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“Modelling disability trajectories over the disease course in Multiple Sclerosis patients”

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DEDICATED WITH EXTREME AFFECTION AND GRATITUDE TO

*my parents Mr. G. Gallo and Mrs. O. De Vincenzi*

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Abstract

Background: Multiple Sclerosis (MS) is a chronic autoimmune disease that attacks the CNS. The immune attack on the CNS cause the damage of a substance, called myelin, which surrounds and protects the nerve fibres. MS is one of the most common causes of neurological disability in young adults. It is well established that axonal injury is a feature of multiple sclerosis (Charcot JM, 1880), that the extent of axonal injury is correlated with the degree of inflammation (Trapp BD, 1998) at least in relapsing multiple sclerosis, and that a close association between inflammation and neurodegeneration might exist in all disease stages of multiple sclerosis (Kutzelnig A, 2005; Frischer J, 2009). However, the interdependence between focal inflammation, diffuse inflammation and neurodegeneration, and their relative contribution to clinical deficits remain ambiguous. Nevertheless, this point is central for understanding the mechanism of tissue injury in multiple sclerosis, which may have an effect on treatment. It has therefore been suggested that disability accrual at later MS stages is primarily driven by neurodegeneration and is largely independent of inflammation. These observations have led to a two-stage hypothesis, with the first stage representing a therapeutic window for modifying disease trajectory, which then becomes uniform in the second stage of disease (Leray E, 2010). This concept was also confirmed in others studies (Scalfari A, 2010; Stys PK, 2012).
Objective: to explore whether disability accrual in moderately and advanced MS is ascribable to the concept of multiple sclerosis as a two-stage disease as proposed by Leray et al. (Leray E, 2010).

Methods: The research was conducted using patients identified through the MS Centre of Montichiari (Brescia, Italy) which is a territory-based centre created in 1980 mainly for patients of Brescia and province. Patients were identified through the territory-based MS Centre of Montichiari (Brescia, Italy) which is a centre created The disability was graded using the Kurtzke Expanded Disability Status Scale (EDSS) and was evaluated through two different neurological disability epochs. In particular, a total of 227 (153 male and 74 female) out of 1442 MS relapsing-remitting patents diagnosed between 1980 and 2016 fulfilled the inclusion criteria for each epoch (pre- and post-EDSS 3). After defining disability milestone and selecting patients (see section 3.2), the EDSS disability trajectory slopes were studied. The outcome of interest were the disability EDSS trajectory slopes in the spam of time prior to and following the EDSS 3 status, that were calculated with a Mixed-Effect Model (MEM) over the pre- or the post-EDSS 3 scores (including the EDSS 3 score in both). The disease progression slope and variability were respectively examined by the F Variance test through the individual EDSS slopes for the pre- and post-EDSS 3 periods. In order to investigate different longitudinal disability trajectories during pre- and post-EDSS 3 epoch, a Latent Class Mixed-effect Model (LCMM) was performed using the fitted EDSS values of the MEM as the dependent variable and the disease duration from MS diagnosis was entered as
covariate in the model. A linear disease duration term was used to specify for the random-effects in the latent process mixed model, i.e., the individual variation around the mean trajectory (of the individual's latent class). Models with one, two, three, etc., latent trajectory classes were fit and to select the best model in term of the number of classes detected, the parsimony seeks minimum values for information criteria (Akaike Information Criterion and, Bayesian Information Criterion) was adopted. Association among classification pre- and post-EDSS 3 epoch was performed using chi-square or Fisher's Exact Test.

The disability trajectories over the disease course were studied, using again, the LCMM including disease duration as covariates with the EDSS disability score as the outcome measure. The linear disease duration were used to specify for the random-effects in the LCMM. After choosing the best model as describe above, univariate analysis was performed using logistic regression, to screen possible determinants of class membership among clinical and demographic characteristics, such as gender, familiarity, group of diagnosis, age at diagnosis, therapy delay, median among EDSS visits, age at on-set and EDSS at diagnosis. Those covariates with a p-value <0.05 were then selected for the multivariate analysis, where the logistic regression model was again used. Differences, with a p-value less than 0.05, were selected as significant.

Results: To find a model that provides the best fit to the data for the pre- and post EDSS 3 epoch, five latent class models were performed increasing the number of classes from 1 until 5.
As regard the per-EDSS 3 epoch, the lowest Akaike Information Criterion (AIC) BIC and AIC were obtained for model with two latent trajectory classes (BIC= -23847.87; AIC= -23912.94). When patients were assigned to the two classes, based on maximum posterior probabilities, there were 197 and 30 patients assigned to class 1 and 2, respectively. The mean of posterior probabilities in each class was 94% and 80%, for class 1 and 2, respectively. As regard distribution of the clinical and demographic characteristics in the two classes, similar gender, familiarity distribution, age at diagnosis and at onset means in two classes were observed, instead the EDSS at diagnosis mean in moderate disability class was greater than that in high disability class (1.83 versus 0.63).

Considering post-EDSS 3 epoch, The lowest BIC and AIC were obtained for model with two latent trajectory classes (BIC= -32285.67; AIC= 32337.04). When patients were assigned to the two classes, based on maximum posterior probabilities, there were 159 and 68 patients assigned to class 1 and 2 (mean of posterior probabilities in each class: 92% and 88%, respectively). As regard distribution of the clinical and demographic characteristics in the two classes, similar gender, familiarity distribution, EDSS at diagnosis mean, age at diagnosis and at onset means in two classes were observed.

The disability trajectory slope mean was 0.08 (SD= 0.04) for pre-EDSS 3 epoch while, a mean of 0.11 (SD=0.23) disability trajectory slope was estimated for the post-EDSS 3 epoch. The graphical evaluation showed that the disability trajectory slopes were differently and highly variable, as
significant evidenced by the F test to compare variances (F test for variance ratio=0.02; p-value<0.0001).

The chi-square test, performed to assay whether observations on two variables were independent of each other, showed no significant association between the pre-EDSS 3 classes and the post-EDSS 3 classes (p-value = 0.6647).

The disability trajectories over the disease course was studied on a total of 881 (602 female; 68.33%) disability EDSS score trajectories over the disease course of patents diagnosed between 1980 and 2016.

Five Latent Class Mixed-effect Model (LCMM) increasing the number of classes from 1 until 5 were performed and the lowest Akaike Information Criterion (AIC) and, Bayesian Information Criterion (BIC) was obtained for model with three latent trajectory classes (AIC= 34984.11; BIC = 35098.86). The graphical evaluation of the plot of the disability EDSS trajectory scores highlighted that two of 3 latent trajectory had very similar graphic trend, so it was decided to aggregate this two similar trajectory in one that it was defined as moderate disability trajectory.

As regard distribution of the clinical and demographic characteristics in the two classes, the age at onset and at diagnosis means were greater in high disability class compared with those in moderate disability class, as significant evidenced by the univariate analysis (p-values: 0.0035 and 0.0023, respectively). The multivariate analysis (Table 11) highlighted the significant effect of age at diagnosis on high disability class membership (p-values=0.0023). In particular, a one-unit increase in age at diagnosis
was associated with an increased chances 5% (OR=1.05) of having high disability class membership.

**Conclusion:** The graphical and analytic evaluation among disability trajectory slopes in pre- and post-EDSS 3 epoch showed that they were differently and highly variable (see section 4.1). The Latent Class Growth Analysis identified two main disability trajectories in both pre- and post-EDSS 3 epoch. No significant association, between the main disability trajectories, was observed using chi-square test (p-value = 0.6647). In contrast to the study of Leray et al. (Leray E, 2010), we have shown that disability trajectories in advanced MS are highly variable as recently showed by Lizak (Lizak N, 2017). Moreover, our results concur that the disability trajectories in advanced disease (post-EDSS 3) is independent of previous disability trajectories (pre-EDSS 3).

Regarding disability trajectories over the disease course, the lowest BIC and AIC was obtained for model with three latent trajectory classes with a mean of posterior probabilities in each class of 55% 61% and 77%, respectively. Two trajectories out of 3 had very similar graphic trend, so it was decided to aggregate this two similar trajectory in one that it was defined as moderate disability trajectory. The next univariate and then multivariate analysis, performed to screen possible determinants of class membership among clinical and demographic characteristics, highlighted the significant effect of age at diagnosis on high disability class membership (p-values=0.0023). In particular, a one-unit increase in age at diagnosis was associated with an increased chances 5% (OR=1.05) of having high disability class membership.
1 Introduction

Multiple sclerosis (MS) is a common, progressive neurodegenerative disease that typically strikes young adults in the prime of their life causing irreversible physical and mental disability. MS represents an immense long term burden for society.

The disease devastates the lives of people with MS and their families, and places an immense long-term burden on society and healthcare system.

The MS affects many young adults worldwide. Globally, the estimated number of people with MS has increased from 2.1 million in 2008 to 2.3 million in 2016. The disease appears to be on the increase, but better reporting and diagnosis may have contributed to this change.

The MS is found worldwide but becomes more common with increasing distance from the equator and is therefore most prevalent in North America, Europe, Australia and other high-income countries. Globally, the median estimated prevalence of MS is 30 people per 100000. Countries with the highest estimated prevalence included Hungary (176), Slovenia (150), Germany (149), USA (135), Canada (133), Czech Republic (130), Norway (125), Denmark (122), Poland (120) and Cyprus (110).

The onset of MS typically occurs between the ages of 20 and 40 years, when individuals are most active and productive in many aspects of their lives and frequently leads to the loss of gainful employment and cognitive impairment is a large contributor to this high rate of unemployment. Since the MS strikes young adults with the potential for many decades of employment and family life, it is the leading cause of non-traumatic
disability among the young and middle-aged in many developed countries: across all age groups in the US, MS is the third most common cause of paralysis and of wheelchair use.

Around two thirds of people with MS are women, a large number of whom are of childbearing age.

Symptoms are distressing and exhausting. The most common types of initial clinical symptoms are sensory (40%), motor (39%), visual (30%) and fatigue related (30%). The range of symptoms experienced depends on the locations of the lesions in the central nervous system (CNS).

The MS is progressive and irreversible. The damage occurs to myelin in the brain and spinal cord. In particular, the immune system mistakenly attacks myelin, disrupting the electrical signals that travel along nerves.

In a healthy person, electrical signals controlling thought processes, movement and bodily functions travel efficiently along the nerve cells within the CNS, composed by brain, spinal cord and optic nerve.

In MS, the body’s immune system wrongly attacks the insulating sheath (made of myelin) that surrounds nerve cells in CNS. The cause of this immune attack is unclear and seems to involve complex interactions between genetics and environmental risk factors. The myelin sheaths become inflamed in small patches (called lesions), which distort or interrupt the electrical signals that travel along nerve fibres.

The immune system does its best to repair the myelin, but eventually the repair process is overwhelmed, the sheath is destroyed ad damage to nerve fibres occurs.
This cycle of immune attacks and repair can proceed undetected until a lesion occurs in an area of the brain responsible for complex functions, which manifests clinically as an attack of symptoms, or enough damage accumulates for clinical symptoms of progressive MS become apparent.

1.1 Multiple sclerosis

The MS is a chronic autoimmune disease that attacks the CNS. The immune attack on the CNS cause the damage of a substance, called myelin, that surrounds and protects the nerve fibres. In fact when any part of the myelin sheath or of nerve fibres is damaged or completely destroyed, nerve impulses traveling to and from the brain and the spinal cord are distorted or interrupted and a large variety of symptoms can occur.

The process of damage of myelin, called demyelination, forms scar tissue (sclerosis), which give the disease its name.

The demyelination cause a break-down of the blood-brain barrier (BBB) with consequent problems for brain cells and spinal cord to communicate with each other.

Demyelination includes cortex and deep grey matter nuclei, as well as diffuse injury of normal-appearing white matter. The mechanisms responsible for the formation of focal lesions in different patients and in different stages of the disease as well as those involved in the induction of diffuse brain damage are complex and heterogeneous.
Damages all occur on a background of inflammatory reaction, composed of lymphocytes and activated macrophages or microglia, and show demyelination, in which axons are at least partly preserved.

Inflammation is dominated by T cells and activated macrophages or microglia. In active lesions this inflammatory process is accompanied by a profound disturbance of the BBB, the local expression of pro-inflammatory cytokines and chemokines as well as of their cognate receptors. In MS, however, the composition of inflammatory infiltrates in the lesions is different. In some cases of acute MS CD8+ T cells, which express granzyme B as a marker of cytotoxic activation, can be seen in close proximity or attachment to oligodendrocytes. Complete demyelination is accompanied by a variability degree of acute axonal injury and axonal loss which in part is counteracted by remyelination. When nerve fibres are damaged, the brain has some ability to re-route signals via undamaged fibres or compensate for the damage. This built-in ‘reserve’ can act as a buffer against cognitive decline at various stages of the disease, but the brain’s ability to ‘buffer’ against damage is finite.

In general, the axons and cell bodies remain intact, despite the absence of myelin. Wallerian degeneration (i.e., axon destruction) may occur in MS, although typically it is described in chronic lesions. Consequently, the pathology in MS may not result in complete cessation of neural transmission, since axons and cell bodies are intact.

Most studies on pathology and pathogenesis have so far concentrated on focal demyelinated lesions in the white matter mainly at the chronic disease stage. This plaque-centred view has recently been challenged by
magnetic resonance imaging (MRI) studies, which revealed a much more widespread and global damage of the brain and spinal cord, in particular in patients at late stages of the disease. Consequently, MS plaques do not localize to discrete systematized fibre tracts but they spread in a centrifugal manner.

The most frequent symptoms are fatigue, numbness of face and body or extremities (arms and legs), vision problems, loss of balance and coordination with associated walking problems and others. Since these symptoms are very general and vary depending on where the cerebral damage has occurred, frequently it takes many years before MS is diagnosed and once it happens it is however very difficult to predict the progression of disease for each patient.

The rarity of MS among Samis, Turkmen, Uzbeks, Kazakhs, Kyrgyzs, native Siberians, North and South Amerindians, Chinese, Japanese, African blacks and New Zealand Maoris, as well as the high risk among Sardinians, Parsis and Palestinians, clearly indicate that the different susceptibilities of distinct racial and ethnic groups are an important determinant of the uneven geographic distribution of the disease.

Prevalence data imply that racial and ethnic differences are important in influencing the worldwide distribution of MS and that its geography must be interpreted in terms of the probable discontinuous distribution of genetic susceptibility alleles, which can however be modifies by environment. Because the environmental and genetic determinants of geographic gradients are by no means mutually exclusive, the race versus place controversy is, to some extent, a useless and sterile debate.
In Italy, during the last 30 years, the frequency of MS in the Italian peninsula and its two major islands, Sicily and Sardinia, has been studied in detail and by means of repeated assessments. The island of Sardinia represents a striking exception to the even distribution of MS in Italy. The most recent survey on large populations confirms the results of previous studies on small populations, indicating that this Italian island has the highest frequency of MS in Mediterranean Europe and one of the highest in the world. The prevalence of MS was 152 per 100 000 in the province of Nuoro in 1994 and 144 in the province of Sassari in 1997. Because of their peculiar genetic structure, Sardinians are probably more susceptible to the disease as compared to other Italians. The genetic distance of Sardinians from most present-day Europeans is second only to Samis and exceeds that of Basques; it is reflected by an unusually high frequency of some blood groups, HLA phenotypes and thalassemia variants that are rare elsewhere. These characteristics reflect several millennia of genetic drift in a small and isolated population.

Environmental factors, anyway, are important. Among the considered environmental factors smoking was found as one of the important MS risk factors. Alcohol, coffee and smoking are connected with high EDSS score. Vitamin D insufficiency (linked with low UV exposition) activated the development of MS. Researchers provided a linear inverse relationship between the risk of MS and the level of education. They found increased risk of MS in women with migraine and probably in people using mobile phones at least for 13 years.
In Denmark besides genetic factors of MS manifestation, a few environmental contributions were investigated. The most important of these were the first infection with Epstein-Barr virus (threelfold enhanced risk of MS) and the child’s lost (a 50% increased risk of developing MS in solitary parents). There were no associations between MS and childhood infections at specific ages, head injuries or exposure to organic solvents. It was possible to estimate the risk of cancer in MS patients. It was reduced by 16% in males and the same as in the background population in females (except breast cancer). The risk of developing MS was increased threefold in patients with diabetes mellitus type I.

As currently only relapsing forms of MS are treatable. An increasing number of disease-modifying therapies (DMTs) that aim to alter the disease course have been approved for treating CIS and relapsing forms of MS. However none has been shown to be effective in treating PPMS or SPMS in its non-relapsing stage.

It is important to diagnose as early as possible so that DMT can be initiated to prevent or delay the onset of further relapses or irreversible disability. Researchers suggest that neuropsychological tests could serve as early diagnostic tools to detect subtle disease progression that may require initiation of DMT.

In recent years, an immunosuppressive regimen followed by the autologous hematopoietic stem cell transplantation (AHSCT) has been a new option for these patients and for other patients affected by several autoimmune disorders as well. It essentially consists in the replacement of defective bone marrow with a healthy and efficacious one. The target
of this treatment is the eradication of auto reactive cells, followed by the infusion of hematopoietic stem cells from previously stored bone marrow or blood cells from the patient (autologous), that either do not contain the autoimmune generating components or that have been purged of them. Stem cells (usually collected from the patient’s peripheral blood prior to conditioning) are reinfused after the conditioning regimen has been completed.

Recently, on June 2016, despite the aggregation observed in some families, pathogenic mutations have remained elusive, Canadian scientists could describe the identification of NR1H3 p.Arg415Gln in seven progressive MS patients from two multi-incident families. The p.Arg415Gln position is highly conserved in orthologs and paralogs, and disrupts NR1H3 heterodimerization and transcriptional activation of target genes. Protein expression analysis revealed that mutant NR1H3 (LXRA) alters gene expression profiles, suggesting a disruption in transcriptional regulation as one of the mechanisms underlying MS pathogenesis. The study indicates that pharmacological activation of LXRA or its targets may lead to effective treatments for the highly debilitating and currently untreatable progressive phase of MS.

Even in the early stages of MS when physical disability is minimal, cognitive impairment can result in a lower health-related quality of life (e.g. greater fatigue, poorer physical well-being), a negative impact upon day-to-day activities and a reduced ability to work.
With the application of neuropsychological tests, evidence is accumulating that cognitive dysfunction is more prevalent and can occur in the course of the disease than had been estimated previously.

The earlier MS can be diagnosed, the earlier treatment can be started - before further deterioration in brain health, relapses or irreversible disability occur.

The total annual direct (medical and non-medical) and indirect costs of MS in Europe has been estimated at 15 euro billion; an average of 36000 euro per person with MS. This is a greater annual cost per person than for other long-term conditions such as asthma, Alzheimer’s disease and diabetes.

1.2 Different stages in multiple sclerosis
Several clinical courses are usually distinguished in MS (Jekyll Island Meeting of MS Society 1995, reported in Lublin FD, 1996) in particular, a first neurological episode of clinical symptoms at the expense of CNS, lasting at least 24 hours, is known as a Clinically Isolated Syndrome (CIS). The CIS stage is caused by inflammation/demyelination in one (mono-focal) or more (multi-focal) sites in the central nervous system and it could represent the first step for a subsequent confirmation of MS diagnosis. In fact not all patients who had a CIS not necessarily go on to develop the MS and this is depending on the similitude of lesions detected with those usually seen in the MS. Sometimes the myelin damage is spotted before any symptoms appear, via a brain scan conducted for another purpose (e.g. headaches). This is MS stage is called Radiologically Isolated Symptoms (RIS); people with RIS are at risk of developing CIS or MS.
To subsequently confirm diagnosis of the MS a series of criterion, defined as McDonald criteria, were developed in 2001 (McDonald WI, 2001) and lately updated in 2010 (Polman CH, 2011). They are based on number of lesions and attacks (relapses) observed in patients (Chapter 2.3).

Once the MS is clinically confirmed the evolution of disease during time is highly variable from one patient to another one and basically it is possible to identify four different form of clinical course.

Among all patients about 85% of them start with a Relapsing-Remitting (RRMS) form in which clearly defined periods, lasting from few days to weeks, of neurological symptoms, called relapse.

Usually, a relapse develops over a few days, before the symptoms plateau and ease off (remit) over the next few weeks or months. Although patients can sometimes be complete recovery from a relapse (particularly early in the disease course), relapses are often associated with a measurable and sustained increase in disability. In the long term, incomplete recovery from relapses may contribute to the stepwise progression of disability. Early on in RRMS, nerve fibres are destroyed and the brain begins to atrophy. When this damage exceeds a certain threshold, the patient stage of progressive disability starts to be called Secondary Progressive MS (SPMS).

In SPMS, disability gets continuously worse (with or without relapses) especially the ability to walk. It takes around 10-20 years for a person with RRMS to develop SPMS on average, and within 20-25 years up to 90% of them will have SPMS. Relapses lead to more pain, restricted mobility, an
increased risk of depressive symptoms, reduced functional ability and a lower health-related quality of life compared with a state of remission.

Clinical and MRI data suggest that inflammation and the formation of new white matter lesions are the substrate for RRMS, while in the progressive phase new inflammatory demyelinating lesions are rare but diffuse atrophy of the grey and white matter and changes in the so-called normal-appearing white matter become prominent. It has been suggested that in the early phase of the disease inflammation is the driving force, whereas the progressive phase may be underlined by a neurodegenerative process, which develops at least in part independent from inflammation.

Around 10% of people with MS have a progressive disease course from the start, with no relapses and progressively worsening. This is known as Primary Progressive MS (PPMS) state. These patients are more likely to be older and male when compared with patients who have relapsing-remitting disease.

A small number of people are classified as having Benign MS (BMS), on the basis of their lack of or slow accumulation of clinically disability. However, this term can be misleading because it does not account for other aspects of the disease and a large proportion of people with MS classified as having BMS end-up becoming disabled. Patient with BMS have better physical disability outcome at 5 years compared to non-BMS cases. However, cognitive impairment frequency and decline over time appeared similar compared to other types of MS. Studies furthermore confirm that cognitive impairment was more accentuated in the BMS patients compared to the RRMS ones, assessed
with a response time test and a percentage of correct responses test. This suggest a silent deterioration of cognitive skills for the BMS that is not usually treated with pharmacological or neuropsychological therapy.

Different stages of the disease suggests that the mechanisms of tissue injury are heterogeneous between patients and stage dependent within the same patient.

The relapses and disease progression of the MS limit everyday activities, restrict participation in society and reduce health-related quality of life at all disease stages.

Despite the devastating impact of the disease, people with the MS have their life expectancy reduced by only 5-10 years. This indicates that most of the MS people live with substantial disability for long time.

1.3 Monitoring disease activity and progression in multiple sclerosis

Currently, there are no symptoms, physical findings or laboratory assessments, which can help to identify the MS disease. No single test is proof-positive for diagnosing multiple sclerosis. Several strategies are used to determine if a person meets the long-established criteria for a MS diagnosis, and to rule out other possible causes. These strategies include a careful medical history, a neurologic exam and various tests including magnetic resonance imaging (MRI), evoked potentials (EP) and spinal fluid analysis. The EP test evaluates the electrical activity of the brain in response to a stimulation of a specific sensory nerve pathway. It is able to detect the slowing of the electrical conduction due to demyelination.
Spinal fluid test analyses a clear, colourless liquid that bathes the brain and spinal cord, cushioning the brain within the skull and serving as a shock absorber for the CNS.

### 1.3.1 Relapses

To monitor the clinical progression and the disease activity several measures of clinical evaluations are available for each MS stage. The clinical relapses represent the most reliable marker of disease activity. A relapse is defined as an episode of objective neurological dysfunction lasting a minimum of 24 hours and occurring more than 28 days from any previous neurological symptoms (Poser CM, 1983).

Several drugs have been approved for the treatment of RR-MS on the basis of the reduction of the total number of relapses occurred over a fixed period of time (Group, 1993; Johnson KP, 1995; Jacobs LD, 1996; PRISM, 1998; Polman CH O. P., 2006; Kappos L, 2010;). This quantity is known as relapse-rate and it is generally expressed as Annualized Relapse-Rate (ARR). The ARR frequently represents the primary outcome in the phase III clinical trials, designed to assess the new drugs efficacy.

Moreover recent works (Sormani MP, 2011; Wang YC, 2011) showed that the effects of the two most used drugs, Interferon (IFN) beta-1A and Natalizumab, on the progression of MS-related disability are largely mediated by the effects seen on relapses.

In clinical trials involving CIS patients the standard primary endpoint is generally represented, instead, by the time at which the first relapse occur for each patients. This primary outcome can be expressed as Time
To First Relapse (TTFR) and will be investigated together with the ARR in this thesis.

### 1.3.2 The Expanded Disability Status Scale (EDSS)

Supervising the disability level of MS patients is frequently used as primary outcome in Phase III trials. Disability or disability changes during time are generally monitored using a standard tool represented by the Expanded Disability Status Scale (EDSS) (Kurtzke JF, 1983).

The EDSS is a scale which ranges from 0 to 10 with increments of 0.5 with higher scores corresponding to a greater level of disability of the patient. Particularly EDSS steps 1.0 to 4.5 refer to people with MS who are fully ambulatory while steps 5.0 to 9.5 are defined by the impairment to ambulation.

Administration time will vary depending upon the condition of the patient and the skill of the examiner. Although the EDSS themselves can be rated in a few minutes, the neurological examination that is needed to make the ratings can take anywhere from 15 minutes to a half-hour. The EDSS are administered in person by a trained examiner, most often a neurologist. However, nurse practitioners with the proper training can also complete the neurological examination and rate the EDSS.

Even if the EDSS is largely used as outcome in clinical trials it has some limits. Firstly, is that it is heavily dependent on locomotors functions while appear to be less sensitive to neurological and cognitive dysfunction. Secondly, the EDSS Scores on the lower end of the scale are more dependent upon nuances in the neurological examination; those in the
middle range are more dependent upon gait, while those in the upper (more impaired) range are also dependent upon activities of daily living. Third, the EDSS is defined in a non-continuous scale but takes on ordinal values with steps of 0.5 and, as consequence, it has been observed that it is more susceptible to jumps along the scale rather than seeing a smooth decline or improvement. Moreover for its ordinal characteristic the difference between one step and the following has different meanings at different EDSS levels.

Despite of same limits, the EDSS scale represents a familiar and widely used albeit imperfect standard, it will probably remain an important part of clinical assessment in the MS for the foreseeable future.

1.4 Evidence for a two-stage disability progression in multiple sclerosis

The MS is one of the most common causes of neurological disability in young adults. It is well established that axonal injury is a feature of multiple sclerosis (Charcot JM, 1880), that the extent of axonal injury is correlated with the degree of inflammation (Trapp BD, 1998) at least in relapsing multiple sclerosis, and that a close association between inflammation and neurodegeneration might exist in all disease stages of multiple sclerosis (Kutzelnigg A, 2005; Frischer J, 2009). However, the interdependence between focal inflammation, diffuse inflammation and neurodegeneration, and their relative contribution to clinical deficits remain ambiguous. Nevertheless, this point is central for understanding the mechanism of tissue injury in multiple sclerosis, which may have an effect on treatment.
Four large cohort studies have explored factors affecting the disability accrual at various stages of the MS and variables associated with early disease progression have been identified (Confavreux C, 2006).

It has therefore been suggested that disability accrual at later MS stages is primarily driven by neurodegeneration and is largely independent of inflammation. These observations have led to a two-stage hypothesis, with the first stage representing a therapeutic window for modifying disease trajectory, which then becomes uniform in the second stage of disease (Leray E, 2010). This concept was also confirmed in others studies (Scalfari A, 2010; Stys PK, 2012).

More recently, the variability and predictability of disability trajectories in the MS were evaluated in a large longitudinal data concluding that, the disability trajectories in moderately advanced MS are highly variable and the disability accumulation in moderately advanced and advanced MS remains substantially driven by inflammatory activity (Lizak N, 2017).

1.5 Aim of the research
In this context, the first aim of this research was to test the Leray hypothesis of MS as a two stage disease, by studying s disability trajectories over the disease course in MS patients using advanced statistical modelling approaches.
2 Materials and methods

2.1 Patients and data collection
Patients were identified through the MS Centre of Montichiari (Brescia, Italy) which is a territory-based centre created in 1980 mainly for patients of Brescia and province. All clinical evaluations of MS patients visited in the centre have been performed by four EDSS-certified neurologists (Capra R, 2017). All the MS outpatients included in the analysis were evaluated at our MS Centre between 1980 and 2016, diagnosed with MS according to the MS diagnostic criteria evolving over time (Inusah S, 2010; Kister I, 2012; Veugelers PJ, 2009) with a relapsing-remitting (RR) course at diagnosis and with age at diagnosis 18-60 years. The first symptom was considered MS onset if objectivized by a specialist and six different period of diagnosis were considered (1980-1990, 1991-1995, 1996-2000, 2001-2005, 2006-2010, 2011+) as described in Capra et al. (Capra R, 2017).

2.2 Disability milestone and inclusion criteria
Disability was graded using the Kurtzke Expanded Disability Status Scale (EDSS) (Kurtzke JF, 1983). For the data analysis on disability accumulation, we took into account two different neurological disability epochs. The first epoch, ranged between the first EDSS visit at diagnosis and EDSS 3 (moderate disability but unrestricted ambulation), instead the second epoch come from EDSS 3 to the last EDSS visit (advanced disability). For each epoch (pre- and post-EDSS 3), the population of patients with clinically definite relapse-diagnosis MS were selected. Moreover, patients must have had an EDSS <3 at the first visit and they must have reached
the step of EDSS 3, confirmed over ≥6 months. Patients had ≥12 months
of prospective follow-up prior to EDSS 3 and at least two visits post-
EDSS 3. A minimum required data set consisted of year of birth, gender,
familiarity, date of the first clinical presentation and diagnosis of MS,
disease course at diagnosis, age at on-set and EDSS at diagnosis. The post-
EDSS 3 epoch was selected to emulate the natural history studies
(Confavreux C, 2006; Lizak N, 2017).

2.3 The Mixed-Effect Model

2.3.1 Introduction

The normal linear model (Fox, 2002),

\[ y_i = \beta_1 x_{1i} + \beta_2 x_{2i} + \cdots + \beta_p x_{pi} + \varepsilon_i \]

\[ \varepsilon_i \sim NID(0, \sigma^2) \]

has one random effect, the error term \( \varepsilon_i \). The parameters of the model
are the regression coefficients, \( \beta_1 + \beta_2 + \cdots + \beta_p \) and the error variance,
\( \sigma^2 \). Usually, \( x_{1i} = 1 \), and so \( \beta_1 \) is a constant or intercept.

For comparison with the linear mixed model of the next section, I rewrite
the linear model in matrix form,

\[ y = X\beta + \varepsilon_i \]

\[ \varepsilon_i \sim NID(0, \sigma^2) \]

where \( y = (y_1, y_2, \ldots, y_p)' \) is the response vector; \( X \) is the model matrix,
with typical row \( x_i' = (x_1, x_2, \ldots, x_p)' \); \( \beta = (\beta_1 + \beta_2 + \cdots + \beta_p)' \) is the
vector of regression coefficients; \( \varepsilon = (\varepsilon_1 + \varepsilon_2 + \cdots + \varepsilon_n)' \) is the vector of
errors; \( N_n \) represents the \( n \)-variable multivariate-normal distribution; \( 0 \) is an \( n \times 1 \) vector of zeroes; and \( I_n \) is the order \( n \) identity matrix.

So-called mixed-effect models (or just mixed models) include additional random-effect terms, and are often appropriate for representing clustered, and therefore dependent, data – arising, for example, when data are collected hierarchically, when observations are taken on related individuals (such as siblings), or when data are gathered over time on the same individuals.

There are several facilities in R and S-PLUS for fitting mixed models to data, the most ambitious of which is the \textit{nlme} library (an acronym for non-linear mixed effects), described in detail by Pinheiro (Pinheiro, 2000). Despite its name, this library includes facilities for fitting linear mixed models (along with nonlinear mixed models), the subject of the present appendix. There are plans to incorporate generalized linear mixed models (for example, for logistic and Poisson regression) in the \textit{nlme} library. In the interim, the reader may wish to consult the documentation for the \textit{glmmPQL} function in Venables and Ripley’s (Venables, 1999) MASS library\(^1\).

Mixed models are a large and complex subject, and I will only scrape the surface here. I recommend Raudenbush and Bryk (Raudenbush, 2002) as a general, relatively gentle, introduction to the subject for social scientists, and Pinheiro and Bates (Pinheiro, 2000), which I have already mentioned, as the definitive reference for the \textit{nlme} library.

---

\(^1\) Version 6.3-2 of the MASS library (or, I assume, a newer version) is required.
2.3.2 The linear Mixed-Effect Model
The linear mixed model (Laird N, 1982; Verbeke G, 2000; Hedeker D, 2006; Fitzmaurice G, 2009) has become a standard statistical method to analyse change over time of a longitudinal Gaussian outcome and assess the effect of covariates on it.

Linear mixed models may be expressed in different but equivalent forms. In the social and behavioural sciences, it is common to express such models in hierarchical form. The \textit{lme} (linear mixed effects) function in the \textit{nlme} library, however, employs the Laird-Ware form of the linear mixed model (after a seminal paper on the topic published by Laird N, 1982):

\[
y_{ij} = \beta_1 x_{1ij} + \beta_2 x_{2ij} + \cdots + \beta_p x_{pij} + b_{i1} z_{1ij} + \cdots + b_{iq} z_{qij} + \epsilon_i
\]

\[
b_{ik} \sim N(0, \varphi_k^2), \text{ Cov}(b_k, b_{kr}) = \psi_{kk},
\]

\[
\epsilon_{ij} \sim N(0, \sigma^2 \lambda_k^2), \text{ Cov}(\epsilon_{ij}, \epsilon_{ijr}) = \sigma^2 \lambda_{ijr}.
\]

Where

- \(y_{ij}\) is the value of the response variable for the \(j\)-th of \(n_i\) observations in the \(i\)-th of \(M\) groups or clusters.
- \(\beta_1, ..., \beta_p\) are the fixed-effect coefficients, which are identical for all groups.
- \(x_{1ij}, ..., x_{pij}\) are the fixed-effect repressors for observation \(j\) in group \(i\); the first regressor is usually for the constant, \(x_{1ij} = 1\).
- \(b_{i1}, ..., b_{iq}\) are the random-effect coefficients for group \(i\), assumed to be multivariate normally distributed. The random effects, therefore, vary by group. The \(b_{ik}\) are thought of as random
variables, not as parameters, and are similar in this respect to the errors $\varepsilon_{ij}$.

- $z_{1ij}, \ldots, z_{qij}$ are the random-effect regressors.

- $\phi_k^2$ are the variances and $\psi_{kk'}$ the covariances among the random effects, assumed to be constant across groups. In some applications, the $\psi$’s are parametrized in terms of a relatively small number of fundamental parameters.

- $\varepsilon_{ij}$ is the error for observation $j$ in group $i$. The errors for group $i$ are assumed to be multivariately normally distributed.

- $\sigma^2 \lambda_{ijj'}$ are the covariances between errors in group $i$. Generally, the $\lambda_{ijj'}$ are parameterised in terms of a few basic parameters, and their specific form depends upon context. For example, when observations are sampled independently within groups and are assumed to have constant error variance, $\lambda_{ijj} = \sigma^2$, $\lambda_{ijj'} = 0 \ (for \ j \neq j')$, and thus the only free parameter to estimate is the common error variance, $\sigma^2$. Similarly, if the observations in a “group” represent longitudinal data on a single individual, then the structure of the $\lambda$’s may be specified to capture autocorrelation among the errors, as is common in observations collected over time.

Alternatively but equivalently, in matrix form,

$$y_i = X_i \beta + Z_i b_i + \varepsilon_i$$

$$b_i \sim N(0, \Psi)$$
\[ \varepsilon_i \sim N_{n_i}(0, \sigma^2 \Lambda_i) \]

where:

- \( y_i \) is the \( n_i \times 1 \) response vector for observations in the \( i \)-th group.
- \( X \) is the \( n_i \times p \) model matrix for the fixed effects for observations in group \( i \).
- \( \beta \) is the \( p \times 1 \) vector of fixed-effect coefficients.
- \( Z_i \) is the \( n_i \times q \) model matrix for the random effects for observations in group \( i \).
- \( b_i \) is the \( p \times 1 \) vector of random-effect coefficients for group \( i \).
- \( \varepsilon_i \) is the \( n_i \times 1 \) vector of errors for observations in group \( i \).
- \( \Psi \) is the \( q \times p \) covariance matrix for the random effects.
- \( \sigma^2 \Lambda_i \) is the \( n_i \times n_i \) covariance matrix for the errors in group \( i \).

Another way to define linear mixed model was proposed by Proust-Lima (Proust-Lima C, 2015). For each subject \( i \) in a sample of \( N \) subjects, let consider a vector of \( n_i \) repeated measures \( Y_i = (Y_{i1}, ..., Y_{ij}, ..., Y_{in_i})^T \) where \( Y_{ij} \) is the outcome value at occasion \( j \) that is measured at time \( t_{ij} \).

We distinguish the time of measurement \( t_{ij} \) from the occasion \( j \) because an asset of the linear mixed model is that the times and the number of measurements can vary from a subject to the other. This makes it possible for example to include subjects with intermittent missing data and/or dropout, or to consider the actual individual time of measurement rather than the planned visit, which in some application can greatly differ.
Following Laird and Ware (Laird N, 1982), we define the linear mixed model as follows:

\[ Y_{ij} = X_{Li}(t_{ij})^T \beta + Z_i(t_{ij})^T u_i + w_i(t_{ij}) + \epsilon_i \]  

(1)

where \( X_{Li}(t_{ij}) \) and \( Z_i(t_{ij}) \) are two vectors of covariates at time \( t_{ij} \) of respective length \( p \) and \( q \). The vector \( X_{Li}(t_{ij}) \) is associated with the vector of fixed effects \( \beta \) and \( Z_i(t_{ij}) \), which includes typically functions of time \( t_{ij} \), is associated with the vector of random-effects \( u_i \). Shapes of trajectories considered in \( X_{Li} \) and \( Z_i \) can be of any type (polynomial (Proust C, 2005), specifically designed to fit the trajectory (Proust-Lima C A. H.-G., 2013), or approximated using a basis of splines).

The vector \( u_i \) of \( q \) random-effects has a zero-mean multivariate normal distribution with variance-covariance matrix \( B \), where \( B \) is an unspecified matrix. The measurement errors \( \epsilon_i \) are independent Gaussian errors with variance \( \sigma^2_\epsilon \). Finally, the process \( w_i(t_{ij})_{t \in \mathbb{R}} \) is a zeromean Gaussian stochastic process (e.g., Brownian motion with covariance \( \text{cov} (w_i(t), w_i(s)) = \sigma^2_w \min(t, s) \) or a stationary process with covariance \( \text{cov} (w_i(t), w_i(s)) = \sigma^2_w \exp(-p|t - s|) \)).

The vector of parameters to estimate is \( (\beta^T, \text{vec}(\beta)^T, \sigma_w, \rho, \sigma_e)^T \) where \( \text{vec} (\beta) \) is the vector of parameters involved for modelling the symmetric positive definite matrix \( B \).
2.4 The Latent class growth analysis

2.4.1 Overview

Researchers in the fields of medical, social and psychological sciences are often interested in modelling the longitudinal developmental trajectories of individuals, whether for the study of personality development or for better understanding how clinical characteristics unfold over time (whether it be days, months, or years). This usually requires an extensive dataset consisting of longitudinal, repeated measures of variables, sometimes including multiple cohorts, and analysing this data using various longitudinal latent variable modelling techniques such as latent growth curve models (MacCallum, 2000). The objective of these approaches is to capture information about inter-individual differences in intra-individual change over time (Nesselroade, 1991).

However, conventional growth modelling approaches assume that individuals come from a single population and that a single growth trajectory can adequately approximate an entire population. Also, it is assumed that covariates that affect the growth factors influence each individual in the same way. Yet, theoretical frameworks and existing studies often categorize individuals into distinct subpopulations (e.g., socioeconomic classes, age groups, at-risk populations). For example, in the field of alcohol research, theoretical literature suggests different classes of alcohol use initiation patterns, e.g., ‘early’ versus ‘late’ onsetters (Hill, 2000). Using Growth Mixture Modelling (GMM) with five different indices of alcohol use (alcohol use disorder, alcohol dependence, alcohol consequences, past year alcohol quantity and frequency, and heavy
drinking), Jackson and Sher (Jackson, 2005) identified four distinct classes for each measure. The results of these studies confirm theoretical contentions that heterogeneity of growth trajectories exist within the larger population. In addition, these findings suggest that describing an entire population using a single growth trajectory estimate is oversimplifying the complex growth patterns that describe continuity and change among members of different groups. Instead, a latent class or growth mixture modelling approach seems to be the most appropriate method for fully capturing information about inter-individual differences in intra-individual change taking into account unobserved heterogeneity (different groups) within a larger population (Jung T, 2008).

A useful framework for beginning to understand latent class analysis and growth mixture modelling is the distinction between person-centred and variable-centred approaches (Muthén, 2000). Variable-centred approaches such as regression, factor analysis, and structural equation modelling focus on describing the relationships among variables. The goal is to identify significant predictors of outcomes, and describe how dependent and independent variables are related. Person-centred approaches, on the other hand, include methods such as cluster analysis, latent class analysis, and finite mixture modelling. The focus is on the relationships among individuals, and the goal is to classify individuals into distinct groups or categories based on individual response patterns so that individuals within a group are more similar than individuals between groups.
2.4.2 The Latent Process Mixed Model

The linear mixed model applies to longitudinal markers that are continuous, have Gaussian random deviations (random-effects, correlated errors and measurement errors) and it assumes that the covariate effects are constant ($\beta$) along the entire range of the marker values. In practice, these assumptions do not hold for many longitudinal outcomes, especially psychological scales. The generalized linear mixed model extends the theory to binary, ordinal or Poisson longitudinal outcomes (Hedeker D, Longitudinal data analysis., 2006); (Fitzmaurice G, Longitudinal data analysis, 2009). In order to study non Gaussian longitudinal markers, we chose another direction by defining a family of mixed models called the latent process mixed models (Proust C J.-G. H., 2006); (Proust-Lima C A. H.-G., Analysis of multivariate mixed longitudinal data: A flexible latent process approach, 2013). Coming from the latent variable framework, this approach consists in separating the structural model that describes the quantity of interest (a latent process) according to time and covariates from the measurement model that links the quantity of interest to the observations.

The latent process $\Lambda_i(t)$ is defined in continuous time according to a standard linear mixed model without error of measurement:

$$\Lambda_i(t) = X_{Li}(t)^T \beta + Z_i(t)^T u_i + w_i(t), \quad \forall \ t \in \mathbb{R}$$

(2)

where $X_{Li}(t), Z_i(t)$ and $w_i(t)$ are defined in section 3.3.2.

In order to take into account different types of longitudinal markers, a flexible nonlinear measurement model is defined between the latent
process and \( w_i(t_{ij}) \) and the observed value \( Y_{ij} \) at the measurement time \( t_{ij} \).

\[
Y_{ij} = H(\overline{Y}_{ij}; \eta) = H(\Lambda_i(t_{ij}) + \epsilon_{ij}; \eta)
\]

(3)

where \( \epsilon_{ij} \) are independent Gaussian measurement errors with variance \( \sigma^2 \), \( H \) is a parameterized link function and \( Y_{ij} \) denotes the noisy latent process at time \( t_{ij} \).

For a quantitative marker, \( H^{-1} \) is a monotonic increasing continuous function. Are currently implemented:

- the linear transformation that reduces to the Gaussian framework of the linear mixed model:
  
  \[
  H^{-1}(Y_{ij}) = \frac{Y_{ij} - \eta_1}{\eta_2}
  \]

- the rescaled cumulative distribution function (CDF) of a Beta distribution: \( H^{-1}(Y_{ij}; \eta) = \frac{h(Y_{ij}; \eta_1, \eta_2) - \eta_3}{\eta_4} \) with \( h(Y_{ij}; \eta_1, \eta_2) = \int_0^{Y_{ij}} x^{\eta_1-1}(1-x)^{\eta_2-1} \frac{dx}{B(\eta_1, \eta_2)} \) is the complete Beta function. For positiveness properties of canonical parameters \( \eta_1^* \) and \( \eta_2^* \) and computation reasons, the Beta distribution is parameterized as follows: \( \eta_1^* = \frac{e^{\eta_1}}{e^{\eta_2(1-e^{\eta_1})}} \) and \( \eta_2^* = \frac{1}{e^{\eta_1(1+e^{\eta_2})}} \). In addition, \( Y_{ij} \) is rescaled in \((0, 1)\) using \( Y_{ij} = \frac{Y_{ij} - \min(Y) + \epsilon_Y}{\max(Y) - \min(Y) + 2\epsilon_Y} \) with the constant \( \epsilon_Y > 0 \) and \( \min(Y) \) and \( \max(Y) \) the (theoretical or observed) minimum and maximum values of \( Y \).
• a basis of quadratic I-splines with m knots: \( H^{-1}(Y_{ij}; \eta) = \eta_0 + \sum_{l=1}^{m+1} \eta_l B^l_i(Y_{ij}) \) with \( B^l_1, \ldots, B^l_{m+1} \) the basis of I-splines (Ramsay, 1988).

For an ordinal or binary marker (with M levels), equation (3) reduces to a probit (cumulative) model with \( Y_{ij} = H(\Lambda_i(t_{ij}) + \epsilon_{ij}; \eta) = M_0 + l \) if \( \Lambda_i(t_{ij}) + \epsilon_{ij} \in [\eta^*_l, \eta^*_{l+1}] \) for \( l = 0, \ldots, M - 1, M_0 \) the minimum value of the marker, \( \eta_0 = \eta^*_1 = -\infty, \eta^*_M = \eta^*_M > +\infty \) and \( \eta^*_1 = \eta_1, \eta^*_1 = \eta_1 + \sum_{j=2}^{l} \eta^*_j \) for \( l < 1 \) to ensure increasing thresholds \( \eta^*_0 < \eta^*_1, \ldots, \eta^*_M < \eta^*_M \) for the noisy latent process.

Latent process mixed models need two constraints to be identified: one on the location of the latent process managed by the intercept effect \( \beta_0 = 0 \) and one for the scale of the latent process managed by \( \sigma^2_\epsilon = 1 \). So the vector of parameters to estimate is \( (\beta^T, vec(B)^T, \sigma_w, \rho, \eta^T)^T \) where \( vec(B) \) is defined in section 3.3.2.

### 2.4.3 The Latent Class Linear Mixed Model

The linear mixed model assumes that the population of N subjects is homogeneous and described at the population level by a unique profile \( X_{Li}(t)^T \beta \). In contrast, the latent class mixed model consists in assuming that the population is heterogeneous and constituted of G latent classes of subjects characterized by G mean profiles of trajectories. Each subject belongs to one and only one latent class so that the latent class membership is defined by a discrete random variable \( c_i \) that equals \( g \) if subject \( i \) belongs to latent class \( g (g = 1, \ldots, G) \). The variable \( c_i \) is latent;
its probability is described using a multinomial logistic model according to covariates $X_{ci}$:

$$
\pi_{ig} = P(c_i = g | X_{ci}) = \frac{e^{\xi_{og} + x_{ciT}^{l} \xi_{1g}}}{\sum_{l=1}^{G} e^{\xi_{ol} + x_{ciT}^{l} \xi_{1l}}} 
$$

(6)

where $\xi_{ol}$ is the intercept for class $g$ and $\xi_{1g}$ is the $q_1$-vector of class-specific parameters associated with the $q_1$-vector of time-independent covariates $X_{ci}$. For identifiability, the scalar $\xi_{og}$ and the vector $\xi_{og} = 0$. When no covariate predicts the latent class membership, this model reduces to a class-specific probability.

The $G$ mean profiles are defined according to time and covariates through latent class specific mixed models. The difference with a standard linear mixed model is that both fixed effects and the distribution of the random-effects can be class-specific. For a Gaussian outcome, the linear mixed model defined in (1) becomes for class $g$:

$$
Y_{ij | c_i = g} = X_{L1i}(t_{ij})^{T} \beta + X_{L2i}(t_{ij})^{T} v_{g} + Z_{i}(t_{ij})^{T} u_{ig} + w_{i}(t_{ij}) + \varepsilon_{ij}
$$

(7)

where $X_{Li}(t_{ij})$ previously defined is splitted in $X_{L1i}(t_{ij})$ with common fixed effects $\beta$ over classes and $X_{L2i}(t_{ij})$ with class-specific fixed effects $v_{g}$. The vector $Z_{i}(t_{ij})$ is still associated with the individual random-effects $u_{i | c_i = g}$ called $u_{ig}$ in equation (7) whose distributions is now class-specific. In class $g$, they have a zero-mean multivariate normal distribution with variance-covariance matrix $w_{g}^{2}B$, where $B$ is an unspecified variance covariance matrix and $w_{g}$ is a proportional coefficient ($w_{g} = 1$ for identifiability) allowing for a class-specific intensity of individual
variability. The auto-correlated process $w_i(t)$ and the errors of measurement $\varepsilon_{ij}$ are the same as in section 3.3.2.

This extension of the linear mixed model also applies to the latent process mixed model described in sections 3.3.2 by replacing the structural model in (2) by:

$$Y_{ij}|_{c_i=g} = X_{L1i}(t_{ij})^T \beta + X_{L2i}(t_{ij})^T v_g + Z_i(t)^T u_{ig} + w_i(t_{ij})$$

(8)

The location constraint for this model becomes $\beta_{01} = 0$ that is the mean intercept in the last class is constrained to 0. The scale constraint remains unchanged. The measurement models remain the same by assuming the heterogeneity in the population only affects the underlying latent process of interest. The vector of parameters to estimate defined in sections 2.1, 2.2 and 2.3 include now also $(\xi_{og}, \xi_{1g}^T)_{g=1,G-1}, v_g^T_{g=1,G-1}, (w_g)_{g=1,G-1})$

2.4.4 Post-fit computations

In the following, the symbol hat (\^) denotes the value of a parameter/vector/matrix/function computed at the maximum likelihood estimates $\hat{\theta}_G$.

2.4.4.1 Maximum Likelihood Estimates

This subsection applies to the four estimation functions. The table of the maximum likelihood estimates along with their estimated standard error are given in function summary. The vector is directly given by function estimates or in output value best. The estimated variance-covariance matrix of the maximum likelihood estimates is given in function VarCov
and in output value V. In the latter, the upper triangular matrix is given as a vector.

The parameters of the variance-covariance matrix of the random-effects are not directly estimated although they are provided in the summaries. The Cholesky parameters used for the estimation are available in output vector cholesky or in function estimates. Estimated standard-errors of the parameters of the variance-covariance matrix are computed in function VarCovRE in the *lccm* package (Proust-Lima C, 2015).

### 2.4.4.2 Posterior classification

In models involving latent classes, a posterior classification of the subjects in each latent class can be done. It is based on the posterior calculation of the class-membership probabilities. It is used to characterize the classification of the subjects as well as to evaluate the goodness of fit of the model (Proust-Lima C, 2015).

- **Class-membership posterior probabilities and classification**

  The posterior class-membership probabilities are computed using the Bayes theorem as the probability of belonging to a latent class given the information collected. In a longitudinal model, they are defined for subject $i$ and latent class $g$ as

  $$
  \pi_{ig}^{(y)} = P(C_i = g|X_{Li}, X_{Ci}, \hat{\theta}_G) = \frac{\pi_{ig}\phi_{ig}(Y_i|C_i = g, \theta_G)}{\sum_{l=1}^{G} \pi_{il}\phi_{il}(Y_i|C_i = l, \theta_G)}
  $$

  In a joint latent class model, the complete information also includes the time-to-event so that for subject $i$ and latent class $g$, the
posterior class-membership probability can also be defined for subject $i$ and latent class $g$ as

$$
\pi_{ig}^{(Y,T)} = P(C_i = g | X_{Li}, X_{Ci}, X_{Si}, Y_i, T_i, E_i, \hat{\theta}_G) = \frac{\pi_{ig} \phi_{ig}(Y_i | C_i = g, \theta_G) e^{\sum_{p=1}^{P}(T_i | C_i = g, \hat{\theta}_G) \prod_{p=1}^{P} \lambda_i(T_i | C_i = g, \hat{\theta}_G)^{1_{E_i=p}}}{\sum_{i=1}^{G} \pi_{il} \phi_{il}(Y_i | C_i = l, \theta_G) e^{\sum_{p=1}^{P}(T_i | C_i = l, \hat{\theta}_G) \prod_{p=1}^{P} \lambda_p(T_i | C_i = l, \hat{\theta}_G)^{1_{E_i=p}}}
$$

A posterior classification can be obtained from these posterior probabilities by assigning for each subject the latent class in which he has the highest posterior class-membership probability ($\hat{c}_i = \text{argmax}_g(\pi_{ig}^{(Y)})$ or $\hat{c}_i = \text{a.argmax}_g(\pi_{ig}^{(Y,T)}).$

---

**Posterior classification**

The posterior classification can be used to assess the goodness-of-fit of the model (for the selection of the number of latent classes for instance) and the discrimination of the latent classes. Many indicators can be derived from it (Proust-Lima C, 2015). The package `lcmm` provides two indicators in the function `postprob`:

- the proportion of subjects classified in each latent class with a posterior probability above 0.7, 0.8 and 0.9. This indicates the proportion of subjects not ambiguously classified in each latent class.

- the posterior classification table as defined in table 2 which computes the mean of the posterior probabilities to belong to the latent class among the subjects classified a posteriori.
in each latent class. A perfect classification would provide ones in the diagonal and zeros elsewhere. In practice, high diagonal terms indicate a good discrimination of the population.

Table 1: Posterior classification table provided in function \textit{postprob}

<table>
<thead>
<tr>
<th>Final class</th>
<th>Mean of the probability of belonging to each class</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\hat{C}_i$</td>
<td>$\hat{\pi}_i^{(1)}$ ... $\hat{\pi}_i^{(g)}$ ... $\hat{\pi}_i^{(G)}$</td>
</tr>
<tr>
<td>1</td>
<td>$\frac{N_1}{N_1} \sum_{i=1}^{N_1} \hat{\pi}<em>i^{(1)}$ ... $\frac{1}{N_1} \sum</em>{i=1}^{N_1} \hat{\pi}<em>i^{(g)}$ ... $\frac{1}{N_1} \sum</em>{i=1}^{N_1} \hat{\pi}_i^{(G)}$</td>
</tr>
<tr>
<td>...</td>
<td>: ... :</td>
</tr>
<tr>
<td>g</td>
<td>$\frac{N_g}{N_g} \sum_{i=1}^{N_g} \hat{\pi}<em>i^{(1)}$ ... $\frac{1}{N_g} \sum</em>{i=1}^{N_g} \hat{\pi}<em>i^{(g)}$ ... $\frac{1}{N_g} \sum</em>{i=1}^{N_g} \hat{\pi}_i^{(G)}$</td>
</tr>
<tr>
<td>...</td>
<td>: ... :</td>
</tr>
<tr>
<td>G</td>
<td>$\frac{N_G}{N_G} \sum_{i=1}^{N_G} \hat{\pi}<em>i^{(1)}$ ... $\frac{1}{N_G} \sum</em>{i=1}^{N_G} \hat{\pi}<em>i^{(g)}$ ... $\frac{1}{N_G} \sum</em>{i=1}^{N_G} \hat{\pi}_i^{(G)}$</td>
</tr>
</tbody>
</table>

2.5 Statistical analysis

Continuous variables are given as means and standard deviations (SD), whereas categorical variables as number and/or percentage of subjects. The outcome of interest were the disability EDSS trajectory slopes in the span of time prior to and following the EDSS 3 status, that were calculated with a Mixed-Effect Model (MEM) over the pre- or the post-
EDSS 3 scores (including the EDSS 3 score in both). The disease progression slope and variability were respectively examined using t test and F Variance test through the individual EDSS slopes for the pre- and post-EDSS 3 periods.

In order to investigate different longitudinal disability trajectories during pre- and post-EDSS 3 epoch, a Latent Class Mixed-effect Model (LCMM) was performed using the fitted EDSS values of the MEM as the dependent variable and the disease duration from MS diagnosis was entered as covariate in the model. A linear disease duration term was used to specify for the random-effects in the latent process mixed model, i.e., the individual variation around the mean trajectory (of the individual's latent class). Models with one, two, three, etc., latent trajectory classes were fit and to select the best model in term of the number of classes detected, the parsimony seeks minimum values for information criteria (Akaike Information Criterion and, Bayesian Information Criterion) was adopted. Correlation among classification pre- and post-EDSS 3 epoch was performed using chi-square or Fisher's Exact Test.

The disability trajectories over the disease course were studied, using again, the LCMM including disease duration as covariates with the EDSS disability score as the outcome measure. The linear disease duration were used to specify for the random-effects in the LCMM. After choosing the best model as describe above, univariate analysis was performed using logistic regression, to screen possible determinants of class membership among clinical and demographic characteristics, such as gender, familiarity, group of diagnosis, age at diagnosis, therapy delay, median
among EDSS visits, age at on-set and EDSS at diagnosis. Those covariates with a p-value <0.05 were then selected for the multivariate analysis, where the logistic regression model was again used. Differences, with a p-value less than 0.05, were selected as significant and data were acquired and analysed in R v3.4.3 software environment (R, 2017).
3 Results

A total of 227 (153 male and 74 female) out of 1442 MS relapsing-remitting patients diagnosed between 1980 and 2016 fulfilled the inclusion criteria for each epoch (pre- and post-EDSS 3). As regard selection criteria, 1077 patients had an EDSS < 3 at the first visit, 329 patients reached EDSS 3, a pre-EDSS 3 prospective follow-up ≥12 months was observed in 253 patients and 239 patients had at least two visits post-EDSS 3. The excluded patients majority had not had reached the EDSS 3 status yet (N=662) following by patients that had an EDSS great than 3 at the first visit (N=247).

The demographic and clinical characteristics of the study participants are summarised in Table 1. Briefly for 621 patients that no reached EDSS 3, 207 were male while 415 were female. The mean of EDSS at diagnosis was 1.22 (SD=0.97) instead, the age at onset and diagnosis means were 30.95 (SD=8.79) and 33.5 (SD=9.18) years, respectively. Seventy-four (32.6%) patients had a MS familiarity. As regard patients that reached EDSS 3, the mean age at diagnosis was 33.5 years (SD=9.18; range = 18.29 : 59.53 years). The means of age at onset was 31.43 (SD=9.59) and the mean of EDSS at diagnosis was 1.67 (SD=1.15). Twenty-five (11.01%) patients had a MS familiarity.
3.1 Classification of EDSS trajectory slopes versus disease duration

To find a model that provides the best fit to the data for the pre- and post EDSS 3 epoch, five latent class models were performed increasing the number of classes from 1 until 5.

As regard the per-EDSS 3 epoch, the Akaike Information Criterion (AIC) and, Bayesian Information Criterion (BIC) were reported in Figure 1 for different models taken into account. The lowest BIC and AIC was obtained for model with two latent trajectory classes (BIC= -23847.87; AIC= -23912.94). When patients were assigned to the two classes, based on maximum posterior probabilities, there were 197 and 30 patients assigned to class 1 and 2 (Table 2; mean of posterior probabilities in each class: 94% and 80%, respectively). The plot of the disability EDSS trajectory slopes with two latent trajectory classes was reported in Figure 2. In particular, moderate disability EDSS trajectory (magenta latent trajectory) and high disability EDSS trajectory (red latent trajectory) were identified.

As regard distribution of the clinical and demographic characteristics in the two classes, similar gender, familiarity distribution, age at diagnosis and at onset means in two classes were observed (Table 3), instead the EDSS at diagnosis mean in moderate disability class was greater than that in high disability class (1.83 versus 0.63).

Considering post-EDSS 3 epoch, the AIC and, BIC were reported in Figure 3 for different models taken into account. The lowest BIC and AIC was obtained for model with two latent trajectory classes (AIC= 32337.04;
BIC= -32285.67). When patients were assigned to the two classes, based on maximum posterior probabilities, there were 159 and 68 patients assigned to class 1 and 2 (Table 4; mean of posterior probabilities in each class: 92% and 88%, respectively). The plot of the disability EDSS trajectory slopes with two latent trajectory classes was reported in Figure 4. In particular, moderate disability EDSS trajectory (orange latent trajectory) and high disability EDSS trajectory (green latent trajectory) were identified.

As regard distribution of the clinical and demographic characteristics in the two classes, similar gender, familiarity distribution, EDSS at diagnosis mean, age at diagnosis and at onset means in two classes were observed (Table 5).

The plot of the disability EDSS trajectory slopes with the selected latent trajectory classes were reported in Figure 5 for pre- and post-EDSS 3 epoch.

The disability trajectory slope mean was 0.08 (SD= 0.04) for pre-EDSS 3 epoch while, a mean of 0.11 (SD=0.23) disability trajectory slope was estimated for the post-EDSS 3 epoch (Table 6). The histogram of the disability trajectory slopes was reported in Figure 6, for the spam of time prior to (panel A) and following to (panel B) the EDSS 3 status. The graphical evaluation showed that the disability trajectory slopes were differently and highly variable, as significant evidenced by the F test to compare variances (variance ratio=0.02; p-value<0.0001).
The distribution among pre- and post- EDSS 3 latent trajectory classes was reported in table 7. The chi-square test, performed to assay whether observations on two variables were independent of each other, showed no significant association between the pre-EDSS 3 classes and the post-EDSS 3 classes (p-value = 0.6647).

### 3.2 Classification of EDSS score trajectories

A total of 881 (602 female; 68.33%) disability EDSS score trajectories over the disease course of patents diagnosed between 1980 and 2016 were studied. The demographic and clinical characteristics of the study participants are summarised in Table 8. Briefly the mean of EDSS at diagnosis was 1.34 (SD=1.03) instead, the age at onset and diagnosis means were 31.08 (SD=9) and 33.71 (SD=9.21) years, respectively. Seventy-seven (32.6%) patients had a MS familiarity. The mean therapy delay was 38.31 months (SD=47.98).

The disability trajectories over the disease course were studied by five Latent Class Mixed-effect Model (LCMM) increasing the number of classes from 1 until 5. The Akaike Information Criterion (AIC) and, Bayesian Information Criterion (BIC) were reported in Figure 7 for different models taken into account. The lowest BIC and AIC was obtained for model with three latent trajectory classes (AIC= 34984.11; BIC = 35098.86). When patients were assigned to the three classes, based on maximum posterior probabilities, there were 198, 633 and 50 patients assigned to class 1, 2 and 3 (Table 9; mean of posterior probabilities in each class was 55% 61% and 77%, respectively). The plot of the disability EDSS trajectory scores with three latent trajectory classes was reported in Figure 8. In particular,
the trajectories of class 1 and 2 (magenta and blue dodger, respectively) had very similar graphic trend, so it was decided to aggregate this two similar trajectory in one that it was defined as moderate disability trajectory.

The new two trajectory classes were reported in Figure 9 and moderate disability EDSS trajectory (blue latent trajectory) and high disability EDSS trajectory (forest green latent trajectory) were identified.

As regard distribution of the clinical and demographic characteristics in the two classes, similar gender, familiarity distribution, period of diagnosis, median among EDSS visits, EDSS at diagnosis and therapy delay in two classes were observed (Table 10). Instead, the age at onset and at diagnosis means were greater in high disability class compared with those in moderate disability class, as significant evidenced by the univariate analysis (p-values: 0.0035 and 0.0023, respectively). The multivariate analysis (Table 11) highlighted the significant effect of age at diagnosis on high disability class membership (p-values=0.0023). In particular, a one-unit increase in age at diagnosis was associated with a increased chances 5% (OR=1.05) of having high disability class membership.
4 Conclusions

The main aim of this research was to explore whether disability accrual in moderately and advanced MS is ascribable to the concept of multiple sclerosis as a two-stage disease as proposed by Leray et al. (Leray E, 2010). The second aim, but not less important, was to study disability trajectories over the disease course in MS patients.

The research was conducted using patients identified through the MS Centre of Montichiari (Brescia, Italy) which is a territory-based centre created in 1980 mainly for patients of Brescia and province. After defining disability milestone and selecting patients (see section 3.2), the EDSS disability trajectory slopes were studied. The graphical and analytic evaluation among disability trajectory slopes in pre- and post-EDSS 3 epoch showed that they were differently and highly variable (see section 4.1). The Latent Class Growth Analysis identified two main disability trajectories in both pre- and post-EDSS 3 epoch. No significant association, between the main disability trajectories, was observed using chi-square test (p-value = 0.6647). In contrast to the study of Leray et al. (Leray E, 2010), we have shown that disability trajectories in advanced MS are highly variable as recently showed by Lizak (Lizak N, 2017). Moreover, our results concur that the disability trajectories in advanced disease (post-EDSS 3) is independent of previous disability trajectories (pre-EDSS 3).

Regarding disability trajectories over the disease course, the lowest BIC and AIC was obtained for model with three latent trajectory classes with
a mean of posterior probabilities in each class of 55% 61% and 77%, respectively. The first two probabilities evidence that the measured EDSS trajectories had poor membership in each latent class. The proportion of subjects could be ambiguously classified in this two latent class. Two trajectories out of 3 had very similar graphic trend, so it was decided to aggregate this two similar trajectory in one that it was defined as moderate disability trajectory. The next univariate and then multivariate analysis, performed to screen possible determinants of class membership among clinical and demographic characteristics, highlighted the significant effect of age at diagnosis on high disability class membership (p-values=0.0023). In particular, a one-unit increase in age at diagnosis was associated with an increased chances 5% (OR=1.05) of having high disability class membership.
5 Tables and Figures

Table 1: The demographic and clinical characteristics of the study participants. The results are expressed as mean with standard deviation or as number of subjects with percentage.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients No Reached EDSS 3 (N=662)</th>
<th>Patients reached EDSS 3 (N=227)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>207 (31.27%)</td>
<td>153 (67.4%)</td>
</tr>
<tr>
<td>Female</td>
<td>455 (68.73%)</td>
<td>74 (32.6%)</td>
</tr>
<tr>
<td>Familiarity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>52 (7.85%)</td>
<td>202 (88.99%)</td>
</tr>
<tr>
<td>Yes</td>
<td>610 (92.15%)</td>
<td>25 (11.01%)</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1980-1990</td>
<td>94 (14.2%)</td>
<td>27 (11.89%)</td>
</tr>
<tr>
<td>1991-1995</td>
<td>28 (4.23%)</td>
<td>34 (14.98%)</td>
</tr>
<tr>
<td>1996-2000</td>
<td>181 (27.34%)</td>
<td>58 (25.55%)</td>
</tr>
<tr>
<td>2001-2005</td>
<td>17 (2.57%)</td>
<td>58 (25.55%)</td>
</tr>
<tr>
<td>2006-2010</td>
<td>198 (29.91%)</td>
<td>44 (19.38%)</td>
</tr>
<tr>
<td>2011+</td>
<td>144 (21.75%)</td>
<td>6 (2.64%)</td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td>33.5 (9.18)</td>
<td>34.39 (9.5)</td>
</tr>
<tr>
<td>Age at onset</td>
<td>30.95 (8.79)</td>
<td>31.43 (9.59)</td>
</tr>
<tr>
<td>EDSS at diagnosis</td>
<td>1.22 (0.97)</td>
<td>1.67 (1.15)</td>
</tr>
</tbody>
</table>
Figure 1: Akaike Information Criterion (AIC) and, Bayesian Information Criterion (BIC) were reported for different models with different latent trajectory classes in the pre-EDSS 3 epoch.

Table 2: Posterior classification table of the model with two different latent trajectory classes are reported for the pre-EDSS 3 epoch.

<table>
<thead>
<tr>
<th>Posterior classification:</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>class1</td>
<td>197.00</td>
<td>30.00</td>
</tr>
<tr>
<td>class2</td>
<td>86.78</td>
<td>13.22</td>
</tr>
</tbody>
</table>

Posterior classification table:

```
--> mean of posterior probabilities in each class
prob1  prob2
class1  0.9397  0.0603
class2  0.1966  0.8034
```
Figure 2: Plot of the disability EDSS trajectory slopes with two latent trajectory classes are reported for the pre-EDSS 3 epoch.

Table 3: Contingency tables of the clinical and demographic distribution characteristics in the two latent classes

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Classification of EDSS disability trajectories</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-EDSS 3</td>
</tr>
<tr>
<td></td>
<td>moderate (magenta)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>134 (87.58%)</td>
</tr>
<tr>
<td>Female</td>
<td>63 (85.14%)</td>
</tr>
<tr>
<td>Familiarity</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>177 (87.62%)</td>
</tr>
<tr>
<td>Yes</td>
<td>20 (80%)</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
</tr>
<tr>
<td>1980-1990</td>
<td>24 (88.89%)</td>
</tr>
<tr>
<td>1991-1995</td>
<td>30 (88.24%)</td>
</tr>
<tr>
<td>1996-2000</td>
<td>48 (82.76%)</td>
</tr>
<tr>
<td>2001-2005</td>
<td>50 (86.21%)</td>
</tr>
<tr>
<td>2006-2010</td>
<td>39 (88.64%)</td>
</tr>
<tr>
<td>2011+</td>
<td>6 (100%)</td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td>34.43 (9.6)</td>
</tr>
<tr>
<td>Age at onset</td>
<td>31.28 (9.74)</td>
</tr>
<tr>
<td>EDSS at diagnosis</td>
<td>1.83 (1.12)</td>
</tr>
</tbody>
</table>
Figure 3: Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) were reported for different models with different latent trajectory classes in the post-EDSS 3 epoch.

Table 4: Posterior classification table of the model with two different latent trajectory classes are reported for the pre-EDSS 3 epoch.

<table>
<thead>
<tr>
<th>Posterior classification:</th>
</tr>
</thead>
<tbody>
<tr>
<td>class1</td>
</tr>
<tr>
<td>N</td>
</tr>
<tr>
<td>%</td>
</tr>
</tbody>
</table>

Posterior classification table:

<table>
<thead>
<tr>
<th>mean of posterior probabilities in each class</th>
</tr>
</thead>
<tbody>
<tr>
<td>prob1</td>
</tr>
<tr>
<td>class1</td>
</tr>
<tr>
<td>class2</td>
</tr>
</tbody>
</table>
Figure 4: Plot of the disability EDSS trajectory slopes with two latent trajectory classes are reported for the post-EDSS 3 epoch.

Table 5: Contingency tables of the clinical and demographic distribution characteristics in the two classes

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Classification of EDSS disability trajectories</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Post-EDSS 3</td>
</tr>
<tr>
<td></td>
<td>moderate (green)</td>
</tr>
<tr>
<td></td>
<td>high (red)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>108 (70.59%)</td>
</tr>
<tr>
<td>Female</td>
<td>51 (68.92%)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Familiarity</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>142 (70.3%)</td>
</tr>
<tr>
<td>Yes</td>
<td>17 (68%)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
</tr>
<tr>
<td>1980-1990</td>
<td>17 (62.96%)</td>
</tr>
<tr>
<td>1991-1995</td>
<td>26 (76.47%)</td>
</tr>
<tr>
<td>1996-2000</td>
<td>39 (67.24%)</td>
</tr>
<tr>
<td>2001-2005</td>
<td>36 (62.07%)</td>
</tr>
<tr>
<td>2006-2010</td>
<td>35 (79.55%)</td>
</tr>
<tr>
<td>2011+</td>
<td>6 (100%)</td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td>33.81 (9.27)</td>
</tr>
<tr>
<td>Age at onset</td>
<td>31.06 (9.42)</td>
</tr>
<tr>
<td>EDSS at diagnosis</td>
<td>1.7 (1.22)</td>
</tr>
</tbody>
</table>
Figure 5: The plot of the disability EDSS trajectory slopes with the selected latent trajectory classes in the pre- and post-EDSS 3 epoch.

Table 6: Descriptive statistics of the disability trajectory slope

<table>
<thead>
<tr>
<th>Statistics</th>
<th>Pre-EDSS 3</th>
<th>Post-EDSS 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min.</td>
<td>0.00</td>
<td>-0.57</td>
</tr>
<tr>
<td>1st quantile</td>
<td>0.04</td>
<td>-0.03</td>
</tr>
<tr>
<td>Median</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>Mean</td>
<td>0.08</td>
<td>0.11</td>
</tr>
<tr>
<td>3rd quantile</td>
<td>0.10</td>
<td>0.25</td>
</tr>
<tr>
<td>Max.</td>
<td>0.23</td>
<td>0.93</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.04</td>
<td>0.23</td>
</tr>
</tbody>
</table>
Figure 6: the disability EDSS trajectory slope histograms in the spam of time prior to (panel A) and following (panel B) the EDSS 3 status.

Table 7: Contingency table among pre- and post-EDSS 3 latent trajectory classes. The Count, Column Percent and Total Percent are reported in each cell.

<table>
<thead>
<tr>
<th>Pre-EDSS 3</th>
<th>Moderate</th>
<th>High</th>
<th>Row Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate</td>
<td>139</td>
<td>58</td>
<td>197</td>
</tr>
<tr>
<td></td>
<td>87.42%</td>
<td>85.29%</td>
<td>86.78%</td>
</tr>
<tr>
<td></td>
<td>61.23%</td>
<td>25.55%</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>20</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>12.58%</td>
<td>14.71%</td>
<td>13.22%</td>
</tr>
<tr>
<td></td>
<td>8.81%</td>
<td>4.41%</td>
<td></td>
</tr>
<tr>
<td>Column Total</td>
<td>159</td>
<td>68</td>
<td>227</td>
</tr>
</tbody>
</table>

Pearson's Chi-squared test  p-value = 0.6647
Table 8: The demographic and clinical characteristics of the study participants (N=881).
The results are expressed as mean with standard deviation or as number of subjects with percentage

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>279 (31.67%)</td>
</tr>
<tr>
<td>Female</td>
<td>602 (68.33%)</td>
</tr>
<tr>
<td><strong>Diagnosis</strong></td>
<td></td>
</tr>
<tr>
<td>1980-1990</td>
<td>44 (4.99%)</td>
</tr>
<tr>
<td>1991-1995</td>
<td>62 (7.04%)</td>
</tr>
<tr>
<td>1996-2000</td>
<td>152 (17.25%)</td>
</tr>
<tr>
<td>2001-2005</td>
<td>201 (22.81%)</td>
</tr>
<tr>
<td>2006-2010</td>
<td>239 (27.13%)</td>
</tr>
<tr>
<td>2011+</td>
<td>183 (20.77%)</td>
</tr>
<tr>
<td><strong>Familiarity</strong></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>804 (91.26%)</td>
</tr>
<tr>
<td>Yes</td>
<td>77 (8.74%)</td>
</tr>
<tr>
<td><strong>Age at diagnosis (years)</strong></td>
<td>33.71 (9.21)</td>
</tr>
<tr>
<td><strong>Age at onset (years)</strong></td>
<td>31.08 (9.0)</td>
</tr>
<tr>
<td><strong>EDSS at diagnosis</strong></td>
<td>1.34 (1.03)</td>
</tr>
<tr>
<td><strong>Median among EDSS visits (days)</strong></td>
<td>180.43 (133)</td>
</tr>
<tr>
<td><strong>Therapy delay (Months)</strong></td>
<td>38.31 (47.98)</td>
</tr>
</tbody>
</table>

Figure 7: Akaike Information Criterion (AIC) and, Bayesian Information Criterion (BIC) were reported for different models with different latent trajectory classes (N=881).
Table 9: Posterior classification table of the model with three different latent trajectory classes are reported for all EDSS scores (N=881).

Posterior classification:
  class1  class2  class3
N 198.00  633.00  50.00
% 22.47  71.85  5.68

Posterior classification table:
  --> mean of posterior probabilities in each class
    prob1  prob2  prob3
class1  0.5530  0.4432  0.0037
class2  0.3689  0.6089  0.0222
class3  0.0604  0.1659  0.7737

Figure 8: The disability EDSS trajectory scores with the three different latent trajectory classes are reported (N=881).
Figure 9: the disability EDSS trajectory scores with the two different latent trajectory classes are reported (N=881).
Table 10: Descriptive statistics with univariate analysis. Characteristic: variable taken into account; OR (95% CI): Odd Ratios with 95% Confidence Interval; p-value: Likelihood Ratio p-value.
*Variables entering the multivariate analysis (see the text for abbreviations and further details).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Descriptive statistics</th>
<th>Univariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>moderate (blue)</td>
<td>high (dark-green)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>258 (92.47%)</td>
<td>21 (7.53%)</td>
</tr>
<tr>
<td>Female</td>
<td>573 (95.18%)</td>
<td>29 (4.82%)</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1980-1990</td>
<td>41 (93.18%)</td>
<td>3 (6.82%)</td>
</tr>
<tr>
<td>1991-1995</td>
<td>59 (95.16%)</td>
<td>3 (4.84%)</td>
</tr>
<tr>
<td>1996-2000</td>
<td>143 (94.08%)</td>
<td>9 (5.92%)</td>
</tr>
<tr>
<td>2001-2005</td>
<td>182 (90.55%)</td>
<td>19 (9.45%)</td>
</tr>
<tr>
<td>2006-2010</td>
<td>226 (94.56%)</td>
<td>13 (5.44%)</td>
</tr>
<tr>
<td>2011+</td>
<td>180 (98.36%)</td>
<td>3 (1.64%)</td>
</tr>
<tr>
<td>Familiarity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>759 (94.4%)</td>
<td>45 (5.6%)</td>
</tr>
<tr>
<td>Yes</td>
<td>72 (93.51%)</td>
<td>5 (6.49%)</td>
</tr>
<tr>
<td>Age at diagnosis (yeas) *</td>
<td>33.47 (9.12)</td>
<td>37.66 (9.86)</td>
</tr>
<tr>
<td>Age at onset (yeas) *</td>
<td>30.85 (8.9)</td>
<td>34.8 (9.91)</td>
</tr>
<tr>
<td>EDSS at diagnosis</td>
<td>1.34 (1.04)</td>
<td>1.32 (0.98)</td>
</tr>
<tr>
<td>Median among EDSS visits (days)</td>
<td>178.47 (95.42)</td>
<td>213.17 (402.84)</td>
</tr>
<tr>
<td>Therapy delay (months)</td>
<td>37.51 (48.27)</td>
<td>48.92 (42.98)</td>
</tr>
</tbody>
</table>
Table 11: Multivariate analysis, the predictor effects on the high disability class. Results are expressed as odds ratio (OR) with 95% confidence interval (95%CI).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>0.01 (0 - 0.04)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td>1.05 (1.02 - 1.08)</td>
<td>0.0023</td>
</tr>
</tbody>
</table>
References


