, M., Weilbach, F.X., Gold, R., Ertl, G., Voelker, W., 2002. Myocardial function in patients with multiple sclerosis treated with low-dose mitoxantrone. Am J Cardiol. 89**,** 1222-5.

Stys, P.K., Zamponi, G.W., van Minnen, J., Geurts, J.J., 2012. Will the real multiple sclerosis please stand up? Nature reviews. Neuroscience. 13**,** 507-14.

Su, K.G., Banker, G., Bourdette, D., Forte, M., 2009. Axonal degeneration in multiple sclerosis: the mitochondrial hypothesis. Current neurology and neuroscience reports. 9**,** 411-7.

Suissa, S., 2008. Immortal time bias in pharmaco-epidemiology. American journal of epidemiology. 167**,** 492-9.

Sumelahti, M.L., Tienari, P.J., Wikstrom, J., Palo, J., Hakama, M., 2001. Increasing prevalence of multiple sclerosis in Finland. Acta neurologica Scandinavica. 103**,** 153-8.

Sumelahti, M.L., Tienari, P.J., Wikstrom, J., Salminen, T.M., Hakama, M., 2002. Survival of multiple sclerosis in Finland between 1964 and 1993. Multiple sclerosis. 8**,** 350-5.

Sumelahti, M.L., Hakama, M., Elovaara, I., Pukkala, E., 2010. Causes of death among patients with multiple sclerosis. Multiple sclerosis. 16**,** 1437-42.

Sun, D., Whitaker, J.N., Huang, Z., Liu, D., Coleclough, C., Wekerle, H., Raine, C.S., 2001. Myelin antigen-specific CD8+ T cells are encephalitogenic and produce severe disease in C57BL/6 mice. Journal of immunology. 166**,** 7579-87.

Sundstrom, P., Nystrom, L., Jidell, E., Hallmans, G., 2008. EBNA-1 reactivity and HLA DRB1*1501 as statistically independent risk factors for multiple sclerosis: a case-control study. Multiple sclerosis. 14**,** 1120-2.

Svenningsson, A., Runmarker, B., Lycke, J., Andersen, O., 1990. Incidence of MS during two fifteen-year periods in the Gothenburg region of Sweden. Acta neurologica Scandinavica. 82**,** 161-8.

Swank, R.L., Lerstad, O., Strom, A., Backer, J., 1952. Multiple sclerosis in rural Norway its geographic and occupational incidence in relation to nutrition. The New England journal of medicine. 246**,** 722-8.

Swanton, J.K., Rovira, A., Tintore, M., Altmann, D.R., Barkhof, F., Filippi, M., Huerga, E., Miszkiel, K.A., Plant, G.T., Polman, C., Rovaris, M., Thompson, A.J., Montalban, X., Miller, D.H., 2007. MRI criteria for multiple sclerosis in patients presenting with clinically isolated syndromes: a multicentre retrospective study. Lancet neurology. 6**,** 677-86.

Sweeney, V.P., Sadovnick, A.D., Brandejs, V., 1986. Prevalence of multiple sclerosis in British Columbia. The Canadian journal of neurological sciences. Le journal canadien des sciences neurologiques. 13**,** 47-51.

Tallantyre, E.C., Bo, L., Al-Rawashdeh, O., Owens, T., Polman, C.H., Lowe, J., Evangelou, N., 2009. Greater loss of axons in primary progressive multiple sclerosis plaques compared to secondary progressive disease. Brain : a journal of neurology. 132**,** 1190-9.

Talman, L.S., Bisker, E.R., Sackel, D.J., Long, D.A., Jr., Galetta, K.M., Ratchford, J.N., Lile, D.J., Farrell, S.K., Loguidice, M.J., Remington, G., Conger, A., Frohman, T.C., Jacobs, D.A., Markowitz, C.E., Cutter, G.R., Ying, G.S., Dai, Y., Maguire, M.G., Galetta, S.L., Frohman, E.M., Calabresi, P.A., Balcer, L.J., 2010. Longitudinal study of vision and retinal nerve fiber layer thickness in multiple sclerosis. Annals of neurology. 67**,** 749-60.

Tartaglia, M.C., Arnold, D.L., 2006. The role of MRS and fMRI in multiple sclerosis. Advances in neurology. 98**,** 185-202.

Tedeschi, G., Lavorgna, L., Russo, P., Prinster, A., Dinacci, D., Savettieri, G., Quattrone, A., Livrea, P., Messina, C., Reggio, A., Bresciamorra, V., Orefice, G., Paciello, M., Brunetti, A., Coniglio, G., Bonavita, S., Di Costanzo, A., Bellacosa, A., Valentino, P., Quarantelli, M., Patti, F., Salemi, G., Cammarata, E., Simone, I.L., Salvatore, M., Bonavita, V., Alfano, B., 2005. Brain atrophy and lesion load in a large population of patients with multiple sclerosis. Neurology. 65**,** 280-5.

Temple, R., 1999. Are surrogate markers adequate to assess cardiovascular disease drugs? JAMA : the journal of the American Medical Association. 282**,** 790-5.

Thompson, A.J., Kermode, A.G., MacManus, D.G., Kendall, B.E., Kingsley, D.P., Moseley, I.F., McDonald, W.I., 1990. Patterns of disease activity in multiple sclerosis: clinical and magnetic resonance imaging study. BMJ. 300**,** 631-4.

Thompson, A.J., Kermode, A.G., Wicks, D., MacManus, D.G., Kendall, B.E., Kingsley, D.P., McDonald, W.I., 1991. Major differences in the dynamics of primary and secondary progressive multiple sclerosis. Annals of neurology. 29**,** 53-62.

Thompson, A.J., Miller, D., Youl, B., MacManus, D., Moore, S., Kingsley, D., Kendall, B., Feinstein, A., McDonald, W.I., 1992. Serial gadolinium-enhanced MRI in relapsing/remitting multiple sclerosis of varying disease duration. Neurology. 42**,** 60-3.

Thone, J., Ellrichmann, G., Seubert, S., Peruga, I., Lee, D.H., Conrad, R., Hayardeny, L., Comi, G., Wiese, S., Linker, R.A., Gold, R., 2012. Modulation of autoimmune demyelination by laquinimod via induction of brain-derived neurotrophic factor. The American journal of pathology. 180**,** 267-74.

Thorley-Lawson, D.A., 2001. Epstein-Barr virus: exploiting the immune system. Nature reviews. Immunology. 1**,** 75-82.

Thorogood, M., Hannaford, P.C., 1998. The influence of oral contraceptives on the risk of multiple sclerosis. British journal of obstetrics and gynaecology. 105**,** 1296-9.

Tintore, M., Rovira, A., Martinez, M.J., Rio, J., Diaz-Villoslada, P., Brieva, L., Borras, C., Grive, E., Capellades, J., Montalban, X., 2000. Isolated demyelinating syndromes: comparison of different MR imaging criteria to predict

conversion to clinically definite multiple sclerosis. AJNR. American journal of neuroradiology. 21, 702-6.
Tintore, M., Rovira, A., Rio, J., Nos, C., Grive, E., Sastre-Garriga, J., Pericot, I., Sanchez, E., Comabella, M., Montalban, X., 2003. New diagnostic criteria for multiple sclerosis: application in first demyelinating episode. Neurology. 60, 27-30.
Tintore, M., Rovira, A., Arrambide, G., Mitjana, R., Rio, J., Auger, C., Nos, C., Edo, M.C., Castillo, J., Horga, A., Perez-Miralles, F., Huerga, E., Comabella, M., Sastre-Garriga, J., Montalban, X., 2010. Brainstem lesions in clinically isolated syndromes. Neurology. 75, 1933-8.
Toledo, J., Sepulcre, J., Salinas-Alaman, A., Garcia-Layana, A., Murie-Fernandez, M., Bejarano, B., Villoslada, P., 2008. Retinal nerve fiber layer atrophy is associated with physical and cognitive disability in multiple sclerosis. Multiple sclerosis. 14, 906-12.
Tomassini, V., Matthews, P.M., Thompson, A.J., Fuglo, D., Geurts, J.J., Johansen-Berg, H., Jones, D.K., Rocca, M.A., Wise, R.G., Barkhof, F., Palace, J., 2012. Neuroplasticity and functional recovery in multiple sclerosis. Nature reviews. Neurology.
Trapp, B.D., Peterson, J., Ransohoff, R.M., Rudick, R., Mork, S., Bo, L., 1998. Axonal transection in the lesions of multiple sclerosis. The New England journal of medicine. 338, 278-85.
Trapp, B.D., Nave, K.A., 2008. Multiple sclerosis: an immune or neurodegenerative disorder? Annual review of neuroscience. 31, 247-69.
Tremlett, H., Paty, D., Devonshire, V., 2005. The natural history of primary progressive MS in British Columbia, Canada. Neurology. 65, 1919-23.
Tremlett, H., Devonshire, V., 2006. Is late-onset multiple sclerosis associated with a worse outcome? Neurology. 67, 954-9.
Tremlett, H., Paty, D., Devonshire, V., 2006. Disability progression in multiple sclerosis is slower than previously reported. Neurology. 66, 172-7.
Tremlett, H., Yinshan, Z., Devonshire, V., 2008a. Natural history of secondary-progressive multiple sclerosis. Multiple sclerosis. 14, 314-24.
Tremlett, H., Zhao, Y., Joseph, J., Devonshire, V., 2008b. Relapses in multiple sclerosis are age- and time-dependent. Journal of neurology, neurosurgery, and psychiatry. 79, 1368-74.
Tremlett, H., Yousefi, M., Devonshire, V., Rieckmann, P., Zhao, Y., 2009a. Impact of multiple sclerosis relapses on progression diminishes with time. Neurology. 73, 1616-23.
Tremlett, H., Zhao, Y., Devonshire, V., 2009b. Natural history comparisons of primary and secondary progressive multiple sclerosis reveals differences and similarities. Journal of neurology. 256, 374-81.
Tremlett, H., Zhu, F., Petkau, J., Oger, J., Zhao, Y., 2012. Natural, innate improvements in multiple sclerosis disability. Multiple sclerosis. 18, 1412-21.
Trojano, M., Avolio, C., Manzari, C., Calo, A., De Robertis, F., Serio, G., Livrea, P., 1995. Multivariate analysis of predictive factors of multiple sclerosis course with a validated method to assess clinical events. Journal of neurology, neurosurgery, and psychiatry. 58, 300-6.

Trojano, M., Liguori, M., Bosco Zimatore, G., Bugarini, R., Avolio, C., Paolicelli, D., Giuliani, F., De Robertis, F., Marrosu, M.G., Livrea, P., 2002. Age-related disability in multiple sclerosis. Annals of neurology. 51**,** 475-80.

Trojano, M., Pellegrini, F., Fuiani, A., Paolicelli, D., Zipoli, V., Zimatore, G.B., Di Monte, E., Portaccio, E., Lepore, V., Livrea, P., Amato, M.P., 2007. New natural history of interferon-beta-treated relapsing multiple sclerosis. Annals of neurology. 61**,** 300-6.

Trojano, M., Lucchese, G., Graziano, G., Taylor, B.V., Simpson, S., Jr., Lepore, V., Grand'maison, F., Duquette, P., Izquierdo, G., Grammond, P., Amato, M.P., Bergamaschi, R., Giuliani, G., Boz, C., Hupperts, R., Van Pesch, V., Lechner-Scott, J., Cristiano, E., Fiol, M., Oreja-Guevara, C., Saladino, M.L., Verheul, F., Slee, M., Paolicelli, D., Tortorella, C., D'Onghia, M., Iaffaldano, P., Direnzo, V., Butzkueven, H., 2012. Geographical variations in sex ratio trends over time in multiple sclerosis. PloS one. 7**,** e48078.

Truyen, L., van Waesberghe, J.H., van Walderveen, M.A., van Oosten, B.W., Polman, C.H., Hommes, O.R., Ader, H.J., Barkhof, F., 1996. Accumulation of hypointense lesions ("black holes") on T1 spin-echo MRI correlates with disease progression in multiple sclerosis. Neurology. 47**,** 1469-76.

Tur, C., Tintore, M., Rovira, A., Nos, C., Rio, J., Tellez, N., Galan, I., Perkal, H., Comabella, M., Sastre-Garriga, J., Montalban, X., 2008. Very early scans for demonstrating dissemination in time in multiple sclerosis. Multiple sclerosis. 14**,** 631-5.

Tutuncu, M., Tang, J., Zeid, N.A., Kale, N., Crusan, D.J., Atkinson, E.J., Siva, A., Pittock, S.J., Pirko, I., Keegan, B.M., Lucchinetti, C.F., Noseworthy, J.H., Rodriguez, M., Weinshenker, B.G., Kantarci, O.H., 2012. Onset of progressive phase is an age-dependent clinical milestone in multiple sclerosis. Multiple sclerosis.

Tutuncu, M., Tang, J., Zeid, N.A., Kale, N., Crusan, D.J., Atkinson, E.J., Siva, A., Pittock, S.J., Pirko, I., Keegan, B.M., Lucchinetti, C.F., Noseworthy, J.H., Rodriguez, M., Weinshenker, B.G., Kantarci, O.H., 2013. Onset of progressive phase is an age-dependent clinical milestone in multiple sclerosis. Multiple sclerosis. 19**,** 188-98.

Tzartos, J.S., Friese, M.A., Craner, M.J., Palace, J., Newcombe, J., Esiri, M.M., Fugger, L., 2008. Interleukin-17 production in central nervous system-infiltrating T cells and glial cells is associated with active disease in multiple sclerosis. The American journal of pathology. 172**,** 146-55.

Uitdehaag, B.M., Ader, H.J., Roosma, T.J., de Groot, V., Kalkers, N.F., Polman, C.H., 2002. Multiple sclerosis functional composite: impact of reference population and interpretation of changes. Multiple sclerosis. 8**,** 366-71.

Ulvestad, E., Williams, K., Vedeler, C., Antel, J., Nyland, H., Mork, S., Matre, R., 1994. Reactive microglia in multiple sclerosis lesions have an increased expression of receptors for the Fc part of IgG. Journal of the neurological sciences. 121**,** 125-31.

Urdinguio, R.G., Sanchez-Mut, J.V., Esteller, M., 2009. Epigenetic mechanisms in neurological diseases: genes, syndromes, and therapies. Lancet neurology. 8**,** 1056-72.

Van Assche, G., Van Ranst, M., Sciot, R., Dubois, B., Vermeire, S., Noman, M., Verbeeck, J., Geboes, K., Robberecht, W., Rutgeerts, P., 2005. Progressive

multifocal leukoencephalopathy after natalizumab therapy for Crohn's disease. The New England journal of medicine. 353, 362-8.
van der Mei, I.A., Ponsonby, A.L., Blizzard, L., Dwyer, T., 2001. Regional variation in multiple sclerosis prevalence in Australia and its association with ambient ultraviolet radiation. Neuroepidemiology. 20, 168-74.
van der Mei, I.A., Ponsonby, A.L., Dwyer, T., Blizzard, L., Simmons, R., Taylor, B.V., Butzkueven, H., Kilpatrick, T., 2003. Past exposure to sun, skin phenotype, and risk of multiple sclerosis: case-control study. BMJ. 327, 316.
van Horssen, J., Brink, B.P., de Vries, H.E., van der Valk, P., Bo, L., 2007. The blood-brain barrier in cortical multiple sclerosis lesions. Journal of neuropathology and experimental neurology. 66, 321-8.
van Oosten, B.W., Lai, M., Hodgkinson, S., Barkhof, F., Miller, D.H., Moseley, I.F., Thompson, A.J., Rudge, P., McDougall, A., McLeod, J.G., Ader, H.J., Polman, C.H., 1997. Treatment of multiple sclerosis with the monoclonal anti-CD4 antibody cM-T412: results of a randomized, double-blind, placebo-controlled, MR-monitored phase II trial. Neurology. 49, 351-7.
van Waesberghe, J.H., Kamphorst, W., De Groot, C.J., van Walderveen, M.A., Castelijns, J.A., Ravid, R., Lycklama a Nijeholt, G.J., van der Valk, P., Polman, C.H., Thompson, A.J., Barkhof, F., 1999. Axonal loss in multiple sclerosis lesions: magnetic resonance imaging insights into substrates of disability. Annals of neurology. 46, 747-54.
van Walderveen, M.A., Kamphorst, W., Scheltens, P., van Waesberghe, J.H., Ravid, R., Valk, J., Polman, C.H., Barkhof, F., 1998. Histopathologic correlate of hypointense lesions on T1-weighted spin-echo MRI in multiple sclerosis. Neurology. 50, 1282-8.
van Walderveen, M.A., Lycklama, A.N.G.J., Ader, H.J., Jongen, P.J., Polman, C.H., Castelijns, J.A., Barkhof, F., 2001. Hypointense lesions on T1-weighted spin-echo magnetic resonance imaging: relation to clinical characteristics in subgroups of patients with multiple sclerosis. Archives of neurology. 58, 76-81.
Veldman, C.M., Cantorna, M.T., DeLuca, H.F., 2000. Expression of 1,25-dihydroxyvitamin D(3) receptor in the immune system. Archives of biochemistry and biophysics. 374, 334-8.
Verdes, F., Petrescu, A., Cernescu, C., 1978. Epidemiologic survey of multiple sclerosis in the Bucharest city and suburban area. Acta neurologica Scandinavica. 58, 109-20.
Viglietta, V., Baecher-Allan, C., Weiner, H.L., Hafler, D.A., 2004. Loss of functional suppression by CD4+CD25+ regulatory T cells in patients with multiple sclerosis. The Journal of experimental medicine. 199, 971-9.
Villard-Mackintosh, L., Vessey, M.P., 1993. Oral contraceptives and reproductive factors in multiple sclerosis incidence. Contraception. 47, 161-8.
Visscher, B.R., Liu, K.S., Clark, V.A., Detels, R., Malmgren, R.M., Dudley, J.P., 1984. Onset symptoms as predictors of mortality and disability in multiple sclerosis. Acta neurologica Scandinavica. 70, 321-8.
Vita, G., Fazio, M.C., Milone, S., Blandino, A., Salvi, L., Messina, C., 1993. Cardiovascular autonomic dysfunction in multiple sclerosis is likely related to brainstem lesions. Journal of the neurological sciences. 120, 82-6.

Vukusic, S., Confavreux, C., 2003. Prognostic factors for progression of disability in the secondary progressive phase of multiple sclerosis. Journal of the neurological sciences. 206**,** 135-7.
Vukusic, S., Van Bockstael, V., Gosselin, S., Confavreux, C., 2007. Regional variations in the prevalence of multiple sclerosis in French farmers. Journal of neurology, neurosurgery, and psychiatry. 78**,** 707-9.
Wallin, M.T., Page, W.F., Kurtzke, J.F., 2000. Epidemiology of multiple sclerosis in US veterans. VIII. Long-term survival after onset of multiple sclerosis. Brain : a journal of neurology. 123 (Pt 8)**,** 1677-87.
Wallin, M.T., Page, W.F., Kurtzke, J.F., 2004. Multiple sclerosis in US veterans of the Vietnam era and later military service: race, sex, and geography. Annals of neurology. 55**,** 65-71.
Wang, W.Z., Olsson, T., Kostulas, V., Hojeberg, B., Ekre, H.P., Link, H., 1992. Myelin antigen reactive T cells in cerebrovascular diseases. Clinical and experimental immunology. 88**,** 157-62.
Warner, H.B., Carp, R.I., 1981. Multiple sclerosis and Epstein-Barr virus. Lancet. 2**,** 1290.
Watts, C., 2004. The exogenous pathway for antigen presentation on major histocompatibility complex class II and CD1 molecules. Nature immunology. 5**,** 685-92.
Waxman, S.G., Craner, M.J., Black, J.A., 2004. Na+ channel expression along axons in multiple sclerosis and its models. Trends in pharmacological sciences. 25**,** 584-91.
Wegner, C., Stadelmann, C., Pfortner, R., Raymond, E., Feigelson, S., Alon, R., Timan, B., Hayardeny, L., Bruck, W., 2010. Laquinimod interferes with migratory capacity of T cells and reduces IL-17 levels, inflammatory demyelination and acute axonal damage in mice with experimental autoimmune encephalomyelitis. Journal of neuroimmunology. 227**,** 133-43.
Weiner, H.L., Guttmann, C.R., Khoury, S.J., Orav, E.J., Hohol, M.J., Kikinis, R., Jolesz, F.A., 2000. Serial magnetic resonance imaging in multiple sclerosis: correlation with attacks, disability, and disease stage. Journal of neuroimmunology. 104**,** 164-73.
Weiner, H.L., 2009. The challenge of multiple sclerosis: how do we cure a chronic heterogeneous disease? Annals of neurology. 65**,** 239-48.
Weinshenker, B.G., Ebers, G.C., 1987. The natural history of multiple sclerosis. The Canadian journal of neurological sciences. Le journal canadien des sciences neurologiques. 14**,** 255-61.
Weinshenker, B.G., Bass, B., Rice, G.P., Noseworthy, J., Carriere, W., Baskerville, J., Ebers, G.C., 1989a. The natural history of multiple sclerosis: a geographically based study. I. Clinical course and disability. Brain. 112 (Pt 1)**,** 133-46.
Weinshenker, B.G., Bass, B., Rice, G.P., Noseworthy, J., Carriere, W., Baskerville, J., Ebers, G.C., 1989b. The natural history of multiple sclerosis: a geographically based study. I. Clinical course and disability. Brain : a journal of neurology. 112 (Pt 1)**,** 133-46.
Weinshenker, B.G., Bass, B., Rice, G.P., Noseworthy, J., Carriere, W., Baskerville, J., Ebers, G.C., 1989c. The natural history of multiple sclerosis: a

geographically based study. 2. Predictive value of the early clinical course. Brain : a journal of neurology. 112 (Pt 6), 1419-28.
Weinshenker, B.G., Rice, G.P., Noseworthy, J.H., Carriere, W., Baskerville, J., Ebers, G.C., 1991a. The natural history of multiple sclerosis: a geographically based study. 3. Multivariate analysis of predictive factors and models of outcome. Brain : a journal of neurology. 114 (Pt 2), 1045-56.
Weinshenker, B.G., Rice, G.P., Noseworthy, J.H., Carriere, W., Baskerville, J., Ebers, G.C., 1991b. The natural history of multiple sclerosis: a geographically based study. 4. Applications to planning and interpretation of clinical therapeutic trials. Brain : a journal of neurology. 114 (Pt 2), 1057-67.
Weinshenker, B.G., Issa, M., Baskerville, J., 1996. Meta-analysis of the placebo-treated groups in clinical trials of progressive MS. Neurology. 46, 1613-9.
Wekerle, H., Kojima, K., Lannes-Vieira, J., Lassmann, H., Linington, C., 1994. Animal models. Annals of neurology. 36 Suppl, S47-53.
Wender, M., Pruchnik-Grabowska, D., Hertmanowska, H., Kowal, P., Zielinska, M., Namysl, I., Marcinkowski, J., 1985. Epidemiology of multiple sclerosis in western Poland--a comparison between prevalence rates in 1965 and 1981. Acta neurologica Scandinavica. 72, 210-7.
Wender, M., Kowal, P., Pruchnik-Grabowska, D., Marcinkowski, J., Hertmanowska, H., Namysl, I., Zielinska, M., 1987. [Multiple sclerosis: its prevalence and incidence in the population of western Poland]. Neurologia i neurochirurgia polska. 21, 33-9.
Westlund, K., 1970. Distribution and mortality time trend of multiple sclerosis and some other diseases in Norway. Acta neurologica Scandinavica. 46, 455-83.
Whitaker, J.N., McFarland, H.F., Rudge, P., Reingold, S.C., 1995. Outcomes assessment in multiple sclerosis clinical trials: a critical analysis. Multiple sclerosis. 1, 37-47.
Willer, C.J., Dyment, D.A., Risch, N.J., Sadovnick, A.D., Ebers, G.C., 2003. Twin concordance and sibling recurrence rates in multiple sclerosis. Proceedings of the National Academy of Sciences of the United States of America. 100, 12877-82.
Willer, C.J., Dyment, D.A., Sadovnick, A.D., Rothwell, P.M., Murray, T.J., Ebers, G.C., 2005. Timing of birth and risk of multiple sclerosis: population based study. BMJ. 330, 120.
Willis, S.N., Stadelmann, C., Rodig, S.J., Caron, T., Gattenloehner, S., Mallozzi, S.S., Roughan, J.E., Almendinger, S.E., Blewett, M.M., Bruck, W., Hafler, D.A., O'Connor, K.C., 2009. Epstein-Barr virus infection is not a characteristic feature of multiple sclerosis brain. Brain : a journal of neurology. 132, 3318-28.
Willoughby, E.W., Paty, D.W., 1988. Scales for rating impairment in multiple sclerosis: a critique. Neurology. 38, 1793-8.
Willoughby, E.W., Grochowski, E., Li, D.K., Oger, J., Kastrukoff, L.F., Paty, D.W., 1989. Serial magnetic resonance scanning in multiple sclerosis: a second prospective study in relapsing patients. Annals of neurology. 25, 43-9.
Wolinsky, J.S., Narayana, P.A., O'Connor, P., Coyle, P.K., Ford, C., Johnson, K., Miller, A., Pardo, L., Kadosh, S., Ladkani, D., 2007. Glatiramer acetate in primary progressive multiple sclerosis: results of a multinational,

multicenter, double-blind, placebo-controlled trial. Annals of neurology. 61, 14-24.
Wolinsky, J.S., Beck, C.A., 2011. The long march to surrogates of meaningful clinical outcomes in MS trials: are we there yet? Neurology. 77, 1658-9.
Wynn, D.R., Rodriguez, M., O'Fallon, W.M., Kurland, L.T., 1990. A reappraisal of the epidemiology of multiple sclerosis in Olmsted County, Minnesota. Neurology. 40, 780-6.
Young, I.R., Hall, A.S., Pallis, C.A., Legg, N.J., Bydder, G.M., Steiner, R.E., 1981. Nuclear magnetic resonance imaging of the brain in multiple sclerosis. Lancet. 2, 1063-6.
Young, J., Quinn, S., Hurrell, M., Taylor, B., 2009. Clinically isolated acute transverse myelitis: prognostic features and incidence. Multiple sclerosis. 15, 1295-302.
Young, P.J., Lederer, C., Eder, K., Daumer, M., Neiss, A., Polman, C., Kappos, L., 2006. Relapses and subsequent worsening of disability in relapsing-remitting multiple sclerosis. Neurology. 67, 804-8.
Yousry, T.A., Major, E.O., Ryschkewitsch, C., Fahle, G., Fischer, S., Hou, J., Curfman, B., Miszkiel, K., Mueller-Lenke, N., Sanchez, E., Barkhof, F., Radue, E.W., Jager, H.R., Clifford, D.B., 2006. Evaluation of patients treated with natalizumab for progressive multifocal leukoencephalopathy. The New England journal of medicine. 354, 924-33.
Zaadstra, B.M., Chorus, A.M., van Buuren, S., Kalsbeek, H., van Noort, J.M., 2008. Selective association of multiple sclerosis with infectious mononucleosis. Multiple sclerosis. 14, 307-13.
Zamvil, S.S., Steinman, L., 1990. The T lymphocyte in experimental allergic encephalomyelitis. Annual review of immunology. 8, 579-621.
Zhang, X., Haaf, M., Todorich, B., Grosstephan, E., Schieremberg, H., Surguladze, N., Connor, J.R., 2005. Cytokine toxicity to oligodendrocyte precursors is mediated by iron. Glia. 52, 199-208.
Zivadinov, R., Weinstock-Guttman, B., Hashmi, K., Abdelrahman, N., Stosic, M., Dwyer, M., Hussein, S., Durfee, J., Ramanathan, M., 2009a. Smoking is associated with increased lesion volumes and brain atrophy in multiple sclerosis. Neurology. 73, 504-10.
Zivadinov, R., Zorzon, M., Weinstock-Guttman, B., Serafin, M., Bosco, A., Bratina, A., Maggiore, C., Grop, A., Tommasi, M.A., Srinivasaraghavan, B., Ramanathan, M., 2009b. Epstein-Barr virus is associated with grey matter atrophy in multiple sclerosis. Journal of neurology, neurosurgery, and psychiatry. 80, 620-5.
Zwemmer, J.N., Bot, J.C., Jelles, B., Barkhof, F., Polman, C.H., 2008. At the heart of primary progressive multiple sclerosis: three cases with diffuse MRI abnormalities only. Multiple sclerosis. 14, 428-30.