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Tobacco smoking and multiple sclerosis: Effects on occurrence, progression and mortality

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Abstract

Multiple Sclerosis is an immune mediated disease of the central nervous system associated with a wide range of mainly irreversible psychological and physical disabilities in young adults. Despite the invaluable knowledge gained from the research into the disease, its aetiology and mechanism of progression are poorly understood. The natural history of multiple sclerosis is complex and there are still many unanswered questions in respect to the risk factors associated with its development and the way that the disease evolves with age. Over the years numerous theories about the disease aetiology have been postulated, but the one that best describe the disease, on the basis of our current understanding, both in terms of susceptibility and progression is the gene-environment hypothesis. According to this hypothesis, multiple sclerosis occurs as the result of an exposure(s) to some unknown environmental factor(s) in genetically susceptible individuals. In multiple sclerosis, it has been hypothesised that tobacco smoking is associated with an increased risk of the disease occurrence and adverse effects on the progression of disabilities. Despite the relatively large amount of data on the adverse effect of smoking on multiple sclerosis risk and clinical course, data from a large population based cohort was lacking. The aim of the current work was to investigate the influence of tobacco smoking on the natural history of the disease from the risk of occurrence to mortality.

In the first part of the investigation, our age- and sex-matched case-control study showed that tobacco smoking is associated with higher risk of disease occurrence. However, we did not observe any association between parental

smoking during patients' childhood and the risk of multiple sclerosis. When investigating the impact of tobacco smoking on the clinical course and prognosis of the disease, our cohort study failed to show any evidence of the influence of tobacco smoking on the risk of progressive onset multiple sclerosis. However, tobacco smoking was associated with more severe disease and significantly higher levels of psychological and physical disability in current smokers. Moreover, tobacco smoking in current smokers was associated with faster disability progression and shorter time to the progressive stage of the disease in patients with relapse onset multiple sclerosis. A significant impact of tobacco smoking on the risk of premature death and patients' life expectancy was also evident in our data where tobacco smoking in our cohort was associated with more than 2.5-fold increase in the risk of premature death and almost 10 years reduction in the patients' life expectancy. Our data also showed that tobacco smoking can account for some of the excess mortality seen in multiple sclerosis patients. A novel finding of our research was that smoking cessation significantly reduced patients' risk of disease progression and premature death. Although the benefits of smoking cessation were greater for patients who stopped at earlier ages, cessation was found to be beneficial at all ages. To our knowledge, this is the first study that showed smoking cessation could potentially be beneficial in reducing the risk of disability progression and premature mortality in patients with multiple sclerosis.

Overall, our findings point toward adverse health impact of tobacco smoking on the clinical course of multiple sclerosis from the occurrence to mortality.

LIST OF OUTPUTS

Articles in professional peer-reviewed journals

Manouchehrinia A, Tench CR, Maxted J, Bibani RH, Britton J, Constantinescu CS. Tobacco smoking and disability progression in multiple sclerosis: United Kingdom cohort study. Brain. 2013 Jul;136(Pt 7):2298-304.

Manouchehrinia A, Weston M, Tench CR, Britton J, Constantinescu CS. Tobacco smoking and excess mortality in multiple sclerosis: a cohort study. Journal of Neurology, Neurosurgery & Psychiatry 2014 doi: 10.1136/jnnp-2013-307187

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My wife Mary has been, always, my support, my joy and my only love, and I cannot thank her enough.

LIST OF ABBREVIATION

ADs	Autoimmune Diseases
AIC	Akaike Information Criterion
ARA	American Rheumatoid Association
BIC	Bayesian Information Criterion
CD MS	Clinically Definite Multiple Sclerosis
CI	Confidence Intervals
CIS	Clinically Isolated Syndrome
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
CSI	Comprehensive Smoking Index
CVD	Cardiovascular Disease
DALY	Disability-Adjusted Life Year
DMT	Disease Modifying Treatment
DSS	Disability Status Score
EAE	Experimental Autoimmune Encephalomyelitis
EBNA	Epstein - Barr virus Nuclear Antigen
EBV	Epstein - Barr virus
ECRHS II	the European Community Respiratory Health Survey II
EDSS	Expanded Disability Status Scale
FDA	Food and Drug Administration
GA	Glatiramer Acetate
GHS	General Household Survey
GLF	General Lifestyle Survey
GNDS	Guy's Neurological Disability Scale
GPRD	General Practice Research Database

HLA	Human Leukocyte Antigen
HR	Hazard Ratio
IFNβ	Interferon Beta
IG	Immunoglobulin
IL	Interleukin
IM	Intramuscular
IMD	Index of Multiple Deprivation
IMR	Incidence Mortality Rates
IQR	Inter Quartile Range
MBP	Myelin Basic Protein
MOG	Myelin Oligodendrocyte Glycoprotein
MRI	Magnetic Resonance Imaging
MS	Multiple Sclerosis
MSIS-29	Multiple Sclerosis Impact Scale
MSQOL-54	Multiple Sclerosis Quality of Life-54
MusiQoL	Multiple Sclerosis International Quality of Life questionnaire
MSSS	Multiple Sclerosis Severity Score
NARCOMS	North American Research Committee on Multiple Sclerosis
NHS	National Health Service
NICE	National Institute of Clinical Excellence
NRES	National Research Ethics Service
NUH	Nottingham University Hospitals
ONS	Office for National Statistics
OR	Odds Ratio
PDDS	Patient Determined Disease Steps
PLP	Proteolipid Protein
PPMS	Primary Progressive Multiple Sclerosis

PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PYLL	Potential Years of Life Lost
QMC	Queen Medical Centre
RA	Rheumatoid Arthritis
REC	Research Ethics Committee
RR	Risk Ratio
RRMS	Relapsing Remitting Multiple Sclerosis
SC	Subcutaneous
SD	Standard Deviation
SMR	Standardised Mortality Ratios
SPMS	Secondary Progressive Multiple Sclerosis
TNF	Tumor Necrosis Factor
VIF	Variance Inflation Factor

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1. Chapter one: Introduction and our hypothesis

1.1   Summary of the chapter

The aim of this chapter is to provide a basic introduction on the key ideas, issues and important findings of studies of multiple sclerosis with particular emphasis on its epidemiology. I kept this chapter as short as possible due to the high volume of the original data in the following chapters. The detailed review of all aspects of the disease is beyond the scope of this thesis. Hence, my summary views here are short and tried to be most relevant to the aim of the thesis. The structure of the chapter is as follows:

The next **section 1.2** will offer some key ideas on some of the well-known theories of multiple sclerosis. **Section 1.3** will very briefly cover some key findings and idea in MS immunology.

Section 1.4 gives a background on studies of multiple sclerosis epidemiology and discuses in a general way some of the key challenges and findings suggested by these studies.

Section 1.5 identifies the key stages in the natural history of the disease and discusses some of the theories behind the multiple sclerosis onset, progression and mortality with particular emphasis on epidemiological findings.

Section 1.6 reviews the disease burden in terms of direct costs of treatment and their cost-effectiveness

Section 1.7 discusses some important features of tobacco smoking in the UK and Nottingham based on findings from national surveys.

Section 1.8 states our aims and hypothesis of the study.

1.2   Multiple Sclerosis (MS):

MS is one of the major health problems in the UK. It is associated with numerous long term complications including difficulty with mobilisation, bowel and bladder problems, cognitive impairments, sexual dysfunction, depression, severe chronic pains, etc. MS has enormous impact on individuals' quality of life and wellbeing. Despite the relatively high prevalence of MS, highly effective interventions for the treatment of MS are lacking. The development of such interventions requires a greater understanding of underlying aetiological mechanisms of the disease.

MS, as it is currently understood, is an immune mediated disorder of the central nervous system (CNS). The most well-known feature of MS pathology is the demyelinated plaques in the white matter of the brain and spinal cord. Clinically, MS is a very variable condition and disease manifestation can range from asymptomatic (subclinical) and relatively benign to somewhat disabling and devastating. Most people with MS experience their first symptoms between the ages of 20 and 40 years and like many of the autoimmune diseases, MS disproportionately affects women more than men. There are three main phenotypes of MS, namely Relapsing Remitting (RR MS), Primary Progressive (PP MS) and Secondary Progressive (SP MS). RR MS accounts for around 85 to 90% of MS cases diagnoses at the disease onset. Studies of the natural history of the disease have shown that of the RR patients, a majority would eventually transit to SP MS characterised by gradual but steady deterioration (Weinshenker, Bass et al. 1989; Lublin and Reingold 1996).

MS epidemiologic studies in migrating populations and recently industrialised populations have shown that there is an increased incidence of MS as individuals adopt a more westernised lifestyle. It may be the pronounced changes in environment, behaviour and lifestyle accompanying globalisation that have resulted in the escalation of MS both in developed and developing nations. Despite the high volume of research investigating the influence of environment on the development and progression of MS, epidemiologic studies of the natural history of the disease are still needed to systematically evaluate the role of environment and life style in MS physiopathology.

1.3 Immunology

The active role of immune system in the pathogenesis of MS is undisputable. Based on our current understanding of the disease, MS is an autoimmune disease inducing organ-specific inflammation (Pouly and Antel 1999; McFarland and Martin 2007). In MS, myelin damage occurs in response to a series of pathological changes initiated by activated peripheral T cells (Pouly and Antel 1999; Huizinga, Linington et al. 2008; Henderson, Barnett et al. 2009). MS shares many similarities with other autoimmune diseases and is one of the most common autoimmune diseases in the world. It has been estimated that approximately 5% of the world population suffer from some sort of autoimmune diseases (Shoenfeld, Selmi et al. 2008). Over the past decade several new developments and technological advances have clarified some of the mechanisms underlying autoimmune diseases. Despite this and some well-known genetic factors, (mainly in HLA region) (Zanelli, Breedveld et al. 2000;

Thorsby and Lie 2005; 2006) most of the causal mechanisms remain to be identified.

Thanks to the pioneer work of Rivers in 1933 (Rivers, Sprunt et al. 1933) many of our current understanding of MS from immunology to therapy are based on data gained from animal models of the disease; experimental autoimmune encephalomyelitis (EAE) (Zamvil and Steinman 1990). EAE is established in numerous species and is developed by immunisation with CNS-derived myelin antigens, such as myelin basic protein (MBP), proteolipid protein (PLP) and oligodendrocyte glycoprotein (MOG), usually with the addition of an immunologic adjuvant, into susceptible animals which ultimately leads to either an acute, chronic, or relapsing-remitting encephalomyelitis that has clinical and pathological similarities with MS. The observation that EAE cannot be transferred by antibodies indicated that MS is likely to be an autoimmune disease mediated by T cells (encephalitogenic T cells) with potential ability to migrate to the CNS and attack the myelin sheath (Crawford, Yan et al. 2004). However, data during the past decades has shown that possibly more factors than originally proposed CD4+ T cells, including B cells, antibodies, and complement, are involved in the development of MS and shaping the disease. In addition to the well-known originally proposed role of CD4+ T cells, there is little doubt that CD8+ T cells also mediate CNS damage. Two closely related cytokines, Interleukin-12 (IL-12) and IL-23 are suggested to play important roles in the mechanisms underlying the differentiations of these T cells (Segal and Shevach 1996; Langrish, Chen et al. 2005). During the past decade several new immunomodulatory treatments, some of which specifically target T cells have emerged. These

immunomodulatory treatments vary in their effectiveness and side-effects. While the result from the clinical trial of anti-interleukin (IL) 12p40 was not successful (Segal, Constantinescu et al. 2008), Alemtuzumab, a monoclonal antibody against CD52, a molecule expressed on many immune cells, has shown high efficacy in reducing relapse rate in MS patients (Investigators, Coles et al. 2008). The proposed mechanism of action of Alemtuzumab in RR MS includes robust depletion of both peripheral lymphocytes and monocytes (Cox, Thompson et al. 2005).

It is clear that activation of T cells with potential for autoreactivity can lead to autoimmunity, but autoimmunity can also occur when the T regulatory (Treg) cells fail to suppress autoreactive T cells. Studies have shown defects in the number and function of Tregs in the peripheral blood of individuals with MS compared with healthy controls (Haas, Fritzsching et al. 2007; Venken, Hellings et al. 2008). No Tregs have also been found at any stage of MS lesions, indicating absence of regulatory mechanism in MS brain (Tzartos, Friese et al. 2008). The CD4+CD25+ regulatory T cell population characterised by the expression of forkhead family transcriptional factor (Foxp3) is shown to be reduced in autoimmune and inflammatory conditions and MS patients (Hori, Nomura et al. 2003; Viglietta, Baecher-Allan et al. 2004; Zhang, Koldzic et al. 2004).

T cells are not the only major players of the MS immunopathology. The elevated level of immunoglobulins (IGs) in the Cerebrospinal fluid (CSF) and presence of B cells in the lesions of MS suggest that B cells are involved in the pathology of the disease (Kabat, Freedman et al. 1950; Biddison, Cruikshank et al. 1998). The role of B cells in the pathogenesis of MS is diverse and B

cells are possibly involve in the disease in many ways (Disanto, Morahan et al. 2012). One likely pathway is throughout local production of pathogenic antibodies leading to demyelination (O'Connor, Appel et al. 2005). In addition, the antigen presentation and cytokine secretion properties of B cells can potentially contribute to the progression of the disease (Bar-Or, Fawaz et al. 2010). Drugs such as Rituximab (an anti-CD20 monoclonal antibody) which specifically target B cells has shown to decrease inflammation and reduced the number of relapses in patients with MS suggesting contributory role of B cells in the progression of MS (Hauser, Waubant et al. 2008).

1.4   Epidemiology

The first published epidemiologic observation of the disease by Davenport in 1922 (Davenport 1922) revealed that the frequency varies across geographical regions. Further research in the epidemiology of the disease in the following years consistently indicated that the incidence and prevalence of MS not only vary in different parts of the world but can also vary within different regions of a country.   The difference in geographical distribution may suggest that environmental factor(s) play roles in aetiology of the disease. Epidemiological studies of MS and in particular those investigating the incidence and prevalence of the disease encounter many difficulties, as there are still many ambiguities about the disease physiopathology. A recent survey of MS incidence and prevalence in the UK have found that the incidence of MS has decreased in the UK by 1.5% (95%CI: 0.99% to 2.07%) per year from 1990 to 2010 time period (Investigators, Coles et al. 2008). These findings contradict

the results from majority of the surveys which have shown an increase in the incidence and consequently prevalence of the disease (Kingwell, Marriott et al. 2013). There are several explanations for this discrepancy. First, authors have mainly used data from the general practice research database which can potentially increase selection bias by excluding patients with benign disease and also introduce misdiagnosis/miscoding of MS. Relatively low positive predictive value (or precision rate of the diagnosis) of around 60% for MS using GPRD has been reported (Jadidi, Mohammadi et al. 2013). Second, the influences of changes in population demographics have not been evaluated by authors. For example, 39% of the population growth of the UK in the year 2012 compared with 2011 was as a result of international migrants (Tzartos, Friese et al. 2008).

It is still unclear whether the incidence of MS is actually increasing or other factors such as increase in MS global awareness (e.g. better and more accurate diagnosis) are responsible for what appears to be an increase of the disease. One possibility which should be noted is that the increase in MS cases from the 1970s onwards can be due to improved diagnosis of MS facilitated by the introduction of magnetic resonance imaging (MRI).

In 1922, Davenport pointed out that the disease affected persons of northern states relatively more common than southern states. This was confirmed in a study of American troops in World War I by Bailey (Bailey 1922). However, only in 1938 did Steiner first proposed that prevalence was associated with regional geographical factors (Steiner 1938). Nearly 30 years later, Ulett related the high disease frequency to northern latitude and cold climate (Ulett 1948) and in 1950, Limburg confirmed the north-south trend using mortality

data (Limburg 1950). In the UK, the number of patients diagnosed with the disease is increased by latitude from 80 in the southern parts to almost 150 per 100,000 in Scotland (Investigators, Coles et al. 2008).

Genetics is a major player of MS aetiology which can also, in part, describe some of the regional variations of MS. A study by Lonergan and colleagues has shown that the HLA DRB1*15 allele associated with MS susceptibility is more common in areas of higher MS prevalence (Cox, Thompson et al. 2005). Overall, the white peoples of north and central Europe are clearly generally more susceptible to the disease. MS is rare among African Caribbean and judging by the prevalence in immigrants and by local experience it is rare in the West Indies (Cabre, Signate et al. 2005). The majority of the data about the prevalence of the disease in African Caribbean comes from studies of USA army veterans (although subject to some biases such as healthy soldier bias) in which identical standards of diagnosis were applied to all races. These studies have shown that the disease is less frequent in African Caribbean than in American Whites (Venken, Hellings et al. 2008) and that in African Caribbean, as in Whites, the disease is less common in the south than in the north. Data from other sources fit this conclusion (O'Connor, Appel et al. 2005). The difference in risk between Africans in Africa and America might be genetic, environmental, or both but an environmental factor similar to those affecting Whites would explain the gradient in the USA. Although studied frequently, the interaction between genetic and environment in MS requires further investigations.

Accurate case ascertainment in surveys of MS is very much handicapped by two features of the disease: first, the lack of an easily carried out specific and

sensitive diagnostic test; second, a gap of several years commonly intervenes between the biological onset, first onset of symptoms and diagnosis. In a disease as chronic as MS there are also problems that are less well recognised, which are associated with the selection of an appropriate population for the computation of incidence and prevalence rates. The normal practice is to use the population of the defined area on a specific date (prevalence day) and to relate to this all patients with the disease living on that day. However, such a population may differ substantially in respect when the disease duration increases, for example, of urban-rural and socio-economic parameters from the population in existence when the same patients experienced the onset of the disease or the causative events preceding the onset.

MS is a disease with an asymmetric distribution in term of the age-incidence, age-prevalence and gender ratio. The majority of surveys agree that MS attacks women more frequently than men and on average in their 30s. Likewise the discrepancy in the onset age, the difference between the genders is not also consistent in different age stratums, however, overall MS gender ratio is at around 2:1 and seems unlikely to provide an etiological clue. It is worth noting that recent surveys suggest an increase in female incidence (Orton, Herrera et al. 2006; Ascherio and Munger 2008). The substantially higher relative risk in women (> 2:1) has also been noted in Hawaii, South Africa and Western Australia, in all of which places the disease is relatively rare.

1.5   Natural history and disease modifiers

Natural history of disease refers to the process of development and progression of a disease in an individual over time. The first phase or biological onset begins with the exposure to the risk factor(s) in a susceptible host. This biological onset would trigger series of asymptomatic pathological changes (during subclinical disease phase or latency period) which as time goes on would eventually lead to the onset of symptoms. At this stage disease would become clinical and symptomatic. Most diagnoses are made during the clinical stage of the disease; however some pathologic changes may be detectable with laboratory, imaging or other screening methods during the latency period such as radiologically isolated syndrome (RIS), incidental MRI findings suggestive of MS (Okuda, Mowry et al. 2009). The process of definite clinical diagnosis can also take years. In the case of MS it has been estimated to be in a range of 0 to 3 years after symptoms onset (Tsai and Lee 2013).
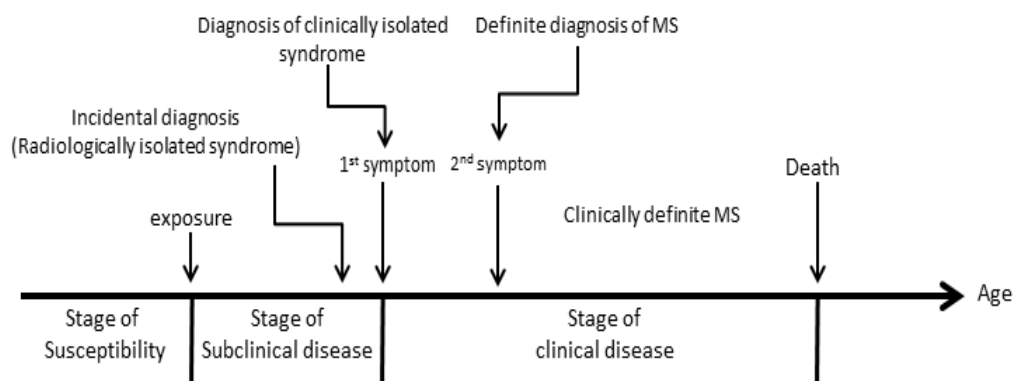
Figure 1-1: MS natural history diagram

There are differences amongst diseases and individuals, in terms of the disease course and its natural history. Environmental factors, genetic and treatment

interventions are amongst the factors which can influence the disease natural history. In MS, many studies have evaluated these factors independently; but identifying and recognising the potential interactions amongst these factors seem crucial. Here, I will briefly discuss some of the important features of MS natural history and some of its modifiers. The effects of tobacco smoking will be discussed in details in later chapters.

1.5.1   MS Occurrence

It is widely accepted that MS susceptibility is mediated by a complex interaction between the unknown environmental and/or behavioural factors that exist in some specific regions of the world in genetically susceptible hosts (Ebers 2008; Handel, Handunnetthi et al. 2010). The complexity of the disease aetiology and natural history has given rise to numerous theories, supported to a higher or lesser degree by evidence, including infectious disease (Ascherio and Munger 2007), autoimmune condition (Hafler and Weiner 1989), vascular disease (Zamboni 2006), psychological abnormality, neurocristopathy (Behan and Chaudhuri 2010), etc. However, accumulated data over the years points toward the plausible theory of MS being an immune mediated disease with variable levels of immune activity in individuals over time and between individuels in a population. Several general mechanisms of autoimmunity have been suggested. These include primary failure of mechanisms of tolerance or secondary failure of the normal tolerance mechanism due to factor(s) such as viral or bacterial infection or abnormality of the target tissue (Anaya 2010).

MS has also been described as a primary neurodegenerative disease (Chaudhuri 2013). For a long time, the clinical course of MS in relapsing onset MS followed by a secondary progressive phase has been thought to characterise the presence of two separate disease course of inflammatory demyelination during RR MS and neuronal degeneration during SP MS. However, recent epidemiologic studies of the natural history of MS (Compston 2006; Confavreux and Vukusic 2006) have found that regardless of the relapsing phase, almost all patients reach the progressive phase at same age. This may indicate that MS is primarily a neurodegenerative disease with variable inflammatory activity in different individuals.

Factors influencing risk of MS are not yet known and can be demographic, environmental and/or genetic. Below, several well-known demographic and environmental factors influencing the risk of MS are discussed.

1.5.1.1 Age and gender effect

The role of gender in MS susceptibility is complex. Like many other autoimmune conditions MS disproportionally affect females almost twice more than males, possibly due to some immunologic differences such as stronger Th1-mediated immune response in females (Schwendimann and Alekseeva 2007). There are also possible contributory roles of X chromosome for higher susceptibility of the disease among females (Bar-Or, Fawaz et al. 2010). Clear understanding of the gender differences in MS requires better understanding of the structural and neurochemical differences in healthy brains, which is yet to be known. Over the years studies have demonstrated that male and female brains are similar in many ways, although, major structural and biological

differences also exist. For example, males have shown to have a larger amygdala and hypothalamus, while women have a larger caudate and hippocampus. Global cerebral blood flow is reported to be consistently higher in females than in males, while global cerebral metabolism is similar. Estrogen and distribution of estrogen and androgen receptors have been suggested to contribute in these regional and biological differences (Cosgrove, Mazure et al. 2007). These sex differences in the brain structure may increase vulnerability to MS which considerably differ in prevalence and symptoms between men and women. Gender ratio in MS varies in different phenotypes of the disease and progressive onset MS is shown to be indiscriminative of gender (Disanto, Morahan et al. 2012). Data has shown that the female:male ratio has increased over the 20th century. Based on the finding from Oslo MS registry from 1910 to 1980, the female to male ratio increased significantly from 1.48:1 to 2.30:1 (Celius and Smestad 2009). These data contradict a recent survey of the general practice research database in the UK which has shown no change in gender ratio over the past two decades in the UK (Investigators, Coles et al. 2008).

Age is another important factor in MS susceptibility and MS is primarily considered a disease of young adults. Studies have shown that the onset of MS to be age-related, dependent on the initial course of the disease. The mean onset age for RR MS has been demonstrated to be in range of 30 years, and of the progressive course in range of 38 to 40 years (Fog and Linnemann 1970; Poser 1978; Confavreux, Aimard et al. 1980; Minderhoud, van der Hoeven et al. 1988; Cottrell, Kremenchutzky et al. 1999). Age also has significant impact on the gender distribution of MS. The female:male ratio differs considerably in

different age groups from 1.4:1 in MS onset before puberty to 3.25:1 in patients with onset after age 11 (Ghezzi, Deplano et al. 1997; Ruggieri, Iannetti et al. 2004; Chitnis, Glanz et al. 2009). At the onset of MS in adulthood the female: male ratio is 2:1 (Rosati 2001; Pugliatti, Rosati et al. 2006). Late onset MS (onset age > 50) has been reported in 0 to 12 percent of the total MS population and unlike adulthood MS, is more frequent in male patients (Bove, Healy et al. 2012). Table **1-1** shows female percentage and onset age of some selected autoimmune diseases.

Table 1-1: approximate female percentage and onset age of some selected autoimmune conditions are shown

	~ female percentage	Onset age
Multiple Sclerosis	65	20-40 (Ghezzi 2004)
Type-1 diabetes	age ≤15: 50	Childhood onset: 5-9 and 10-14 (2000)
	age >16: 40	Adulthood onset: 25-61 (Nishimura, Obayashi et al. 2000)
Grave's disease	>85	30-60 (Lantz, Abraham-Nordling et al. 2009)
Thyroiditis	95	20-40 (Furszyfer, Kurland et al. 1972)
Systemic lupus erythematosus	88	65% 16-55 (Ballou, Khan et al. 1982)
		20% <16 (Font, Cervera et al. 1998)
		15% >55 (Font, Cervera et al. 1998)
Rheumatoid arthritis	75	30-55 (Deal, Meenan et al. 1985)
Crohn's disease	53 (Montgomery, Wakefield et al. 2003)	Bimodal onset age distribution.
		Peak at 20 and 50 (Rose, Roberts et al. 1988; Haug, Schrumpf et al. 1989)
Uveitis	50	45-65 male
		>65 female (Gritz and Wong 2004)
Sjögren's Syndrome	94	40-75 (Pillemer, Matteson et al. 2001)

except as referenced, female percentage from Jacobson et al. (Jacobson, Gange et al. 1997)

1.5.1.2 Geography and ethnicity

The cumulative evidence that mainly comes from Western countries indicates that MS is present in most ethnic groups but is more prevalent in white Caucasians with far lower rates appearing in tropical regions (Ebers and Sadovnick 1993). For a long time MS geographical distribution had been the most prominent epidemiological clue of MS aetiology (Agranoff and Goldberg 1974). However, reports of low MS incidence between certain ethnic groups in several hot geographical spots of MS draw attention to the contributory role of ethnicity and genetics in risk MS. The variations in the prevalence of MS suggest that geography, genetics and ethnicity interact in some complex ways. For example, regardless of the geography and the place of living, hardly any documented cases of MS have been reported in some ethnic groups, such as Maoris (Skegg, Corwin et al. 1987). In epidemiology "ethnicity" is a complex term, as it refers not only to the biological differences between individuals possibly with different genetic traits, but most importantly points towards distinct health beliefs and behaviours. Therefore "ethnicity" encompasses a range of factors and characteristics, from biology to health beliefs and behaviours. Despite its importance, epidemiologic studies of MS have not appreciated the ethnicity fully in its epidemiological concept. Many of these studies are only limited to categorising patients into some distinct groups of ethnical categories such as Whites vs. Blacks, etc. regardless of their lifestyle and/or health beliefs. The other issue in the epidemiological studies of MS is the presence of predominantly white Caucasian population. The studies of the epidemiology of MS require more between ethnic groups comparisons as this may break the confounding due to highly correlated exposures and outcomes

observed in conventional white Caucasian MS populations. Thus in MS, simple demonstrations of ethnic differences may provide aetiological clues.

Geography is suggested to be a better determinant of human genetics than ethnicity (Manica, Prugnolle et al. 2005). However, factors such as individuals' adaptations to the new country's' lifestyle should be considered when discussing geographical distribution. Geography may also be an indirect indicator of distinct lifestyles. This was clearly demonstrated by Swank et al. who found that incidence of MS differs significantly between coastal communities with high consumption of fish and low consumption of saturated animal fats and inland population in Norway with a substantially different dietary habits (Swank, Lerstad et al. 1952). In this case geographical distribution clearly and indirectly points toward a different life style chosen by the coastal communities.

Valuable knowledge in MS epidemiology has been provided by the studies of migrations of ethnic groups to new locations. Migrants in a new geography usually bring their genetic disease risk and within a few generations adopt the new country's lifestyle. Studies of migrants have clearly shown the importance and mutability of health behaviours, compared with genetic and geographical factors (Marmot and Syme 1976). In a classical example of such studies Marmot and Syme classified 3809 Japanese-Americans in California based on their level of adaptation to the host country and showed that despite the higher prevalence of coronary heart disease amongst Japanese-Americans living in the US compared with Japanese in the Japan, the most traditional group of Japanese-Americans in the US had prevalence as low as that observed in Japan. Thus, we may need to accept that studies of migrants in MS may not

provide us definite clue to the aetiology of MS unless some essential confounding factors such as migrants' level of adaptation in the adopted country, migrants' socioeconomic status in the origin and adopted country, etc. are taken into account. It is also important to note that migrants are a selective proportion of a population usually with better state of health which is required for migration and may not necessarily represent the population in the country of origin. Hence, results from the studies of migrants in MS should be interpreted cautiously.

Research has shown a significant impact of age at migration in the susceptibility to MS. The study of French West Indies population in the islands of Martinique and Guadeloupe between 1990 and 2000,showed higher incidence of MS for individuals who migrated to the French continent before 15 years of age (107.2, 95%CI: 52.7 to 161.7) whilst the incidence of MS was similar to locals (20.3, 95%CI: 12.7 to 27.9) for those who migrated after adolescence (Cabre 2007). Study of children of Caribbean migrants living in England also found the contributory role of migration age (Dean and Elian 1997). Although informative, the influence of age at migration may well be due to the fact that normally younger people are at higher risk of adverse health behaviours and psychiatric disorders upon immigration (Patterson, Kyu et al. 2013). Such migration would have resulted in major changes in environmental exposures and lifestyle changes in the migrants such as lack of exposure to sunlight during childhood or adopting a more westernised life (Phadke 1987). However, the role of genetic predisposition should not be neglected as some ethnic groups of northern latitude such as the Inuit and some

coastal communities are relatively insusceptible to the disease, regardless of where they live (Swank, Lerstad et al. 1952; Kuroiwa and Kurland 1982).

Studies have linked individuals' deficiency of vitamin D to the onset of MS (Munger, Zhang et al. 2004; Munger, Levin et al. 2006). This may partially explain why people of northern altitude show higher susceptibility to MS (Ramagopalan, Handel et al. 2011). It has been shown that MS occurs more frequently in people with low vitamin D levels (Munger, Zhang et al. 2004) and patients with MS have low serum vitamin D levels compared with healthy controls (Ekestern and Lebhart 2004). Vitamin D has shown to regulate the expression of the HLA-DRB1*1501 which is the genetic association with MS in Northern Europeans (Stromberg, Martensson et al. 2003). Studies have also found pronounced effect of vitamin D on the immune system in MS patients. Correale and colleagues have shown that Vitamin D significantly increase the number of CD4+CD25+ T regulatory cells (Sadovnick 2013)

The month of birth has also been suggested as a contributing factor to the onset of MS with fewer births occurring in cases with MS in November (Willer, Dyment et al. 2005; Disanto, Chaplin et al. 2012; Mackenzie, Morant et al. 2014). However, a recent study by Fiddes and colleagues suggested that the correlation between month of birth and incidence of MS is likely to be influenced by other factors such as year and place of birth than month of birth alone (Fiddes, Wason et al. 2013). Nevertheless, sunlight exposure and vitamin D deficiency cannot fully explain increasing incidence of MS in some Middle Eastern countries as well as east-west incidence rate of MS in the United States (Sadovnick and Ebers 1993; Etemadifar and Maghzi 2011; Deleu, Mir et al. 2012).

1.5.1.3 Health behaviours and lifestyle

It is frequently reported that lifestyle factors are correlated with MS onset with varying degrees of association. There have been a great interest in the role of dietary habits on the risk of MS, although, no definite scientific proof for the influence of dietary intake on risk of MS has been found (Coo and Aronson 2004). Amongst all dietary habits, caffeine and alcohol are widely used substances with some well-known effects on the CNS. It has been hypothesised that consumption of alcohol and caffeine, which are more evident in the western countries where MS is also more prevalent is associated with higher risk of MS . Despite the results from experimental studies which have shown that ethanol can alter the autoimmune activity in animal models of MS (Kuchroo, Martin et al. 1993; Steinman 2001), several case-control studies have failed to show any significant association between alcohol and caffeine consumption and risk of MS (Massa, O'Reilly et al. 2013).

Epidemiological comparisons of autoimmune disorders in a population of Greenland with the matched controls from Denmark provided the first evidence of the protective effects of omega-3 polyunsaturated fatty acids (PUFAs) in risk of some autoimmune disorders (Kromann and Green 1980). In general, studies showed that communities that consume diets high in animal fatty acids have higher incidence of MS (Lauer 1994; Esparza, Sasaki et al. 1995). However, several population-based case-control studies consistently reported no association between specific diet and risk of MS (Tola, Granieri et al. 1994; Zhang, Willett et al. 2000).

Infection (mainly viral) is another commonly studied proposed environmental factor involve in the development of MS. The Epstein-Barr virus (EBV) has been suggested to play a role in MS aetiology (Ascherio and Munch 2000; Ascherio and Munger 2010). The most consistent and important finding in the studies of EBV and risk of MS is that approximately 99.5% of MS patients are reported to be seropositive for EBV infection in contrast with 94.2% of healthy control populations (Ascherio, Munger et al. 2001). The significantly higher number of patients who are seropositive for EBV has also been found in paediatric MS patients where only 42% of the healthy children were seropositive for EBV compared with 83% of MS patients (Alotaibi, Kennedy et al. 2004). It is still unclear how EBV infection can lead to development of MS in small fraction of all infected individuals but data suggest that the age of infection is an important factor for determining MS susceptibility. It has been reported that people with MS are more than 2-fold more likely to report past infectious mononucleosis (a marker of late EBV infection) than unaffected controls (Thacker, Mirzaei et al. 2006). It is also possible that an abnormal response to EBV infection in MS patients (e.g. late infection) is a consequence of the disease rather than its cause. In principle, the substantially high prevalence of EBV seropositivity in children and adults with MS and the fact that the risk of the disease increases significantly with high levels of EBV antibody titers years before the onset of MS (DeLorenze, Munger et al. 2006) suggest a possible contributory role of EBV in aetiology of MS. Studies have also found an interaction between EBV and smoking. This is discussed in detail in chapter three.

Sexual practice and risky health behaviours were suggested as potential factors which could influence the risk of MS onset (Hawkes 2002; Hawkes 2005). It is postulated that some geographical distribution of MS can be explained by some unknown exogenous factor(s) which can be transmitted via sexual intercourse. For example, an epidemic of 42 MS cases which was observed by Kurtzke in the Faroe Islands during the Second World War and the decades after that (Kurtzke, Hyllested et al. 1993) was attributed to the era when the Faroese residents came into contact with (assumed) infected British troops possibly via sexual intercourse. In contrast, no association was found between sexual habits before the disease onset and risk of MS in a case-control study of Danish population (Lidegaard and Svendsen 2008).

1.5.2 Progression

Like the disease onset, the mechanism underlying progression of the disease is yet to be identified. Evidence from the natural history studies of the disease suggested two mechanisms of progression in MS. First: accumulation of disabilities over time by means of series of relapses followed by partial remissions and second: gradual worsening of the symptoms which can occur with or without relapses. Almost all of the currently available treatments in MS aim to reduce the frequency of relapses with no effective treatment yet identified for stopping or at least slowing down the progression of the disease in the SP MS or PP MS patients. Despite the relatively high number of patients with progressive MS (secondary or primary), the underlying mechanism or factor(s) associated with the risk of progressive MS are not known.

RR MS is recognised by its inflammatory features while progressive course is mainly characterised by axonal damage and suggested to be of neurodegenerative nature (Bjartmar, Kinkel et al. 2001; DeLuca, Williams et al. 2006; Trapp and Nave 2008). It is unlikely that MS is a clear cut multistage disease in terms of physiopathology as evidences of acute axonal damage in early MS lesions have also been reported frequently (Ferguson, Matyszak et al. 1997; Trapp, Peterson et al. 1998; Kornek and Lassmann 1999; Tallantyre, Bo et al. 2010). It seems MS is a complex mixture of both inflammatory and neurodegenerative components which are vary in degree of activity as disease progress. Here in this section I will provide a summary of demographic and environmental factors and treatment interventions with potential influence on the clinical course and progression of the disease in MS.

1.5.2.1 Age and gender effects

Age is perhaps the most important factor in the progression of diseases. The risk of occurrence and progression of many diseases changes with increase in individuals' age. In MS, the interaction between patients' age and disease progression is somehow complex. It is well known that older age at the onset of MS is associated with progressive onset MS and poor clinical outcomes (Confavreux, Aimard et al. 1980). Each year increase in onset age of MS is shown to be associated with significant increase in the risk of having progressive onset MS (Manouchehrinia, Tench et al. 2013). Therefore, PP MS is characterised by a significantly higher mean age at the onset of the disease compared with RR MS. Age at the disease onset has been reported to be

around 40 years for PPMS vs. 30 years in RR MS (Ebers 2004; Tremlett, Paty et al. 2005). The significant impact of age is also evident in the risk of developing progressive phase in relapsing onset MS patients. Onset at age 40 and 50 years could double and triple the risk of developing SP MS, respectively, in relapsing onset MS (Scalfari, Neuhaus et al. 2011). Comparison of age at the time of transition to progressive phase in RR MS and onset age in PP MS has shown a modest but significant difference. It has been estimated that the onset of progressive phase (in those with relapsing onset MS) is at the average age of 43 years. This is almost 2 years older than onset age in PP MS (Tremlett, Zhao et al. 2009). However, the difference could be diminished if factors such as treatment interventions and disease modifiers with potential influence on the natural history of the disease are taken into account.

The influence of gender on the progression of the disease is relatively clear. Although females are shown to be more susceptible to the disease, male patients present with more aggressive clinical course. Examinations of the clinical course of MS in males and females clearly indicate a significant disadvantage of males in reaching higher disability scores and SP MS (Confavreux and Vukusic 2006; Scalfari, Neuhaus et al. 2013). Gender has also been shown to influence the initial clinical course of the disease. Male patients are shown to have higher risk of PP MS than female patients. In comparison with the typical female:male ratio of 2:1, the frequency of females is lower in PP MS and estimated to be around 1.3:1 (Cottrell, Kremenchutzky et al. 1999). It is noteworthy that with a proper adjustment for the onset age,

the ratio tends to approach the conventional 2:1 (Noseworthy, Paty et al. 1983).

1.5.2.2 Disease phenotype

It is somehow difficult to recognise many differences between the principal clinical phenotypes of MS in terms of pathology, cause, etc.. Conversely, there are major and distinct differences in the clinical presentation of MS phenotypes. The clinical course of the disease, particularly at its onset, provides the concept behind the categorisation of the disease into its major phenotypes. Relapsing onset MS accounts for 85 to 90% of the disease phenotypes diagnosed at the disease onset. The remainder present with progressive onset (Lublin and Reingold 1996). Unlike relapsing onset MS in which courses of exacerbations are followed by partial (rarely complete) remissions, progressive onset MS is characterised by uninterrupted progression of the disease with or without exacerbation.

Despite this pronounced difference at the onset of the disease many patients with relapsing onset MS will eventually transit to the progressive phase which is almost identical in features with progressive onset phenotype. It appears MS occurs in 2 discrete stages in terms of clinical manifestation of the disease. Studies have shown that disability progression in the progressive phase of the disease can be independent of the disease activity in the relapsing phase as measuring by estimating times to some disability score milestones (Leray, Yaouanq et al. 2010; Scalfari, Neuhaus et al. 2010). In addition, examination of age at different disability milestones has shown no meaningful differences between relapsing and progressive onset MS (Confavreux and Vukusic 2006;

Scalfari, Neuhaus et al. 2011). It seems unlikely that any of the phenotypes of MS as currently understood provides us any clue to the mechanism underlying the transition to the progressive phase of the disease or strongly indicates a particular phenotype of any particular cause.

1.5.2.3 Treatment

Disease modifying treatments (DMTs) are available to reduce the frequency and severity of relapses in RR MS. It has been hypothesised that this reduction in the frequency of relapses could ultimately postpone or reduce the future disability progression. First-line DMTs for the treatment of RR MS include four interferon beta (IFNB) products; intramuscular (IM) interferon beta-1a (IFNβ-1a IM) (Avonex®), subcutaneous (SC) IFNβ-1a, (Rebif®), IFNβ-1b (Betaseron® or Betaferon®, and Extavia®), and glatiramer acetate (GA) (Copaxone®). In addition to the conventional DMTs, natalizumab, and fingolimod, the first oral agent, have been more recently introduced in the management of MS and are generally considered as the second-line treatments.

Furthermore, Teriflunomide (Aubagio) has been approved in some palaces and BG-12 (dimethyl fumarate) and Alemtuzumab (Lemtrada / Campath) may be approved in the US and Europe in the near future. The second line treatments are largely recommended for patients with a highly active course of MS who have had unsatisfactory response to the first-line treatments

1.5.2.3.1   First-line treatments:

Double-blind, placebo-controlled randomised trials have shown that RR MS patients taking DMTs experienced a modest but significant improvement. An

average one-third reduction in exacerbation rate was observed in treated patients compared with placebo group in the first clinical trials of interferon-1b (1993; Paty and Li 1993; 1995). Progression of lesion burden was non-significant compared with the baseline MRI scan in the high dose treatment arm of interferon-1b trial. Trials of IM and SC interferon-1a also showed significant decrease in relapse rate (Clanet, Radue et al. 2002; Durelli, Verdun et al. 2002; Panitch, Goodin et al. 2002) and progression of disability in terms of expanded disability status scale (EDSS) score (Jacobs, Cookfair et al. 1996). EDSS score was also improved or remained unchanged in the pivotal clinical trial of GA in the active treatment group compared with those receiving placebo(Johnson, Brooks et al. 1995). Effectiveness of DMTs in RR MS patients was confirmed in the extensions of the previous trials and long-term follow-ups (2001; Ford, Johnson et al. 2006). The average reduction in relapses for all pivotal trials was around 30%. GA showed a slightly lower reduction in relapse rate (29%)(Johnson, Brooks et al. 1995); however, it showed equal efficacy to IFNβ-1a and IFNβ-1b in head to head trials (Mikol, Barkhof et al. 2008; O'Connor, Filippi et al. 2009). In addition to studies conducted to determine the efficiency of first-line treatments in RR MS, four large-scale trials have evaluated the effects of early treatment on delaying the conversion of clinically isolated syndrome (CIS) to definite MS. Results from all four trials indicated that first-line treatments significantly delay the development of clinically definite MS and can decrease new lesion formation (Jacobs, Beck et al. 2000; Comi, Filippi et al. 2001; Comi, Martinelli et al. 2009; Kappos, Freedman et al. 2009).

1.5.2.3.2   Second-line treatments:

Recent clinical trials have shown promising results in patients with RR MS who received second-line treatment. In the FREEDOMS trail, average reductions in relapse rate of 54% and 60% were reported in the lower dose (0.5mg) and higher dose (1.25mg) fingolimod treatment groups, respectively, compared to placebo. The reduction in the progression of disability was also significantly greater in the treatment groups by 30% and 32% respectively compared to placebo (Kappos, Radue et al. 2010). Results from two-year AFFIRM trial also showed that natalizumab was associated with an average reduction in relapse rate of 67% compared with placebo. A significant improvement was also seen on the quality of life assessed in the study (Polman, O'Connor et al. 2006). Despite this, the main question remains whether reduction in the frequency of relapses can stop or postpone later disabilities in the course of the disease.

1.5.2.4 Environment and lifestyle

Individuals' lifestyle and the environment they live in have pronounced effects on their state of health and wellbeing. As an example, it has been suggested that relapses are more common in the springtime and least common in the winter in Switzerland and higher frequency of relapse was observed in Arizona and Cleveland (Ohio) in the summer time (Sibley and Foley 1965; Wuthrich and Rieder 1970; Bamford, Sibley et al. 1983). There are few proposed environmental and lifestyle factors associated with progression in MS. These include patients' vitamin D status, tobacco smoking, (which will be discussed in details in the following chapters), alcohol and coffee consumption, diet, etc.

The effect of vitamin D on the progression of the disease is perhaps one of the most commonly studied environmental factor associated with progression in MS. Studies have looked at the potential protective role of vitamin D in reducing the frequency of relapses, disability progression and measures of disease activity on MRI scans. Reports from these studies have yielded some mixed results in terms of the magnitude of the effect. While many of these studies have found a significant increase in MRI activities and relapse rate in individuals with lower levels of serum vitamin D (Mowry, Krupp et al. 2010; Loken-Amsrud, Holmoy et al. 2012; Runia, Hop et al. 2012), many other have failed to show any beneficial effects of the vitamin D on measured clinical outcomes of MS including relapse rate and disability progression (Mowry, Krupp et al. 2010; Kampman, Steffensen et al. 2012; Soilu-Hanninen, Aivo et al. 2012). An issue here is that while some of the findings are statistically significant, the change has little clinical significance. For example, in a study by Mowry et al. an increase in disability score of 0.04 (95%CI: -0.091 to -0.003) was found per 10 ng/mL lower vitamin D in a 4-year follow-up period (Mowry, Waubant et al. 2012). This could mean that for a period of 20 years, 10 ng/mL higher vitamin D volume can only reduce disability progression by 0.2 score. It is unlikely that this score can have a significant contribution to most of the disability score already accumulated after 20 years and even more unlikely to have pronounced effect on patients' quality of life. In addition, a study by Zivadinov and colleagues (Zivadinov, Treu et al. 2013) has shown that the effects of sun exposure on MRI measures can be independent of patients vitamin D status.

A limitation in the studies in which the influence of vitamin D on the clinical course of MS has been investigated, is the possibility of reverse causality where it is not clear whether low vitamin D levels causing the higher levels of disability or disability affecting vitamin D levels by preventing patients to be physically active and get sufficient sun light. Another possibility in such studies is the presence of interaction and/or effect modification. For example, ethnicity has been shown to have an influence on factors associated with vitamin D levels in individuals with MS (Amezcua, Chung et al. 2012). Nevertheless, the influence of vitamin D in the progression of MS deserves further investigation as most studies do not address the influence of vitamin D in progressive MS.

Infections and in particular upper respiratory tract infections have found to trigger exacerbations in MS (Sibley, Bamford et al. 1985; Andersen, Lygner et al. 1993; Panitch 1994). A study by Correale and colleagues has shown the impacts of both viral and bacterial infections on relapse rate, disease activity on MRI scan and T cell activation in relapsing onset patients during the first 2 weeks after the clinical onset of infection. (Correale, Fiol et al. 2006). They pointed out that relapses which were triggered by systemic infection were more severe and longer. Diet is one of the most commonly studied factors influencing the progression in MS. However, dietary interventions have shown no association with disability progression in MS (Farinotti, Vacchi et al. 2012).

1.5.2.5 Comorbidity

MS is a lifelong chronic disease often associated with a range of comorbid conditions complicating the disease and the choice of therapeutic interventions

(Marrie, Horwitz et al. 2008). Although the presence of one or more comorbid diseases may result in less desirable clinical outcome and in the case of MS delay the diagnosis (Marrie, Horwitz et al. 2009), comorbidity may facilitate identifying the at risk population and underlying mechanisms more robustly, bearing in mind that the concept behind epidemiology is that diseases are not randomly distributed amongst individuals in a population. Amongst a range of comorbidities associated with MS, autoimmune diseases (ADs) are of particular interest as they may share some immunological similarities which could facilitate identifying the underlying mechanism of MS as an immune mediated condition. The coexistence of some ADs in MS patients has been investigated and studies have reported an inverse association with Rheumatoid Arthritis (Somers, Thomas et al. 2006; Nielsen, Frisch et al. 2008) and positive association with autoimmune thyroid disease (Sloka, Phillips et al. 2005; Munteis, Cano et al. 2007). The frequency of rheumatoid arthritis reported to be in range from 1% to 4.5%. Thyroid disease can occur in about 9% of MS population, significantly more than what would have been observed in the general population (Jacobs, Wende et al. 1999; Niederwieser, Buchinger et al. 2003; Barcellos, Kamdar et al. 2006). Cases of patients with both MS and systemic sclerosis, autoimmune hepatitis, myasthenia gravis have also been reported in smaller scales in some case-control studies (Achari, Trontelj et al. 1976; de Seze, Canva-Delcambre et al. 2005; Pelidou, Tsifetaki et al. 2007). Accurately defining the association of MS and other ADs based on the incidence and/or prevalence data can be limited due to possible sources of error in the rate calculation and the fact that the standards and criteria may vary significantly among different studies performed. A problem arises from

variations in diagnostic criteria. One classic example of such a problem is clearly demonstrated in a study by O'Sullivan and Cathcart (O'Sullivan and Cathcart 1972) comparing the prevalence of rheumatoid arthritis (RA) using New York Rheumatoid Association and the American Rheumatoid Association (ARA) diagnosis criteria. The survey showed significant variation in the results of the population of Sudbury from 3.8% for women and 1.3% for men by ARA criteria to 0.5% for women and 0.1% for men by New York criteria. This notable variation in diagnostic criteria may also have significant effects on our evaluation of the associations of MS with other ADs. In addition to the presence of publication bias, mainly affecting negative results, many of the studies investigating the association of MS with other ADs were conducted as population surveys and only some of the estimates in the current literature are drawn from MS specific population-based cohorts. These have resulted in substantial differences in the rate estimated and significant between-study heterogeneity. There are also limitations in generalisability of the results due to such factors as geography. Therefore most of our current estimations of the incidence and prevalence rates and consequently the association of MS and other ADs may be over- or underestimation of the real-world data. Furthermore treatment interventions may influence the rates greatly. One treatment may increase the risk of particular comorbid AD and another treatment can keep the concurrent AD hidden in its preclinical stage. For example, interferon β has been shown to modify the clinical course of RA (Alsalameh, Manger et al. 1998). Cases of sclerosing skin disorder while receiving interferon β have also been reported in MS patients (Hugle, Gratzl et al. 2009).

Comorbidity can potentially complicate and limit the process of prescribing rational medicinal therapy. However, information drawn from studies looking at treatment response in MS patients with concomitant comorbid diseases can also be invaluably informative. Despite the relatively high interest in the association of MS with other ADs, little knowledge exists about their impact on treatment decisions, treatment responses and MS clinical outcomes. Data are mixed regarding the frequency of asthma and allergies in MS patients (Monteiro, Souza-Machado et al. 2011) and no study has looked at the association of asthma and disability progression in patients with MS.

MS is often associated with psychiatric disorders. Amongst the range of psychiatric symptoms in people with MS, major depression is the most commonly reported comorbid condition with reported life-long prevalence between 25 to 50% (Feinstein 2002; Feinstein 2004). As one would expect, depression has been shown to decrease the quality of life in MS patients but no evidence of its effect on MS clinical course has been found yet (Amato, Ponziani et al. 2001; Koch, Uyttenboogaart et al. 2008; Goksel Karatepe, Kaya et al. 2011). Unlike depression visual and vascular comorbidities were associated with higher disability scores. In a study by Marrie and colleagues visual comorbidity was associated with 1.47 (95%CI: 1.37 to 1.59) times higher risk of mild visual disability (Marrie, Cutter et al. 2011).

Vascular comorbidities and in particular hypertension are also amongst common comorbid conditions in MS patients. The frequency of hypertension has been reported to be about 30% in MS patients (Marrie, Rudick et al. 2010) which is similar to what has been reported from the general population data (Hajjar, Kotchen et al. 2006). In another study Marrie and colleagues examined

the effects of vascular comorbidity on the risk of ambulatory disability. They found that any vascular comorbidity whether present at the time of diagnosis or any time after MS onset was associated with almost 50% increase risk of ambulatory disability (Marrie, Rudick et al. 2010).

Despite its clinical relevance and importance, the effects of comorbidities on MS progression seem to be poorly investigated. Better identifying comorbidities commonly associated with disease can not only increase patients' quality of life but may also be helpful in identifying the pathways behind the aetiology of the disease. It is also important to establish the effects of MS on comorbidities if patients' wellbeing is to be improved. For example, a large study of cancer risk in MS patients has shown larger tumour sizes at cancer diagnosis in MS patients compared with the matched controls (Kingwell, Bajdik et al. 2012).

1.5.3 Mortality

MS is usually associated with range of mainly irreversible and progressive disabilities but not considered as a lethal disease. Life span in patients with MS is generally estimated to be 5 to 10 years shorter than general population primarily due to complications arise from the disease (Sadovnick, Ebers et al. 1992; Bronnum-Hansen, Koch-Henriksen et al. 2004). MS survival from the onset or diagnosis of the disease has been reported to be in a range from 24 to 43 years with some studies showing an increase in MS survival (Phadke 1987; Riise, Gronning et al. 1988; Wallin, Page et al. 2000; Bronnum-Hansen, Koch-Henriksen et al. 2004; Hirst, Swingler et al. 2008). It is hard to accurately

comment on possible trends in MS survival as many of the studies investigating MS survival did not compare MS mortality rates with that of general population. Hence, the reported increase in MS survival may the result of increased life expectancy in the general population.

The large population-based study of Danish MS registry has shown that the excess mortality in MS patients (relative to general population) has almost halved during the past 50 years (Bronnum-Hansen, Koch-Henriksen et al. 2004). However the more recent study of the large British Columbia cohort could not find any evidence of improvement in MS excess mortality over time (Kingwell, van der Kop et al. 2012).

1.5.3.1 Incidence and rates

Studies have shown a significant decrease in the mortality incidence rates over time. From 1951-1958 to1967-1973 time periods incidence mortality rates have decreased in Scotland (from 3.0 to 2.1), Switzerland (from 2.2 to 1.8) and France (from 2.2 to 0.8) and have increased or remained stable in New Zealand (from 1.2 to 1.1), US (from 0.9 to 0.8) and Finland (from 0.9 to 0.6) (Massey and Schoenberg 1982). Whether MS mortality rates are changing is debatable but the absolute mortality rates reported in most of these studies have no value in establishing the trend in MS survival as they were not compared to the rates from the general population. Furthermore the potential impact of calendar and birth cohort effects should be examined intensively.

Despite the evidence regarding increased absolute MS survival and decreased incidence of mortality amongst MS patients, two probably the largest MS survival studies estimated that MS patients have almost 3-fold increased

mortality rates relative to the general population (Bronnum-Hansen, Koch-Henriksen et al. 2004; Kingwell, van der Kop et al. 2012). These findings clearly shows that the reported decrease in incidence of MS mortality can be a direct reflective of the increase in general life expectancy in the general population during the past decades. Similar to these, in Wales, it was found that MS patients were 2.8 times more likely to die prematurely relative to the general population (Hirst, Swingler et al. 2008). In the UK it was estimated that patients with MS have 3.5-fold increased mortality (Lalmohamed, Bazelier et al. 2012). Evidence is consistent with regard to the standardised mortality ratios (SMR), although lower mortality rates have also been reported. For example, French data showed excess mortality ratio of 1.8 which is considerably lower than that reported from the studies stated above (Leray, Morrissey et al. 2007). Methodological differences, length of the follow-up, sample size and differences in the medical practice may explain parts of this discrepancy in the results.

1.5.3.2 Cause of death and potential risk factors

Studies have shown that more than 50% of MS patients die from the complication of the disease. Regardless of several possible biases (misdiagnoses and underreporting) which can be present in the data acquired from the death certificates (Malmgren, Valdiviezo et al. 1983; Midgard, Riise et al. 1996), results are almost consistent regarding the percentage of patients who die from MS-related causes. Major causes of death in MS patients include: respiratory (mainly pneumonia), sepsis (mainly urosepsis), cardiovascular disease and cancers. Cases of suicide have also been reported in MS patients and are shown to be more frequent in MS compared with general

population (Stenager, Stenager et al. 1992). Data regarding the incidence of cancer in MS are mixed but in general the overall risk of cancer in MS patients is either reduced or is at the level of the general population (Sadovnick, Eisen et al. 1991; Koch-Henriksen, Bronnum-Hansen et al. 1998; Kingwell, Bajdik et al. 2012). However, it is not clear whether mortality rates due to cancer is also at the level of rates in the general population.

There are potential risk factors associated with increased mortality rates in MS patients. It has been shown that female patients with MS have higher mortality ratio than males, relative to the general population (Poser, Kurtzke et al. 1989; Wallin, Page et al. 2000; Grytten Torkildsen, Lie et al. 2008).

Life-style, environmental risk factors and treatment interventions can also influence the mortality rates. For example, a hypothetical cohort of MS patients with a significantly higher number of smokers than the general population is expected to have more death due to cardiovascular disease or cancer and hence higher SMR's. The difference between the proportion of smokers in MS and the general populations may explain some of the discrepancy seen in the studies comparing cancer-related mortality ratios in MS patients to those of the general population (Bronnum-Hansen, Koch-Henriksen et al. 2004; Grytten Torkildsen, Lie et al. 2008). Amongst the all life-style risk factors, cigarette smoking is a significant health risk which has previously been linked to more severe disease in MS patients (Hernan, Jick et al. 2005; Hawkes 2007; Di Pauli, Reindl et al. 2008; Sundstrom and Nystrom 2008; Healy, Ali et al. 2009) and has recently been reported to be associated with a significant decrease in people life span (Huxley and Woodward 2012; Sakata, McGale et al. 2012). A recent survey of MS patients in the UK has

shown a significant influence of cigarette smoking on the risk of death in MS patients compared with the referent subjects (Lalmohamed, Bazelier et al. 2012). Despite the relatively large number of studies investigating mortality in MS, the influence of life-style factors, mainly cigarette smoking, on mortality rates in MS patients remained to be examined.

1.5.3.3 Gender effect

Contrasting the general perception towards higher mortality ratio amongst male patients with MS, studies have fail to show any advantage of female patients in terms of excess mortality when the mortality rates in MS cohorts are compared with that of the general population (Poser, Kurtzke et al. 1989; Wallin, Page et al. 2000; Kingwell, van der Kop et al. 2012). It appears that when compared to the general population female patients have higher mortality ratios than males.

1.5.3.4 Treatment effects

Data is very limited regarding the potential influence of treatments on mortality rates in MS patients. Many of the survival studies in MS predate the treatment era or have very few percentage of their population exposed to the DMTs. Accurately commenting on the potential beneficial role of DMTs in MS has some limitations. The most pronounced and important bias is lack of randomisation. For example, in the UK relapsing patients only have access to the DMTs if they can walk independently (at least 100 metres without assistance) and have had at least two clinically significant relapses in the last two years. Such criteria can potentially put patients with active disease on treatments and introduce selection bias to the cohort. Recently, Goodin and

colleagues estimated the survival rate and examined the effects of treatment in the patients who participated in the first pivotal clinical trial of IFNβ-1b. In their study the risk of death was almost halved for patients receiving IFNβ-1b treatment, at either dose, compared with placebo group (Goodin, Reder et al. 2012). Cause of death was concluded to be MS related in 78% of patients with some excess MS related death recorded in placebo group compared with the active treatment arm (Goodin, Ebers et al. 2012). There are issues that need to be addressed as far as the results are concerned. First, with the mean age at the onset of 31 and disease duration of 9 years, after only 21 years of follow up the mean age of the cohort reaches 60 at the time of study. With the reports of survival with MS of up to 78 years in North America, 60 seems a remarkably young age to precisely comment on the effects of treatment on survival in MS. The actual mean age at the time of death reported in the study was 51.7 (±8.7) years. Second and most importantly, the effects of treatments after the trial have completely been ignored. Nevertheless, additional work into the covariates and underlying causes of death in this cohort of patients is needed to systematically comment on the effects of treatment on MS survival.

1.6   Disease Burden

To assess the burden of a disease, both mortality and morbidity are taken into account using the disability-adjusted life year (DALY). DALY is a time-dependent variable which combines years of life lived with disability or state of health less than ideal health and potential years of life lost due to premature mortality (PYLL). MS is a very disabling disease with an average age at the

onset of 32 year old during the primary productive time of life. It is usually associated with a range of severe impairments and disabilities, which can negatively influence patients' quality of life (Handel and Ramagopalan 2010). In addition to the vast range of comorbidity associated with the disease, MS is also frequently associated with premature mortality. On average it has been estimated that patients with MS live 5 to 10 years less than the general population (Scalfari, Knappertz et al. 2013). Considering that many of these patients spend a significant proportion of their lives with restricting impairments and disabilities and given the fact that MS is a relatively prevalent disease particularly in the Europe and North America the overall burden of the disease is considerable. The total cost of MS combines direct and indirect costs. Direct costs usually represent the costs of resources used to treat the disease or its symptoms. While indirect costs represent the value of production lost due to the disease.

1.6.1 Cost of the disease

Many studies have evaluated the cost of MS in various countries. While the costs varies largely between studies and in different parts of the world, the general findings imply that indirect costs account for the majority of the disease costs and also costs increase significantly as the disease progress. Costs of MS (direct and indirect) can increase by nearly twofold in patients with EDSS score 3.5 to 6 compared with those with EDSS score ≤ 3, from £7,273£ to £12,875 per patient per year (Kobelt, Berg et al. 2006).

1.6.2 Cost of treatment

It has been estimated that 34 % of total costs (direct and indirect) of MS can be attributed to DMTs (Kobelt, Berg et al. 2006). MS drug expenditure has increased significantly during the past years (Schafer, Gunderson et al. 2010). From 2006 to 2010, the average wholesale price of IFNβ-1a increased by approximately US$18,000. The price of GA has also increased by about US$25,000 for the same time period. The acquisition cost of all DMTs increased in 2011 as competition increased with the introduction of natalizumab and fingolimod. Fingolimod was approved by the FDA in September 2010, with an average wholesale price of around US$57,000 per year. The FDA approval of second-line treatments means that substitution of natalizumab and fingolimod for one of the most commonly used DMTs would add around US$10,000–15,000 a year to treatment costs. In the UK, patients had access to DMTs only after NHS and the drug manufacturers agreed to the risk-sharing scheme that provides the drugs on the basis that they meet certain clinical and cost-effectiveness outcomes. However, it has been argued that even cost reductions by 67% in the UK achieved by the scheme would be unlikely to make DMTs cost-effective (Boggild, Palace et al. 2009). Table 1-2 compares the price of treatments in the UK, US and Denmark.

Table 1-2: Comparison of drug costs per patient per year

Drug	UK [a]	US 2009 [d]	US 2012 [e]	Denmark 2012 [f]
IFNβ-1a IM	£9,061	$US 26,916	$US 45,878	€ 17,436
IFNβ-1b SC	£7,259	$US 29,532	$US 45,953	€ 14,990
IFNβ-1a SC	£9,088 (44 µg: £12,068)	$US 28,008 (44 µg)	$US 43,865 (44 µg)	€ 8,511 (44 µg: €11,611)
GA SC	£6,650	$US 27,396	$US 51,762	€ 17,523
Natalizumab	£14,730 (2007)[b]	$US 28,400	$US 46,535	€ 26,889
Fingolimod (0.5 mg)	£19,169 (2011)[c]	--	$US 55,776	€ 32,430

[a] source: NICE. TA32 Beta interferon and glatiramer acetate for the treatment of multiple sclerosis; 2002.
[b] source: NICE. TA127 Multiple sclerosis - natalizumab: costing template
[c] source: NICE. Multiple sclerosis (relapsing-remitting) - fingolimod
[d] source: Drug Topics Red Book.
[e] source: http://www.destinationrx.com/
[f] source: Danish medicines agency, Price period: 14.05.2012 - 27.05.2012

1.7   Tobacco smoking in the UK; prevalence and general features

Tobacco smoking is a major risk factor for many diseases and is the biggest preventable cause of premature death accounting for nearly 6 million deaths worldwide (WHO 2011). Tobacco smoking will continue to be the biggest cause of premature death during the 21st century with approximately 1 billion smoking-related deaths (Jha 2009). It causes billions of dollars of economic damage each year (Allender, Balakrishnan et al. 2009). Cigarette smoke contains roughly 4,000 compounds, some of which are highly toxic with significant negative impacts on human tissues.

It is important to identify the problem and develop appropriate strategies to reduce both the incidence and the prevalence of this major public health issue. Here I provide an overview of the general patterns of cigarette smoking in the UK. The main source of data presented here for smoking prevalence in the UK is the General Lifestyle Survey (GLF), formerly known as the General Household Survey (GHS), published by the Office for National Statistics (ONS). The GLF is a national survey covering adults aged 16 and over living in Great Britain. Since 1998 the survey has included a core section of questions on smoking, drinking and drug use. Based on the 2011 data, 20% of adults (20% of men and 19% of women) reported smoking (current smokers) an average of 12.7 cigarettes a day which is similar to 2009 where 21% of adults reported smoking. Smoking prevalence was decreased significantly in 2010 compared with the 39% in 1980. The proportion of never smokers or only occasional smokers has been rising steadily, from 43% in 1982 to 55% in

2010. Filter cigarettes are the most common type of cigarettes smoked, although there have been substantial increases in the numbers smoking hand-rolled tobacco since 1990. Probably cost is the most influential factor in this trend. There was a substantial numerical difference in the prevalence of smokers in different age groups and those aged 20 to 24 (28%) and 25 to 34 (26%) continue to have the highest prevalence of cigarette smoking. In 2010, 5% of children aged 11 to 15 reported smoking at least one cigarette a week, while 25% of them have tried smoking at least once. Smoking prevalence was different amongst people with different demographic characteristics and was higher in the routine and manual socio-economic group than managerial and professional group. There were also geographical differences in the prevalence of smoking. Table below compares geographical distribution of smoking in the East Midlands and England.

Table 1-3: data from: the Integrated Household Survey.

	England (benchmark)	East Midlands	Nottingham	Nottinghamshire	Derbyshire	Leicestershire
Smoking prevalence - routine & manual group (%)	30.3	29.5	32.6	32.1	30.2	22.4
Smoking Prevalence (total) (%)	20.0	19.8	23.3	19.7	18.6	17.1
Smoking status at time of delivery (%)	13.2	15.7	18.5	18.2	15.6	11.3
Smoking attributable mortality (per 100,000)	210.6	209.2	294.3	211.5	207.9	170.2
Lung cancer registrations (per 100,000)	45.8	56.3	70.1	46.3	44.6	39.7
Deaths from lung cancer (per 100,000)	37.7	36.7	57.3	38.1	34.5	30.4
Deaths from chronic obstructive pulmonary disease (per 100,000)	25.8	24.7	40.0	26.0	24.0	18.9

1.8   The present investigation, aims and the hypothesis

Improving the health and well-being of the population is a moral imperative and is essential for stability and development. The vast efforts and resources in science and technologies have given us the opportunity to reduce disease and improve health in our population. Much of an individual's lifestyle behaviour has implications on health and illness status and the degree of physical and mental wellbeing an individual can enjoy. Engagement in health-risk behaviours such as tobacco smoking increases the likelihood of development of diseases such as cardiovascular disease (CVD) and various cancers. However, the roles of lifestyle habits in MS are yet to be confirmed. In the current work I investigate risk factors with potential impact on MS to provide foundation and knowledge for the future research and also to inform efforts for making effective interventions in MS possible.

Chapter two: Introduction to the cohort and data collection

2.1 Summary of the chapter

The aim of this chapter is to describe our study procedure and to lay the foundation and justification for the data used in all the following chapters. I acknowledge that information on outcomes and covariates in each analysis was not always available (missing data), resulting in slightly different numbers of patients in each analysis.

In the next section, **section 2.2**, I will give a brief description about the Nottingham City and the MS registry at the Nottingham University hospital (QMC).

Section 2.3 will provide a summary of our assumption at the beginning of the study and the rationales behind our sample size and data collection.

Section 2.4 describes our study procedure.

Section 2.5 summarises our inclusion and exclusion criteria.

Section 2.6 provides information on our ethical considerations.

Section 2.7 and **section 2.8** define the procedures taken for the data collection and the types of clinical outcomes used in our study.

In the final section, **section 2.9**, study's response rate, general features and demographic characteristics of our cohort are presented.

2.2 Nottingham city and Nottingham university hospital MS registry

Nottingham (**52.9700° N, 1.1800° W)** is a city and unitary authority in the East Midlands of England in the traditional county of Nottinghamshire. The total population of Nottingham city was 305,680 in 2011 with more than 70% of the population being white. Over the past decade Nottingham has faced pronounced changes in terms of population characteristics. The following lines provide basic counts of Nottingham City residents based on their answers to the 2011 Census (The 2011 Census programme, Office for National Statistics). In 2011, 50% of the City's population were aged 30 or under with an average age of 34.8. This made Nottingham the fourth youngest city outside London. Most likely the two universities are the principal reason for the high proportion of young people in the City. In total, 19.5% of Nottingham's population was born outside the UK. Nottingham has seen an increase in the number of people of mixed or multiple ethnic groups (from 3.1% to 6.7%) as well as a significant fall in the proportion of White population of the City since 2001. There has been a large numerical increase in the Black African and Pakistani groups. Now, Nottingham has the third highest proportion of people of mixed ethnicity outside London. 18.1% of Nottingham's population reported health problems or disabilities in 2011 census which is slightly lower than the national average of 18.4%. However, amongst people of working age which are usually young adults, 14.2% of people had health problems or disabilities compared to 12.7% nationally. Nottingham has a higher proportion than nationally of residents with no qualifications. As a result Nottingham residents are less likely to be in

managerial or professional occupations and are more likely to have jobs in routine, semi routine and lower supervisory or technical occupations.

Nottingham University Hospitals NHS Trust (NUH) is based in the heart of Nottingham city on three separate sites around the city providing health care for residents of Nottingham and across the East Midlands region. Queen's Medical Centre is one of the largest hospitals in the UK with currently more than 1300 beds. In partnership with the University of Nottingham the trust has achieved a national and international reputation for many of its specialist services, including stroke, renal, neurosciences, cancer services and trauma.

2.3 Power of the study

In England, there are an estimated 120,000 people with MS (Investigators, Coles et al. 2008). National surveys in England estimated that around 20% of British adults aged over 18 smoke tobacco products regularly. Our previous survey in the centre suggested that the prevalence rate of current smokers at the time of disease onset and/or diagnosis is around 29% (Manouchehrinia, Tench et al. 2013). With 95% confidence interval and to have a margin of error less than 5%, sample size was calculated according to:

$$n = z\frac{p\,(1-p)z^2}{ME^2}$$

Where ME is the margin of error, P is the prevalence rate (29%) and z is the z-score (1.96 for 95% confidence intervals).

Based on the above assumptions, a sample size of 620 was required to compare the prevalence of smoking between our MS cohort and England general population. We anticipated a response rate of about 45 to 55% based on the previous research in our centre and in the UK. Based on this response rate and the sample required for the study, it was estimated that questionnaire should be sent to at least 1400 MS patients to achieve the sample required for the study. We expected the average participants' age in our study to be around 50 years, with a 2:1 female:male split.

2.4   Selection of patients

Participants were identified and recruited from Nottingham University Hospital MS clinics database. The study subjects consist of those enrolled in the Queen's Medical Centre MS clinic registry. Patients in this registry are referred from in and around Nottingham. For inclusion in the study a patient must have diagnosis of MS made by the neurologist and be > 18 years of age. Patients in the registry are seen routinely in the clinics and undergo neurological examination. The examination usually includes estimation of disability score, reports of comorbidity and treatment interventions. The patients in this study were those whose baseline visit was between 1994 and 2012. Patients recruited into the registry undergo an extensive medical evaluation with standardised reporting of history, physical evaluation, Imaging and laboratory investigations. Eligible patients were contacted by letter to inform them of the study. The patient information sheet and questionnaire booklets were included with the letter. A separate consent form was also

included in the package as the study involved reviewing participants' MS clinic medical notes. The medical notes in this clinic are different from those on GP's database and consist of records of extensive neurological evaluation with standardised reporting of history, physical evaluation, imaging and laboratory investigations. Some participants were recruited on their MS clinic appointment in order to reduce the data collection time, postal costs and environmental impact. Efforts were made to keep this at minimum to reduce any unforeseen biases which could arise from clinic recruitments. If the patients wished to take part in the study they were asked to sign the consent form and complete the questionnaires and return them by the prepaid envelope provided. The patients' information sheet prepared by the research team gave the participant adequate information regarding all aspects of the study and information pertaining to participation in the study. The research team and chief investigator contact detail were included for patients to peruse and contact the research team. In the patient information sheet it was explained to the potential participant that entry into the study is entirely voluntary and that their treatment and care will not be affected by their decision. It was also explained that they can withdraw at any time.

2.5 Inclusion and exclusion criteria

2.5.1 Inclusion criteria

- Clinically definite MS diagnosed at least 12 months beforehand.

- Adults: aged between 18 and 90 years old.

- Men and women.

2.5.2   Exclusion criteria

Patients with current or concomitant illness that would interfere with the individual's ability to complete the study results were excluded. Furthermore, children, prisoners, and those unable to give consent were not to be included.

2.6   Ethical Considerations

As the study did not involve any potential harmful treatments or any sort of invasive interventions or lifestyle alterations, the main ethical issue was confidentiality of data and adequate and proper data storage. Confidentiality of data were addressed by coding all patient data and arranging appropriate secure storage of paper and electronic data (which were labelled with coded ID only). The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, 1996; the principles of Good Clinical Practice, and the Department of Health Research Governance Framework for Health and Social care, 2005. All participating researchers adhered to ICH-GCP and good clinical governance guideline. The study was conducted at the Nottingham University Hospital and all the paper data were kept in a secure and locked storage at the Division of Clinical Neurology. All the data analysis and electronic data were conducted and stored on the University of Nottingham's computers and were password protected. The password was issued to the chief investigator and the research team only. The study was initiated after the protocol, consent forms and participant information sheets received approval from the Research Ethics Committee (REC), and the respective National Health Service (NHS) Research &

Development (R&D) department. The study was approved by the NRES East Midlands Ethics Committee- Derby-1 and Nottingham university hospital R&D office.

2.7   Data collection and questionnaire booklet

Data used in our study were collected from patients' medical records, a prospective cohort database started in year 2000 by Professor Constantinescu (Edwards and Constantinescu 2004) and a questionnaire booklet designed specifically for the study as we aimed to collect additional data which was not exist on the database and was not part of the patients' routine care to enhance our 'snap shot' of each patient's smoking status as well as their level of disability.

Since year 2000, more than 4070 EDSS scores were recorded in the prospective cohort database. This represents almost four EDSS scores per patient, which were estimated by a neurologist during patients' routine clinic visits.

The recorded EDSS scores in the database and new updated scores obtained from the medical records were then used for the time series analysis. When estimating the time to EDSS score milestones 4 and 6, extra care was taken to ensure that the year which was recorded as the time in which patients reached the scores is a true value, no EDSS scores 4 and 6 are recorded before this year and the score is either sustained or escalated in the following years. Due to the uncertainty about the date in which some of the patients escalated to EDSS

scores 4 and 6, 12% to 15% of the patients were dropped from the time-series analysis.

In case of a gap between two scores, the median year between the scores were recorded as the year of reaching that particular score. For example, if a patient had an EDSS score of 3.0 in year 2005 and 5.0 in year 2007, the year 2006 was chosen as the year in which the patient has reached EDSS score 4.

Four validated, self-report questionnaires were combined and used in our study. In the questionnaire booklet, patients were asked to answer a series of questions regarding any history of asthma, allergy, eczema, appendectomy and tonsillectomy. In order to be able to conduct a case-control study investigating the potential contributory role of smoking in development of MS, patients in our registry were asked to answer the exact questions obtained from the England health survey 2010. Specifically for the asthma, smoking and allergy part, patients were asked to complete a series of questionnaires in the booklet format containing questions from following questionnaires:

- Questions from the European Community Respiratory Health Survey II (ECRHS II)
- Questions from General Household Survey and England Health Survey 2010

The level of patients' disability was measured via two validated, self-report questionnaires. Booklet contains following questionnaires:

- Multiple Sclerosis Impact Scale (MSIS-29)
- Patient Determined Disease Steps (PDDS)

There are several patient oriented outcome measures in MS including Guy's Neurological Disability Scale (GNDS), Multiple Sclerosis International Quality of Life questionnaire (MusiQoL), Multiple Sclerosis Quality of Life-54 (MSQOL-54), etc. We selected MSIS-29 and PDDS scores for several reasons. First, number of items and time required for answering the questions by patients. Due to the high number of the questions already included in the first part of the questionnaire, we had to use the most informative outcomes with relatively few number of items. Second, good responsiveness to clinical change and possibility of continuing evaluation of individual patients in form of a longitudinal follow-up and third, being easy to administer with minimal contact required with patients (due to the high number of participants).

MSIS-29 and PDDS scores are responsive patient-based outcome measures covering a broad range of domains of MS- and health-related quality of life. The two questionnaires were used to improve our understanding of the impact of MS and to increase the generalizability of our evaluation as they have been widely used in epidemiologic studies of MS. In addition MSIS-29 gave us the ability to measure the psychological impairment (in more details than conventional measurements such as the Short Form (36) Health Survey) in our sample population and investigate the impact of smoking on patients' physical and psychological wellbeing.

A copy of the questionnaire booklet is shown in appendix 1.

2.8 Measurements

2.8.1 Clinical outcomes

In order to measure the effects of tobacco smoking on the progression of disability and severity of the disease in individuals with MS, we used range of validated clinical outcomes. The clinical outcomes used included EDSS, PDDS, MSIS-29 scores and Multiple Sclerosis Severity Score (MSSS). The measures are extensively discussed in the literature and briefly discussed below.

2.8.1.1 EDSS

EDSS was developed from the formerly known Disability Status Score (DSS) in 1983 (Kurtzke 1983). DSS was developed in 1955 (Kurtzke 1955) and used in the first randomised, placebo-controlled, double-blind trial of MS (1957). Like DSS, EDSS is based on the neurologic examination of seven Functional Systems (FS): Pyramidal (P), Cerebellar (Cll), Brain Stem (BS), Sensory (S), Bowel & Bladder (BB), Visual (V), Cerebral or Mental (Cb). Unlike DSS, EDSS measures twenty levels of impairment from 0 to 10 in 0.5 increments (except that no intermediate score of 0.5 exists between 0 and 1). Regardless of all shortcomings attributed to the scale, EDSS is still the gold standard in measurements of neurological deficit in MS.

2.8.1.2 MSSS

MSSS was proposed in 2005 by Roxburgh and colleagues to assess the severity of MS by aggregating EDSS score and disease duration using the clinical data from 9892 mainly European MS patients (Roxburgh, Seaman et

al. 2005). MSSS is different from the simple progression index score (EDSS divided by disease duration) as it corrects EDSS for disease duration by comparing each individual's EDSS score with the distribution of EDSS scores in individuals with similar disease duration. Hence, MSSS is capable of measuring disease severity in MS using single EDSS scores. MSSS can be generated using either local (study dataset) or global (data from 9892 patients) EDSS scores. For the propose of this study we generated the global MSSS score where the EDSS scores from our sample population were compared to the larger sample of 9892 MS patients as this first, increases the generalizability of our results and second, the disease severity in our cohort could be compared with that of a larger MS population.

2.8.1.3 MSIS-29

The MSIS-29 is a 29-item patient-reported scale for measuring physical and psychological functioning impact of MS. It has two subscales a 20-item physical impact scale (questions 1 to 20) and a 9-item psychological scale (questions 21 to 29). The scale was generated in 2001 by Hobart and colleagues using traditional psychometric methods (Hobart, Lamping et al. 2001). The MSIS-29 has been comprehensively compared with a range of other scales and has shown validity to be used in clinical trials and studies of MS.

2.8.1.4 PDDS

The PDDS was adapted from a physician administered scale called disease steps (Hohol, Orav et al. 1995). The PDDS is a patient-reported measure of disability developed and widely used by the North American Research

Committee on Multiple Sclerosis (NARCOMS). Although there is no direct correspondence between scores in EDSS and PDDS, studies have shown a significant correlation of EDSS and PDDS scores (Learmonth, Motl et al. 2013).

2.9 Response rate, general features and demographic characteristics of our cohort

2.9.1 Response rate

The questionnaire was sent to 1404 patients. Figure 2-1shows patients' postcode plotted on a map to visualise the geographical distribution of our sample population. The response rate was calculated as the total number of questionnaires sent to the patients divided by the total number of questionnaires received. The clinical and demographic data were collected from 1246 eligible patients. By March 2013, overall 681 questionnaires were returned and their responders were qualified to participate in the study on the basis of our inclusion criteria. This gave us response rate of 48.4%. Mean age in non-respondents was 53 (SD ±11.03) with 2:1 female:male ratio. Non-respondents were significantly more likely (P < 0.001) to live in more deprived geographical areas than respondents as measured by the index of multiple deprivation (IMD 2007). The IMD is a relative measure of deprivation in a particular geographical area which can be used to compare the extent of deprivation across local authorities and different groups of patients. Many surveys have found non-respondents to be from lower socio-economic status (Bakke, Gulsvik et al. 1990). Our findings confirm this, however, little is known about non-respondents and impact of socio-economic status in surveys of MS. Differences in socio-economic status between our respondents and non-respondents can potentially influence our estimates of the prevalence of smoking and level of disability as surveys of tobacco smoking in the general population has constantly shown that the prevalence of smoking is significantly higher in more deprived areas of the country (Hiscock, Bauld et

al. 2012). There is also a possibility of reverse causality where disability due to MS can lead to patients being from lower socio-economic class. As is evident from our survey, not only are non-respondents representing more patients of lower socio-economic status but they are significantly more disabled compared with respondents. Hence, our estimates of the prevalence of smoking may be slightly lower than the true prevalence. In general, non-respondents were more likely to be off treatment, have slightly longer disease duration (2 years) and be more disabled (0.5 EDSS score). Baseline characteristics of 1246 MS patients are summarised in Table 2-1.

Nevertheless, the general demographic and clinical features of our cohort of respondents were similar to those reported from other MS cohorts. For example with regard to the disease phenotype, 58% of our bout onset patients had transited to SP MS after median 20 years of follow-up which is almost similar to the reported percentage of 66 (after median 23 years of follow-up) in the London Ontario cohort (Scalfari, Neuhaus et al. 2010). Slightly more than 10% of our patients were diagnosed with PP MS which was also consistent with the reports from other MS cohorts (Kingwell, van der Kop et al. 2012).

Table 2-1: summaries of baseline characteristics of respondent and non-respondent patients.

	Respondent	Non-respondent	
Age (mean(SD))	52.89 (±11.33)	52.88 (±11.03)	P = 0.99
Sex (female percentage)	71.47%	69.8%	P = 0.56
Disease phenotype (%)			
RR MS	57.2%	50.7%	P = 0.12
SP MS	33.3%	38.7%	
PP MS	9.4%	11.1%	
DMT (%)	54%	40%	P < 0.001
Disease duration (mean(SD))	19.28 (±10.44)	21.37 (±10.16)	P = 0.001
Latest EDSS score	5.5 (3.5 to 6.5)	6 (3 to 6.5)	P = 0.03
IMD (mean (SD))	16.7 (±11.83)	21.35 (±14.93)	P < 0.001

DMT: disease modifying treatment
EDSS: expanded disability scale status
IMD: index of multiple deprivation.
SD: standard deviation
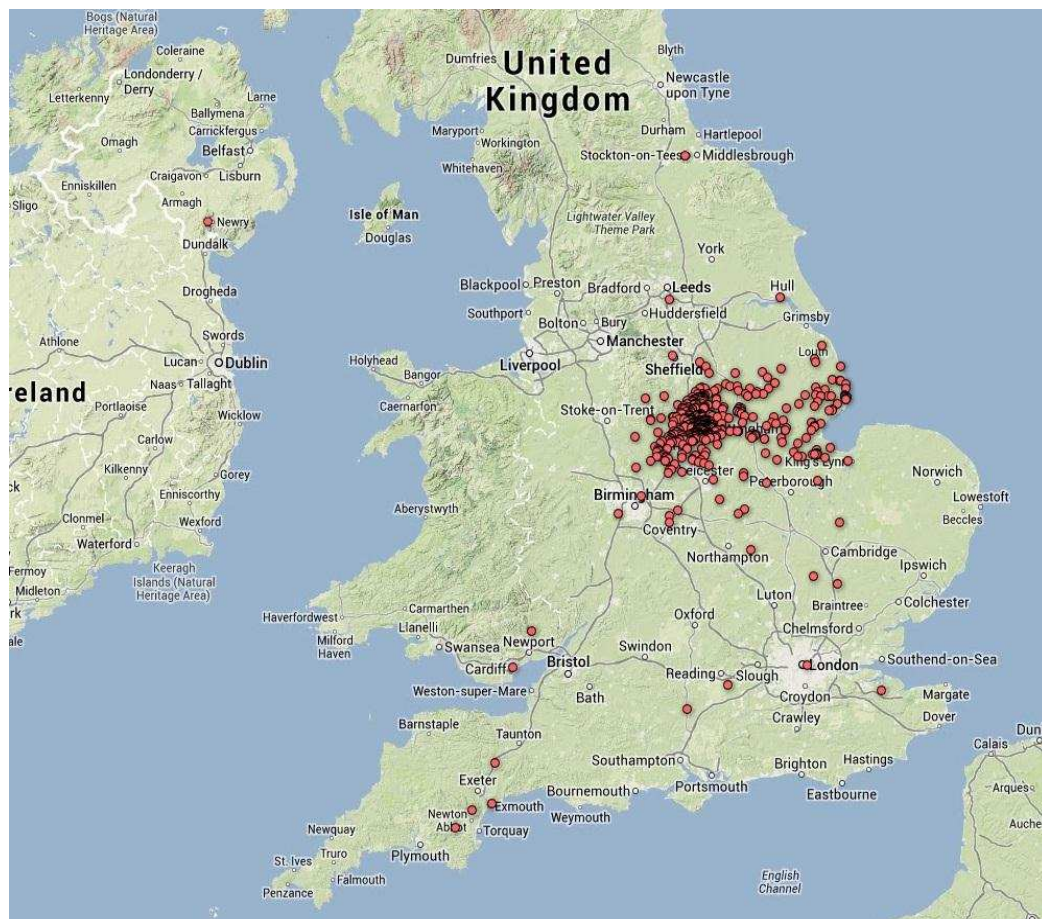
Figure 2-1: patients' postcode plotted on a map to visualise the geographical distribution of our sample population

2.9.2   Demographic and general MS characteristics

From the total of 1246 cases 69.6% were female. Average age at the time of study was 53.4 (SD ±11.55). The majority of the patients were RR MS (51.2%), 10% had PP MS and 37.6% had been diagnosed as SP MS (Figure 2-2). Forty six percent of the patients had been exposed to DMT for at least one year. The mean age at the onset of MS was 32.7 (SD ±10) and mean duration from the date of the first manifestation of the disease was 20.6 (SD ±10.4) years.
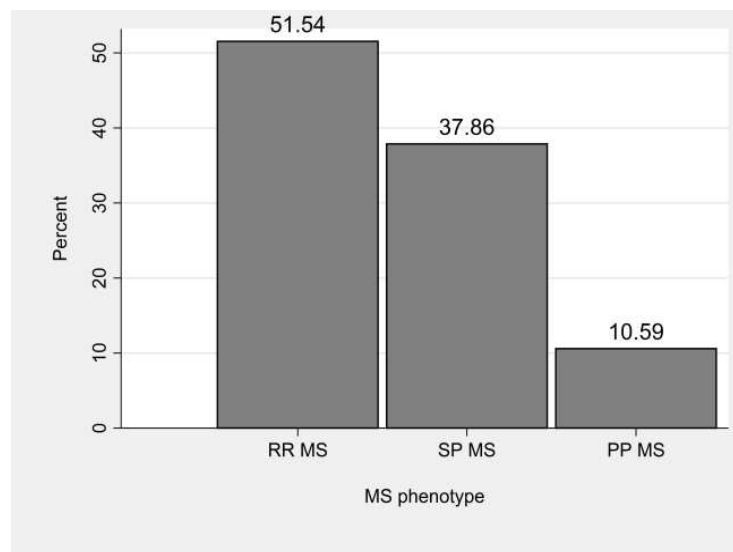
Figure 2-2: Bar chart showing the frequency of MS phenotypes in our sample population

2.9.3 General features

2.9.3.1 Asthma and allergy

Data with regard to coexistence of asthma and allergic diseases and MS is contradictory. Traditionally, it was believed that the two conditions are mutually exclusive one mediated by Th2 and the other Th1 cells. In 2004, an epidemiologic survey of more than 650 MS patients in our centre found an increased susceptibility of MS patients to asthma and all atopy compared with the general population (Edwards and Constantinescu 2004). In contrast, a non-age and –sex matched study by Oro and colleagues reported lower prevalence of allergic disease in a population of 24 patients with MS compared to 18 controls (Oro, Guarino et al. 1996). Further epidemiologic study by Tremlett and colleagues on 306 MS patients obtained from general practitioner data base in Wales showed an inverse association between asthma and MS and no

association to any Th1associated disease (Tremlett, Evans et al. 2002). Besides the biological plausibility of coexistence of MS and asthma and its effect on the disease progression and its potential therapeutic interference, the association between MS and asthma remains controversial. A systematic review and meta-analysis by Monteiro and colleagues (Monteiro, Souza-Machado et al. 2011) has shown that there is no evidence of an association between asthma and MS (OR: 0.83; 95%CI: 0.48 to 1.44). On the other hand, it has been shown that MS patients and their families have an increased susceptibility to autoimmune diseases (Broadley, Deans et al. 2000). In the current work, we investigated the prevalence of asthma in our MS population and compared it to the prevalence in the general England population. The prevalence rate of asthma in England population was obtained from the Health Survey for England 2010.

In our survey, 12.3% (n = 84) of the patients reported a previous history of asthma confirmed by their GPs (Figure 2-4). Mean age at the onset of asthma was 18.8 (SD ±14.5). The percentage of asthma was not significantly different by gender in our population (76% female with asthma vs. 71% female without asthma, P = 0.2). The majority of MS patients with asthma presented with relapse-onset MS compared to MS patients without asthma (96.5% of MS patients with asthma vs. 89.5% of MS patients without asthma, P = 0.048). The mean age at the onset of MS was 30.5 (SD ±9.2) in patients with asthma compared with 33.9 (SD ±9.9) patients without asthma (P = 0.003). 15.2% and 19.7% of MS patients with asthma reported a history of asthma in their fathers and mothers, respectively. 45.8% of MS patients with asthma were receiving treatment for their asthma and 28.2% of them reported at least one attack of

asthma in the past 12 months. Overall, 66.6% of MS patients with asthma received at least one year of DMT (median 2 years) compared with 54% of the whole cohort (P = 0.01). 1.1% of MS patients with asthma lived in the farm, 19.5% lived in a small village, 42.5% lived in a small town, 25.3% lived in a suburb of a city and 11.5% lived inner city when they were under the age of five years. This is compared with 4.5%, 24.9%, 32.1%, 31.5% and 7% in MS patient without asthma (P = 0.08) (Figure 2-3).
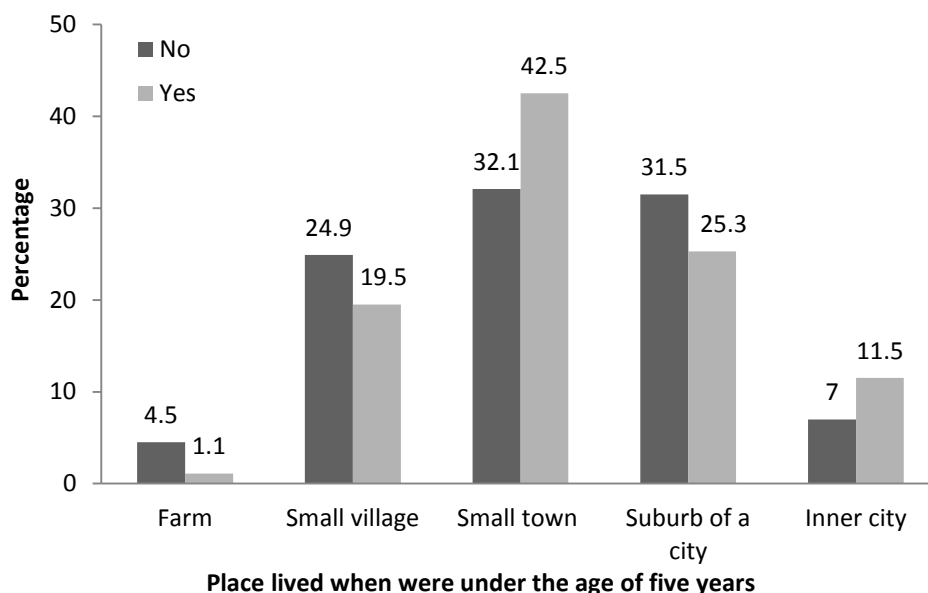
Figure 2-3: percentage of patients by the place of living under the age of five years by asthma status.

35.2% of MS patients reported a previous history of eczema or any kind of skin allergy, 27.1% reported history of hay fever or nasal allergy and 19.8% reported that they have previously had an itchy rash that was coming and going for at least 6 months.
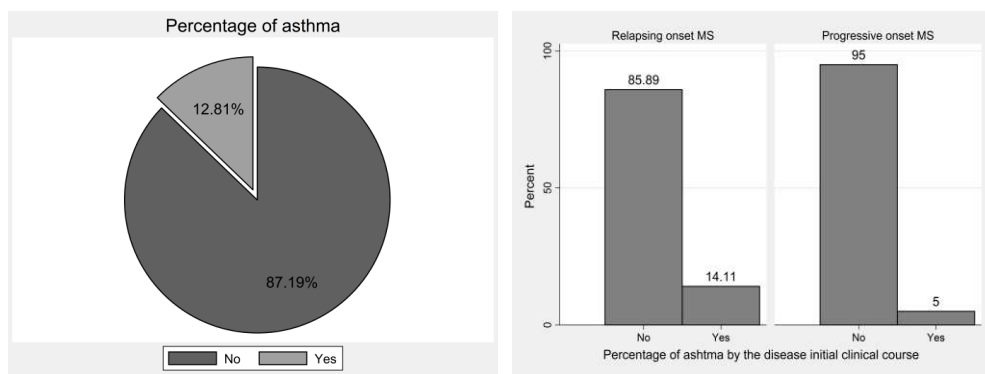
Figure 2-4: Left: percentage of MS patients who reported history of asthma. Right: percentage of asthma in the MS patient cohort stratified by the initial clinical course

Our cohort's 12.3% prevalence rate of asthma was significantly lower than 16.1% prevalence rate in the England general population before matching for sex and age (χ^2 (1) = 5.43, P = 0.02). Age at the first attack of asthma was significantly older amongst people with MS compared with their counterparts in the England general population (16.4 vs. 18.8; P < 0.001). This was predominantly caused by older age at the onset of asthma in female patients with MS (19.4 in females vs. 16.5 in males, P < 0.001). In order to investigate the difference in the prevalence rate of asthma between MS patients and the general population, we performed a sex and age matched case-control study. For each case of asthma in MS patients we randomly selected 2 exact age and sex matched from over 14,000 participants in the Health Survey for England 2010. We made sure that our MS patients answered the exact questioned asked from the controls in the Health Survey for England 2010.

Logistic regression was then used to measure the likelihood of asthma in MS patients compared to their matched controls. Our regression model failed to show any association between occurrence of MS and asthma (OR: 0.83, 95%CI: 0.64 to 1.09, P = 0.19). Nor adjustment of the model for smoking

status (ever- vs. never-smoked) neither for parental smoking did not change the risk of asthma in MS patients (OR: 0.84, 95%CI: 0.63 to 1.11, P = 0.22). The only significant risk factor for asthma was age. In our MS population (without controls) each year increase in age was associated with 4% reduction in the risk of asthma (OR: 0.96, 95%CI: 0.94 to 0.98, P < 0.001). In MS patients, the risk of asthma was higher in females but did not reach significance level (OR: 1.26, 95%CI: 0.73 to 2.1, P = 0.4). Separate logistic regression models with one covariate only (excluding age and sex which were present in all the models) were run to measure the effects of individual and parental smoking on the likelihood of asthma in MS patients. Table 2-2 summarises the results of these logistic regression models. As shown in the table, no evidence of any association between asthma in people with MS and individual and parental smoking was found.

Table 2-2: Results of conditional logistic regression models

	Odds ratio	P-value	95% Confidence interval
MS	0.83	0.19	0.64 to 1.09
Ever-smoking *	1.12	0.62	0.69 to 1.81
Regular smoking *	0.98	0.94	0.62 to 1.55
Father smoking *	0.93	0.77	0.58 to 1.49
Mother smoking *	0.99	0.98	0.62 to 1.59
ever-smoking †	1.08	0.74	0.66 to 1.75
father smoking	0.92	0.76	0.55 to 1.53
mother smoking	1.02	0.93	0.61 to 1.70

First model investigates the likelihood of having asthma in MS patients and their exact age and sex matched controls from the England general population.
* MS patients only. Results obtained from four separate statistical models all adjusted for age and sex.
† One logistic regression model adjusted for age and sex. In MS patients only

2.9.3.2 Tobacco smoking status

Detailed smoking data was obtained through the questionnaire. From the 681 cases who returned questionnaires and had completed the smoking part of the questionnaire, 62.5% reported that they have tried tobacco products at some points during their life (Figure 2-5). 51.1% of the patients reported having smoked at least 20 packs of cigarettes or 12 oz (360 grams) of tobacco in a lifetime, or at least one cigarette per day or one cigar a week for one year which is defining regular smoking in our study. 35.2% of regular smokers had given up smoking while 16% reported current tobacco consumption. Smoking status was different at the time of the disease onset. At the time of the onset of the disease 18% and 33% of individuals were ex-smoker and current smokers, respectively (Figure 2-6). The percentages of non-, ex- and current smokers were significantly different between genders. At the time of study 40.4%, 45% and 14.5% of males were non- ex- and current smoker compared with the 52%, 31.2% and 16.5% of females, respectively (P = 0.003) (Figure 2-7). Mean age at the start of regular smoking was 17.5 (SD ±4.4). Our patients smoked for an average duration of 22.8 (SD ±13.4) years with average smoking intensity of 18.7 (SD ±12.5) cigarettes per day.
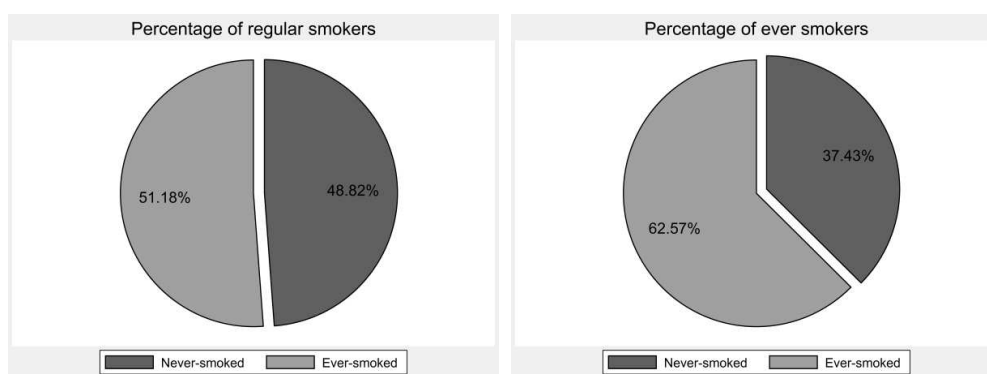
Figure 2-5: Left: percentage of regular smokers. Right: percentage of ever-smokers including patients who has never smoked regularly
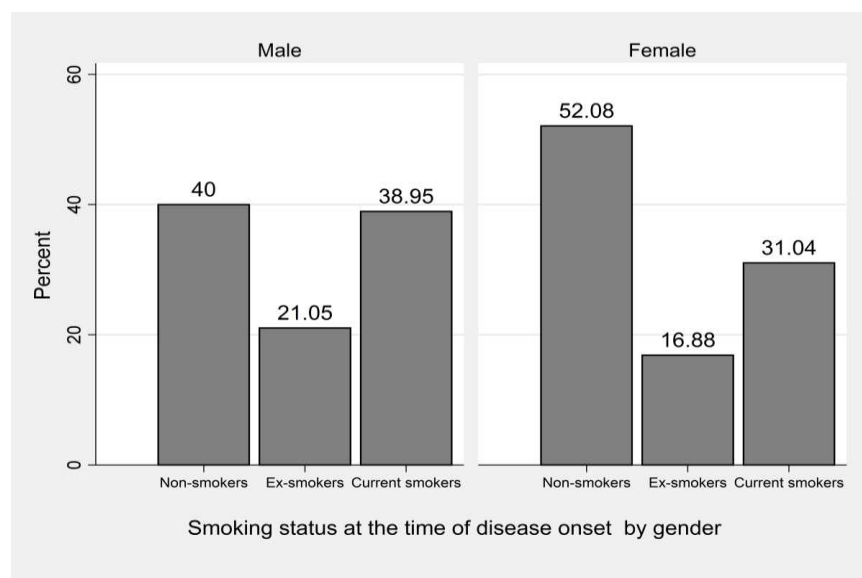
Figure 2-6: percentage of smoking status at the time of the onset of the disease stratified by gender
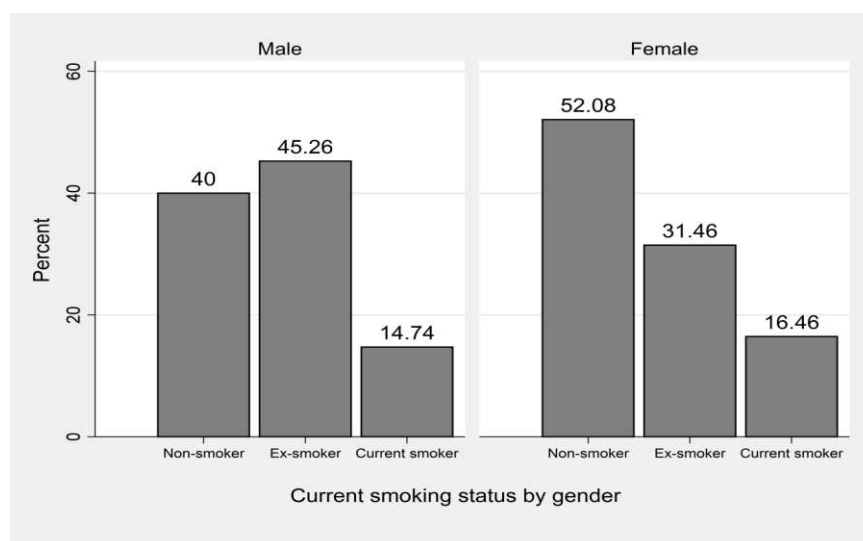
Figure 2-7: percentage of current smoking status stratified by gender

2.9.3.3 Disability

Table 2-3 shows the mean (SD) and median (IQR) of measured disability scores in our sample population. EDSS score and MSSS were available for 1245 patients while PDDS and MSIS-29 were available in 681 cases.

Table 2-3: Summaries of disability and severity scores

	Mean (SD)	Median (IQR)
EDSS	4.94 (±2.1)	6 (3 to 6.5)
MSIS-29	83 (±29.4)	85 (60 to 106)
20-item physical impact scale (questions 1 to 20)	58 (±22.5)	62 (41 to 76)
9-item psychological scale (questions 21 to 29).	24 (±9.4)	23 (16 to 31)
PDDS	3.93 (±2.3)	4 (2 to 6)
MSSS	4.91 (±2.6)	5.09 (2.4 to 7.1)

EDSS: expanded disability scale status
IQR: interquartile range
MSIS-29: multiple sclerosis impact scale
MSSS: multiple sclerosis severity score
PDDS: Patient Determined Disease Steps
SD: standard deviation

2.10 Comments

The aim of this chapter was to give an introduction to our cohort setting and characteristics. However, some very interesting facts were found when data was being analysed. Our study of the prevalence of asthma in patients with MS and its comparison with the prevalence rate of the England general population is the largest study of its kind to date. Here we found that the prevalence of asthma in patents with MS is not significantly different from that of the England general population. Our finding contradict the results from a previous survey in our centre which showed a significantly higher prevalence of asthma in MS patients (Edwards and Constantinescu 2004) and the results from a survey in Wales which showed reduced prevalence of asthma in patients with MS (Tremlett, Evans et al. 2002). However, our result is compatible with the results from the meta-analysis by Monteiro and colleagues (Monteiro, Souza-

Machado et al. 2011). The exact reason for this discrepancy needs further investigations but matching strategies and characteristics of both cases and controls may be responsible for this in part. The significant lower prevalence rate of asthma in our MS population compared with the England general population before matching for age and sex ($P = 0.02$) was disappeared when patients and controls were matched for age and sex. This very clearly shows the influence of demographics and in particular age on the results. Nevertheless, use of DMTs and steroids in MS patients should be considered as these drugs and in particular IFN-β can alleviate asthma symptoms (Traynor, Alexander et al. 2003).

Tobacco smoking in our MS population showed some unique characteristics. The percentage of current smokers at the time of the disease onset was 39% in males and 31% females. This seems significantly higher than what would have been expected if the percentage was similar to that of England general population. Potential contributory rule of smoking on the risk of MS will be investigated in a matched case control study in the following chapter.

Chapter three: Smoking and MS. Effects on the occurrence of MS

3.1 Summary of the chapter

Epidemiologic studies point toward an influence of tobacco smoking on the risk of MS. In this chapter we present our finding from a matched case-control study with regard to the potential effects of individuals' and parental smoking on the risk of MS. Our main objectives here were to identify whether parental smoking during childhood and individuals' smoking later in life have any influence on MS susceptibility.

In the **section 3.2** a comprehensive review of the previous literature is presented.

Section 3.3 describes our methodology and our approach for conducting a matched case-control study.

Our findings are presented in **section 3.4** and the next section, **section 3.5** contains a brief discussion on the influence of tobacco smoking on the risk MS.

3.2 Background

Early Research:

The association between cigarette smoking and the risk of MS development was first suggested by Antonovsly et al. in 1965 (Antonovsky, Leibowitz et al. 1965). In their retrospective case-control study, ever smoking (prior to disease onset) was associated with an increased risk of developing MS (OR = 1.4, P < 0.02) among ever smokers (n = 106) compared with never smokers matched for age, sex and region of birth. This represented the first positive results on the influence of smoking on MS risk, but, in view of the lack of confidence intervals and a small sample size it was hard to scientifically rule out the possibility of some inaccuracies.

In 1966 an investigation in North England by Simpson et al. (Simpson, Newell et al. 1966) raised the possibility of a gender effect in terms of disease susceptibility and smoking. Simpson suggested that female smokers have a higher risk of developing MS than male smokers. The study was carried on 584 "probable" MS cases (233 males and 351 females) and found no difference between the intensity of smoking in cases compared with age-matched controls.

In 1993 a retrospective case-control study was performed in the UK by Villard-Mackintosh et al. (Villard-Mackintosh and Vessey 1993) as part of a study investigating the association of oral contraceptive pills with the risk of MS (Oxford Family Planning Association Study). This incident case study on 63 new MS patients (female only) found a borderline significant association between the intensity of smoking and the risk of MS (P = 0.05). However, the

suggested relative risk of 1.8 (95% CI: 0.8 to 3.6) for current smokers (more than 15 cigarettes per day) and the relative risk of 1.5 (95% CI: 0.6 to 3.3) for ex-smokers compared with non-smokers were not significant. The study was repeated after five years (the Royal College of General Practitioners' Oral Contraception Study) on the 114 incident MS cases, yielding almost similar and no significant results (Thorogood and Hannaford 1998).

In 2001, significant results on the association of tobacco smoke and MS risk was suggested in a case-control study (education-, age- and sex-matched) by Ghadirian et al. (Ghadirian, Dadgostar et al. 2001). In their study, data from a year prior to MS diagnosis were collected from 197 incident MS subjects from Montreal. Data analysis showed an odds ratio of 1.6 (95% CI: 1.0 to 2.4) for ever-smokers compared with never smokers. A significant trend between the number of cigarette consumption and the risk of MS was also suggested where it was shown that compared with never-smokers, the risk of MS for cases who smoked 20-40 cigarettes per day was almost twice with an odds ratio of 1.9 (95% CI: 1.2 to 3.2) and was even higher for cases with intensity of > 40 per day with an odds ratio of 5.5 (95% CI: 1.7 to 17.8). However, results from this study should be interpreted cautiously as the large confidence interval (1.7 to 17.8) means that there is a large uncertainty about the true value perhaps due to the small numbers of cases with smoking consumption of more than 40 cigarettes per day. The study also used the smoking data from a year before the MS diagnosis while it is well-known that in many of MS patients, disease onset occurs several years before the diagnosis date.

Data from two on-going cohorts of US female nurses (the Nurses' Health Study (NHS) and the Nurses' Health Study II (NHS II)) were examined by

Hernan et al. (Hernan, Olek et al. 2001) aiming to find an association between tobacco consumption and risk of MS. Of 121,700 female nurse registered in the 1976 cohort, and of 116,671 in the 1989 cohort overall 315 incident cases of MS were identified (181 cases in the NHS (127 definite and 54 probable) and 134 (103 definite and 31 probable) in the NHS II). After adjusting for age, statistical analysis showed an increased risk of MS in both cohorts for ever-smokers compared with never-smokers with a pooled relative ratio of 1.6 (95% CI: 1.2 to 2.1). Furthermore, the study revealed a borderline significant level ($P = 0.05$) of the MS risk increased by the number of cigarettes consumed. Repeated analyses with definite cases of MS further increased the pooled relative risk of 1.4 (95% CI: 1.0 to 2.0) for ex-smokers to 1.9 (95% CI: 1.3 to 2.8) for smokers of ≥ 25 pack-years. However, the study was limited to females only.

The early epidemiologic studies investigating the influence of smoking on the occurrence of MS encountered major limitations. Small sample size, restricted demographic characteristics (females only) and lack of appropriate matching approaches are amongst some of the limitations of these earlier studies. In addition many of these surveys were conducted when the MRI as the main MS diagnostic tool was not routinely available and hence the diagnosis may have been subjected to bias. The quality of studies conducted after year 2000 has been substantially improved with fewer limitations and improved methodological approaches. These studies are presented below.

Recent Findings:

The association between cigarette smoking and risk of MS has been the focus of several case-control and population based studies after year 2000. A large population based study by Riise et al. (Riise, Nortvedt et al. 2003) in 2003 in Norway showed higher risk of developing MS (self-report diagnosis) for ever-smokers compared with never-smokers with a rate ratio of 1.81 (95% CI: 1.1 to 2.9; P = 0.014).

In 2005, in the second attempt, Hernan et al. (Hernan, Jick et al. 2005) in a prospective nested case–control study of 201 definite MS cases and 1913 age- and sex-matched controls found an odds ratio of 1.3 (95% CI: 1.0 to 1.7) for ever-smokers compared with never-smokers. The risk was found to be similar for both RR MS and PP MS.

The first meta-analysis investigating the association of smoking and MS development risk was undertaken by Hawkes et al. (Hawkes 2007). Their pooled analysis of six qualified previous studies (two included 100% women) indicated a risk ratio of 1.24 (95% CI: 1.04 to1.48) for the increased risk of MS after smoking.

In 2009, Jafari et al. (Jafari, Hoppenbrouwers et al. 2009) conducted a family based matched case control study in order to assess the influence of smoking on the risk of MS using unaffected siblings as controls in multiplex MS families. Analysis of 136 MS patients from 106 multiplex MS families compared with their 204 unaffected siblings showed no significant risk of MS for ever-smokers compared with never-smokers (OR 1.09; 95%CI: 0.68 to 1.73). Although the overall differences were not significant, the study found

slightly higher risk of MS in the groups with more smoking consumption, longer smoking duration and also in female patients. The study offered enhanced genetic and environmental matching of controls, due to using siblings from the same family as the control group. However, one important limitation of such studies is the presence of similar smoking behaviours within families. The findings of this study are of particular importance as they may point to a stronger genetic or environmental confounder(s) overriding smoking effects.

Role of Nicotine:

The role of nicotine, as the major component of tobacco smoke, in the development of MS was questioned in some studies. In one of the studies undertaken by Hedstrom et al. in 2009 (Hedstrom, Baarnhielm et al. 2009), the risk of MS by using tobacco and/or Swedish snuff (smokeless tobacco) was assessed in the patients with clinically definite MS. The study population was comprised of 902 MS cases and 1,855 age, sex and residential area matched controls from Sweden. As expected, the study suggested an odds ratio of 1.5 (95% CI: 1.3 to 1.8) for ever-smokers compared with never-smokers. Interestingly, a protective effect of Swedish snuff and decreased risk of MS was found in the snuff-takers of more than 15 years who had never-smoked (OR: 0.3; 95% CI: 0.1 to 0.8; $P = 0.02$). These findings were confirmed in a study by Carlens et al. a year later in 2010 and a more recent study by Hedstrom and colleagues in 2013 (Carlens, Hergens et al. 2010; Hedstrom, Hillert et al. 2013).

Further experimental studies have also supported the neuroprotective properties of nicotine. It has been shown that treatment with nicotine can significantly reduce the disease activity and inflammation in EAE (Naddafi, Reza Haidari et al. 2013). The pathway in which nicotine enters the body and the form of used tobacco, in this case smokeless tobacco, seem to be important factors when the association of smoking and MS risk is questioned.

Interaction between smoking and other risk factors

Since both genetic and environmental factors including smoking displayed low or at best modest associations with MS risk, the hypothesis of gene-environmental interaction was tested in 2010 when combined effects and potential interactions of three well-known risk factors for MS; smoking, EBV exposure (as assessed by anti-EBNA antibodies), and HLA-DRB1*1501 were assessed by Simon et al. (Simon, van der Mei et al. 2010). 442 cases and 865 controls in this study were those from three previous case control studies. While the anti-EBNA titers were significantly higher in ever-smokers with MS, the risk of MS for ever-smokers was only significant among the cases with high anti-EBNA titers. The study also suggested that smoking is unlikely to influence the association of HLA-DR15 and MS risk, a result which is in contrast with the results from a study by Hedstrom et al. (Hedstrom, Sundqvist et al. 2011) in which a significant interaction between smoking, HLA-DR15 and risk of MS was found. In this case control study (843 cases, 1209 controls) undertaken in Sweden, the potential interaction of smoking and two human leukocyte antigen genes, presence of DRB1*15 and absence of A*02, was

assessed. The odds ratio for ever-smokers compared with never-smokers in the group with neither of genetic risk factors was similar to those from previous studies (OR: 1.4; 95%CI: 0.9 to 2.1)). Non-smokers with both genetic risk factors were 4.9 (95%CI: 3.6 to 6.6) and ever-smokers with both genetic risk factors were 13.5 (95%CI: 8.1 to 22.6) times more likely to have MS compared with never-smokers with neither of the genetic risk factors.

In 2011 Palacios and colleagues (Palacios, Alonso et al. 2011) compared the gender rate ratio of MS incidence with that of smoking data. They showed that the gender ratio of MS is correlated with the gender ratio of smoking and that smoking is one of the factors responsible for the difference in female:male ratio of MS. The study compared the cross-country data from each country birth cohorts and smoking statistics (in depth for Canada and Denmark). Under the assumption that both males and females have equal increase in the risk of MS an overall incidence rate ratio of 1.50 (95% CI: 1.17 to 2.01) of MS for ever-smokers was suggested in the cross-country data analysis.

Summary:

In summary, there is ample epidemiological evidence that tobacco smoking is a significant risk factor in the development of MS. However, it should be noted that smoking may mark out a certain group of population with an increased risk to develop MS by means of lifestyle. In this regard, the validity of the association is not supported by the relationship between other diseases known to be related to smoking such as lung cancer and risk of MS. Surprisingly, it has been found that the risk of lung cancer is reduced in MS patients (Handel,

Joseph et al. 2010). The recent study by Riise et al. (Riise, Kirkeleit et al. 2011) on the Norwegian workers gave weight to the conclusion, reached in some but not all the previous studies, that smoking itself in fact explains the higher risk of MS . The study found a marked inverse association between the level of education and the risk of MS. Their statistical analysis showed a rate ratio of 0.43 (95% CI 0.27 to 0.66) for workers with a graduate degree compared to workers with elementary school only. They could also find an inverse association between the level of education and the risk of colorectal cancer, bronchus/lung cancer and mortality due to vascular diseases for all of which smoking is a risk factor. To enable a better interpretation of the association between education and the risk of MS the study data were compared to the data from the general population and it has been shown that in 2009 only 7% of those with a graduate education were regular smokers, compared to 26% of those with only an elementary school education. Hence, smoking may explain much, although not all, of the association between MS and education. Table 3.1 contains the summery of some of the studies reviewed here.

The exact mechanism in which smoking alters the immune system is not clear. Chronic exposure to tobacco smoke has been shown to alter a wide range of immune functions including reduction and inhibition in production of proinflammatory cytokines (TNFα- , IL-1, IL-6, IL-8) (Chen, Cowan et al. 2007; Mortaz, Lazar et al. 2009). Chronic exposure to tobacco smoke has also been shown to be associated with Th17 and Treg imbalance in mice (Wang, Peng et al. 2012) and patients with Psoriasis (Torii, Saito et al. 2011). Data with regard to the effects of smoking on the development of some autoimmune

conditions such as rheumatoid arthritis is strong. Although not entirely known, possible mechanisms include the ability of tobacco smoke to augment auto-reactive B cells, stimulation of the proliferation of peripheral T-lymphocytes (Kingwell, Marriott et al. 2013) and production of free radicals.

In the current work we examined the hypothesis of smoking being a risk factor for MS in our sample population and investigated whether parental smoking during childhood can increase the risk of MS.

Table 3-1: Summary of some of the studies investigating the association of cigarette smoking and risk of MS

Author/Year	Sample (Case/Control)	Odds Ratio (OR) or Risk Ratio (RR)	Study Type
Antonovsly/1965	241/61	1.4 OR (95% CI: 1.05 to1.86)	Retrospective, case-control
Simpson/1966	584/ 1958	Not stated	Case-control
Villard-Mackintosh/1993	63/-	1.8 (95% CI: 0.8 to 3.6)	Prospective cohort incident study
Thorogood/1998	114/56	1.2 RR (95% CI: 0.8 to 1.8)	Prospective, cohort incident cases.
Ghadirian/2001	197/202	>15/day 1.4 RR (CI: 0.9 to 2.2) Ever smoked 1.6 OR (CI:1.0 to 2.4)	Incident case-control
Hernan/2001	315 / 128,638	1.6 RR (95% CI: 1.2 to 2.1)	Prospective cohort incident study
Riise/2003	87/23,312	1.81 RR (95% CI 1.1 to 2.9).	Case-control population base
Hernan/2005	201/1,913	1.3 OR (95% CI: 1.0 to 1.7)	Prospective nested case–control study,
Mikaeloff / 2007	129/1038	2.12 (95% CI: 1.43 to 3.15)	Population-based, case-control study
Hawkes / 2007	---	1.24 RR (95% CI: 1.04 to 1.48)	Meta-analysis
Sundstrom/2008	109/208	2.9 OR (95% CI: 1.3 to 6.3).	Matched case-control study
Jafari/2009	136/204	1.09 OR (95% CI: 0. 68 to 1.73)	Family-based matched case-control study
Hedstrom/2009	902 / 1,855	1.4 OR (95% CI: 1.2 to 1.7) for Male 1.8 OR (95% CI 1.3 to 2.5) for Female	Population-based case-control study
Simon/2010	442 / 865	1.7 OR (95% CI: 1.1 to 2.6) among those with high anti-EBNA titers	3 case-control studies and a nested case-control
Handel/2010	3,052 / 457,619	1.48 RR (CI 1.35 to 1.63)	Meta-analysis
Carlens/2010	214 / 277,777	1.9 RR (95% CI, 1.2 to 3.1)	Cohort incident case-control study
Alonso/2011	394/394	Female: 6.48 OR (95% CI:1.46 to 28.78) Male 0.72 OR (95% CI: 0.31 to 1.68)	Case–control study
Palacios / 2011	---	1.50 OR (95%CI: 1.17 to 2.01)	Cross-Country and within-country birth cohort analysis
Hedstrom/2011	843/1209	1.4 OR (95%CI: 0.9 to 2.1) 4.9 OR (95%CI: 3.6 to 6.6) 13.5 OR (95%CI: 8.1 to 22.6)	Case-control Study
Sundqvist/2013	552/625	1.30 OR (95%CI: 1.03 to 1.64)	Case-control Study

3.3 Methodology

In order to measure the influence of tobacco smoking on the risk of MS occurrence, we conducted a case-control study with cases recruited from the Nottingham MS registry. In anticipation of including controls from a population-based data, questions from the Health Survey for England 2010 questionnaire were included in the study questionnaire booklet (questions; 6, 6.6, 6.7, 7.5 and 7.6).

Obtaining controls from a population-based data not only increased the accuracy of our estimates, but also reduced the efforts and costs of finding exact matches for a large number of patients. Doing this, we ensured that identical questions were answered by both cases and controls in our study. In the questionnaire patients were specifically asked whether they have ever smoked tobacco product (cigar, cigarette and pipe) and also whether their father and/or mother did smoke regularly during their childhood.

3.3.1 Study population

Study population included patients with definite diagnosis of MS with complete detailed smoking history obtained via the questionnaire booklet and population-based matched controls (matched for age, sex) randomly obtained from the participants in the Health Survey for England 2010. We aimed to match two controls for each MS case from the England general population. Analyses were conducted while controlling for area of residence. Area of residence was defined as the East Midlands health authority.

3.3.2 Statistical analysis

The association of individuals and parental tobacco smoking with MS occurrence was estimated using conditional logistic regression for matched case–control data. Using identification number and conditional logistic regression model enabled us to match each case of MS to its exact age and sex controls. A logistic regression model was also used when matching for residential area as not enough healthy controls were available for matching (only 1350 controls from East Midlands were available from the Health Survey for England 2010). This logistic regression model was adjusted for sex, age and residential area. Multivariable analysis was performed after initial models for each of the confounders. Models were controlled for sex, age and area of residence. In the models investigating the association of parental smoking and MS occurrence, individual's smoking history was also taken into account. All statistical analyses were performed with Stata 11 (StataCorp. 2009. Stata Statistical Software: Release 11. College Station, TX: StataCorp LP).

3.4 Findings

A total of 676 cases of MS with fully detailed smoking history with 1349 exact age and sex matched controls were included in the model (Table 3-2). Ten patients had started smoking after the onset of MS and, were excluded from further analysis.

Table 3-2: Mean age and number of cases and id matched controls by gender.

		Male	Female	Total
Control	Number	386	963	1349
	Age (mean)	54.55	52.30	52.94
Case	Number	193	483	676
	Age (mean)	54.55	52.26	52.93

3.4.1 Individual smoking

Compared with MS patients, controls started smoking at slightly but significantly (P = 0.02) older age. The average age at the start of smoking was 17.5 in controls compared with 16.8 in MS patients. More than half (51.1%) of the MS patients reported a history of regular smoking compared with the 37.1% of the randomly selected controls in the general population ($\chi^2 = 48.8$, P < 0.001). The percentage of ever-smokers including those who had never smoked regularly was also significantly higher in our MS population compared with the controls (62.6% vs. 42.7, $\chi^2 = 100.8$, P < 0.001).

As expected, females were more likely to develop MS (OR: 1.94, 95%CI: 1.62 to 2.32, P < 0.001). We found that regular smokers were 64% (OR: 1.64, 95%CI: 1.35 to 1.99, P < 0.001) more likely to develop MS than non-smokers. Ever-smoking (including non-regular smokers) was associated with 44% (95%CI: 1.19 to 1.74, P < 0.001) increase in risk of MS. When controls were limited to the East Midlands health authority the MS risk was increased to 2.13 (95%CI: 1.65 to 2.75, P < 0.001) for regular smokers and 2.14 (95%CI: 1.73 to

2.65, P < 0.001) for ever-smokers. Of note, the latter estimates are based on non-matched controls and with fewer numbers of individuals.

3.4.2   Parental smoking during childhood

We found no association between parental smoking during patients' childhood and risk of MS occurrence. Parental smoking showed a significant influence on our patients smoking habits. MS patients were 52% (OR: 1.52, 95%CI: 1.11 to 2.08, P = 0.008) and 51% (OR: 1.51, 95%CI: 1.10 to 2.07, P = 0.009) were more likely to become regular smokers if the father or mother smoked regularly during the subjects childhood, respectively. The risk was further increased to 85% (OR: 1.85, 95%CI: 1.26 to 2.73, P = 0.002) when both parents smoked regularly during the subject's childhood. In our age and sex matched case-control population, the risk of MS development (accounting for individual's smoking status) was 0.88 (95%CI: 0.73 to 1.07, P = 0.22) if the mother smoked regularly during the subjects' childhood. Father's regular smoking during subjects' childhood showed protective effect against developing MS (OR: 0.79; 95%CI: 0.65 to 0.96, P = 0.02), however became insignificant when the model was controlled for mother's smoking status (OR: 0.82; 95%CI: 0.67 to 1.01, P = 0.07).

3.5 Discussion

This large UK-based cohort study found and confirmed that tobacco smoking is associated with a significant increased risk of MS onset. No evidence of an association between exposure to parental smoking during childhood and MS

occurrence was found. The lack of association with exposure to parental smoking during childhood may indicate that second hand exposure is unlikely to represent a significant risk of MS amongst offspring of smokers. Our results contradict with the results from the previous case–control study by Mikaeloff and colleagues (Mikaeloff, Caridade et al. 2007) which found a positive association between parental smoking at home and risk of childhood-onset MS. We could not investigate the influence of tobacco smoking on the risk of childhood-onset MS as only 17 individuals in our population had their first attack of MS before age 18 years. A case-control study by Montgomery and colleagues (Montgomery, Bahmanyar et al. 2008) found no association between MS risk and maternal smoking during pregnancy. This is somehow in line with the results from our study as many mothers who have smoked during pregnancy are likely to continue to smoke after pregnancy, during their offsprings' childhood (Cnattingius, Akre et al. 2006; Janson, Kunzli et al. 2006). While the association of individuals' smoking and risk of MS has been extensively established, surprisingly none of the two previous studies investigating the role of parental smoking in MS onset has controlled for the significant contributory role of individuals' smoking habits. Parental smoking may play an indirect behavioural role in the development of MS as children of smokers are more likely to initiate smoking later in life (Hill, Hawkins et al. 2005). In our analysis father's smoking showed some protective effects against MS when tested alone. We could not find any explanation for this but the effects diminished when the model was controlled for mother's smoking status. Indeed, better measures of parental smoking are required to systematically rule out the potential effects of parental smoking in MS.

Another case-control study by Hedstrom and colleagues (Hedstrom, Baarnhielm et al. 2011) has found that exposure to environmental tobacco smoke increases the risk of MS by 30% (95%CI: 1.1 to 1.6). These findings are in contradiction with our results. Demographic differences may explain the discrepancy.

Our results here with regard to individuals' smoking are consistent with the previous studies showing modest role of tobacco smoking on MS risk. It seems, though currently it is not fully understood, that the timing and type of exposure to tobacco smoke is an important factor in the risk of MS (Salzer and Sundstrom 2013).

The selection of controls is a major challenge for conducting a case-control study. We chose our controls from the England general population, which gave us the ability to choose two exact age and sex matched controls for each case. A potential bias may result from the 50% non-respondent rate among cases as our non-respondents were from more deprived areas which are often associated with higher smoking prevalence. Therefore, our risk estimates here may be lower than the actual estimates. However, non-respondents had similar baseline characteristics to the cases used in our study with no statistically significant differences in age and sex distribution (see Table 2-1). Nevertheless, the general demographic and clinical features of our cohort of respondents were similar to those reported from other MS cohorts. For example with regard to the disease phenotype, 58% of our bout onset patients had transited to SP MS after median 20 years of follow-up which is almost similar to the reported percentage of 66 (after median 23 years of follow-up) in the London Ontario cohort (Scalfari, Neuhaus et al. 2010). Slightly more than

10% of our patients were diagnosed with PP MS which was also consistent with the reports from other MS cohorts (Kingwell, van der Kop et al. 2012). (Table **2-1**). The basis of the underlying mechanism between smoking and MS risk is unclear but various mechanisms have been postulated. These include increase risk of infection through immune suppression or stimulation amongst smokers and elevation of nitric acid (Hernan, Jick et al. 2005). Some studies suggested a dose-response effect of smoking on MS risk (see introduction). Our estimated odds ratios in the current study showed higher risk for regular smokers compared with ever-smokers including those who have never smoked regularly. This shows the potential influence of duration and/or intensity of smoking on the risk of MS.

It is very unlikely that tobacco smoking is the only factor playing a role in the complex aetiology of MS. The magnitude of the effects of smoking on MS risk is modest, which may indicate potential interactions between tobacco smoking, other environmental factors and genetics. For example, smoking has been shown to be associated with higher levels of Epstein–Barr virus antibodies (Nielsen, Pedersen et al. 2007) and genetic susceptibility (the presence of HLA DR15*15 and absence of HLA-A*02) has been shown to influence risk on MS (Hedstrom, Sundqvist et al. 2011).

3.6 Conclusion

In this case-control study we found that individuals' tobacco smoking but not parental smoking during childhood is associated with increased MS susceptibility. A dose response effect may also exist.

Chapter four: smoking and MS. Effects on disability progression and disease severity

4.1 Summary of the chapter

Smoking is an avoidable exposure that as shown in the previous chapter and previously has been linked with an estimated 50% increased risk of developing MS. However, it is not entirely clear whether smoking also influences the clinical course of the disease. The few studies addressing this issue having yielded conflicting results. Hence, it is important to identify the problem and design appropriate strategies.

Our main objective here was to identify whether patients smoking habits have any influence on the clinical outcomes of MS.

In the **section 4.2** a comprehensive review of the previous literature is presented.

Section 4.3 describes our methodology and our approach for conducting the study.

Our findings are presented in **section 4.4** and the next section, **section 4.5** contains a brief discussion on the influence of tobacco smoking on the risk of disability progression and higher disease severity.

4.2 Background

Signs and symptoms of MS are vary from time to time and can change in severity and duration. MS can result in physical disability and/or cognitive impairments. Table 4-1 summarises some of the most common signs and symptoms of MS.

Table 4-1: summary of the most common signs and symptoms of MS

Visual	Afferent Pupillary Defect, Optic Neuritis, Diplopia, Nystagmus, Ocular Dysmetria, Internuclear Ophthalmoplegia
Motor	Paresis, Paraparesis, Hemiparesis, Paraplegia, Hemiplegia, Spasticity, Spasms, Cramps, Restless Leg Syndrome, Foot drop, Dysfunctional Reflexes
Sensory	Paraesthesia, Neuralgia, Neuropathic and Neurogenic pain, L'Hermitte's, Proprioceptive Dysfunction, Trigeminal Neuralgia
Coordination and balance	Ataxia, Intention tremor, Dysmetria, Vertigo, Speech Ataxia, Dystonia,
Bowel, Bladder and Sexual	Frequent Micturition, Urgency, Bladder Spasticity, Flaccid Bladder, Erectile Dysfunction, Constipation,
Cognitive and language	Depression, Cognitive dysfunction, Dementia, Mood swings, euphoria, Bipolar syndrome, Anxiety, Aphasia, Dysphasia
other	Fatigue, Uhthoff's Symptom, Sleeping Disorders

Since the pioneer work of Courville in 1964 who first proposed the adverse effects of smoking on MS progression (Courville, Maschmeyer et al. 1964), studies have reached conflicting results regarding the potential influence of smoking on MS clinical course. Measuring the effects of smoking on individuals' health requires careful and accurate measurements of first, the outcomes used in the studies and second: individuals' lifelong smoking

history. Many epidemiologic studies of smoking in MS are subject to two inherent biases that may lead to under- or over-estimation of this association. First is the causality bias which may occur when patients start or stop smoking due to symptoms and second, a healthy smoker bias which suggests that current smokers have better state of health compared with ex-smokers (Montgomery, Hassan et al. 2013). This observation can be explained by the fact that many of ex-smokers probably quit smoking because of the worsening of their symptoms. As a consequence, the remaining current smokers are those who have experienced fewer smoking-related symptoms or worsening of their MS. Hence, the relationship between smoking and MS may be biased by selection of those current smokers which are more healthy and continued to smoke. In addition, epidemiological studies of MS have used variety of different outcomes which toughens reporting any firm conclusion.

In 2005, Hernan and colleagues (Hernan, Jick et al. 2005) investigated the risk of transition to SP MS in 179 cases of clinically definite MS from General Practice Research Database (GPRD) in the UK followed for a median duration of 5.3 years. In their study overall 20 patients (15 smokers and 5 non-smokers) transited to SP MS during the cohort follow up time. Time to the date at the onset of SP MS was assessed using Cox proportional hazard regression analysis which revealed a hazard ratio of 3.6 (95%CI: 1.3 to 9.9) for ever-smokers compared with never-smokers. In contrast with the results from the study by Hernan, in 2007 Koch et al. (Koch, van Harten et al. 2007) assessed the effects of smoking on the transition to SPMS in 364 MS patients in the Netherlands. The study outcomes comprised time to EDSS scores 4 and 6 and development of SP MS. Cox proportional hazard regression model was used to

estimate the risk of developing SP MS and assessing the time to EDSS scores 4 and 6 comparing ever- and never-smoking groups, and could not find any significant influence of cigarette smoking on disease progression in patients with MS. In a study by Di Pauli et al. (Di Pauli, Reindl et al. 2008) in Austria, the risk of developing Clinically Definite MS (CD MS) for smokers was assessed in 129 patients with the diagnosis of CIS. At the end of 3 years of follow up, 44 smokers and 36 non-smokers developed CD MS. Comparison of time to CD MS in the two groups suggested a hazard ratio of progression to CD MS of 1.83 (95%CI: 1.2 to 2.8) for smokers compared with non-smokers (in case all the missing data coded as never-smokers). Ever-smokers also had a higher number of lesions on T2-weighted MRI scans (Hazard ratio = 1.20, 95%CI: 1.10–1.32), but the comparison of EDSS scores after 3 years in the two smoking groups did not show any statistically significant difference (P = 0.9).

Adverse effects of cigarette smoking on the clinical course of the disease were supported in a study by Sundström and colleagues (Sundstrom and Nystrom 2008) which showed that MS patients who ever smoked are more likely to present with the progressive onset MS at the time of diagnosis. In this study risk of progression to SP MS was also compared in 122 incidence cases of MS with three different smoking statuses. They assessed the effects of smoking in patients with an early smoking start age ($\leq$ 15), late start ($\geq$ 15) and never smokers. Higher rate of progressive disease among ever-smokers compared with never-smokers was found which was more prominent in smokers with an early smoking start age. Additionally, the risk of transition to SP MS was

higher among smokers who were also more likely to have PP MS at the time of diagnosis.

The largest study to date assessing the adverse effects of smoking on the clinical course of MS was undertaken by Healy and colleagues in 2009 on 1465 MS cases (Healy, Ali et al. 2009). Despite the significantly higher EDSS and MSSS scores in smokers at the baseline, no sign of any change in EDSS scores was observed. However, weak evidence of higher T2 hyperintense lesion volume (P = 0.02) in MRI scan of smokers compared with never-smokers and higher risk of transition to SP MS (2.5 HR 95%CI: 1.42 to 4.41) for smokers after mean follow up duration of 3.29 years were found. Unfortunately, the authors did not include a control group of smokers without MS to facilitate the assessment of whether smoking has effects on brain imaging measures of smokers without the disease. In a 3-year prospective cohort study in Australia (Pittas, Ponsonby et al. 2009) smoking was positively associated with an increase in MSSS score. It was shown that current smoking is not associated with the possibility of developing a relapse in RR MS patients while smoking was associated with an increased risk of PP MS at MS onset.

Smoking is reported to be associated with some MRI markers of disease activity or progression such as increased in number of contrast-enhancing lesions, number of T2 lesion volume, number of T1 lesion volume, lateral ventricle volume, third ventricle width and decreased brain parenchymal fraction (Zivadinov, Weinstock-Guttman et al. 2009). Evidence of more severe disease (Gholipour, Healy et al. 2011), shorter time to walking aid (D'Hooghe M, Haentjens et al. 2012) and higher relapse rate (Mowry, Waubant et al. 2012) in smokers have also been suggested in some studies.

Almost all of the studies which have investigated the association of cigarette smoking and MS progression suggest adverse influences of smoking on the progression of the disease and accumulation of disabilities. There are still some unsettled questions with regard to the smoking and its effects on MS clinical outcomes. For example, the role of age at the smoking initiation, potential beneficial effects of smoking cessation and the effects of intensity and duration of smoking have not been examined yet. One of the constraints to doing research in MS is the lack of standardised definitions. For the studies suggesting the effects of smoking on the transition to SP MS, identifying and categorising cases correctly is essential to be certain about the results. Most of the previous studies lack proper duration of follow up. The fact that EDSS score did not change in two of the studies may indicate the inability of the studies to identify the influence of the smoking on the disease course when the follow up period is relatively short. This brief review shows that there is a preeminent need for further population based epidemiologic studies. Current evidence seems inadequate to systematically accept the role of tobacco smoke in MS.

The average annual cost to the National Health Service of £30,263 per individual makes MS one of the most costly conditions in the United Kingdom (Kobelt, Berg et al. 2006; Orme, Kerrigan et al. 2007; Manouchehrinia and Constantinescu 2012). Given the fact that MS is a life-long chronic disease and with estimated prevalence rates between 84 and 203 per 100,000 population in the United Kingdom alone (Ford, Gerry et al. 1998; Rothwell and Charlton 1998), the impact of MS, in terms of the strain on health services as well as

cost, is considerable. It is therefore important to identify means to prevent the onset, and slow the progression of MS.

Smoking is an avoidable exposure that, as shown here, has been linked with an estimated 50% increased risk of developing MS in case-control studies (Antonovsky, Leibowitz et al. 1965; Ghadirian, Dadgostar et al. 2001; Hernan, Olek et al. 2001; Riise, Nortvedt et al. 2003; Hawkes 2007; Hedstrom, Baarnhielm et al. 2009; Simon, van der Mei et al. 2010; Hedstrom, Sundqvist et al. 2011; Riise, Kirkeleit et al. 2011). However, it is not entirely clear whether smoking also influences the clinical course of the disease, the few studies, discussed above, addressing this issue having yielded conflicting results. The results of a recent meta-analysis of these studies examining the role of smoking in disease progression fell short of statistical significance and showed high heterogeneity (Handel, Williamson et al. 2011). The possible correlation between smoking and disease progression in MS is of particular interest in view of reports on a negative correlation between smoking and some neurodegenerative conditions (e.g. Parkinson's disease (Checkoway, Powers et al. 2002)) and some autoimmune disorders (e.g. Ulcerative colitis (Boyko, Koepsell et al. 1987)). The evaluation of the magnitude of the effect of cigarette smoking on the clinical course of MS may help to determine underlying disease mechanisms and is important, as studies have reported a high percentage of smokers amongst MS patients (Koch-Henriksen, Bronnum-Hansen et al. 1998; Marrie, Cutter et al. 2009). Here we examine the effects of smoking on the disability progression and explore the potential benefit of smoking cessation using data from a well-documented, substantial, clinical cohort of patients with MS.

Table 4-2: Summaries of some of the studies investigating the impact of smoking on disease outcomes

Author/Year	Sample size	Results	Outcome of interest	length of follow-up (mean or median)
Emre / 1992	21 cases / 11 controls	--	--	--
Hernan / 2005	179 cases	3.6 HR (95%CI: 1.3 to 9.9)	progression to SPMS	5.3 years
Koch / 2007	364 cases	0.97 HR (95%CI: 0.65 to 1.46) 1.11 HR (95%CI: 0.63 to 1.97) 0.93 HR (95%CI: 0.66 to 1.33) 0.88 HR (95%CI: 0.61 to 1.28)	age at the progression to SPMS age at the progression to PPMS time to EDSS 4.0 time to EDSS 6.0	--
Di Pauli / 2008	129 cases	1.8 HR (95%CI: 1.2 to 2.8)	Conversion to clinically definite MS	3 years
Sundstrom / 2008	122 cases	2.4 HR (95%CI: 0.96 to 6.0)	Risk for progressive disease	6 years
Healy / 2009	1465 cases	2.5 HR (95%CI: 1.42 to 4.41) P = 0.02 Not Significant	progression to SPMS T2 hyperintense lesion volume EDSS progression	3.29 years
Pittas / 2009	198 cases	0.34 (95%CI: 0.28 to 0.66) 0.41 (95%CI: 0.03 to 0.85) 0.99 (95%CI: 0.41 to 1.58)	increase in mean MSSS 0 to 1 Pack-Year increase in mean MSSS 1 to 2 Pack-Year increase in mean MSSS 2 ≥ Pack-Year	909 days
Zivadinov / 2010	368 cases	P = 0.004 Increased, P = 0.001 Increased, P = 0.009 Increased, P = 0.003 Decrease, P = 0.047 Increased, P = 0.001 Increased, P = 0.023	Increased EDSS score number of contrast-enhancing lesions number of T2 lesion volume number of T1 lesion volume brain parenchymal fraction lateral ventricle volume third ventricle width	Cross-sectional

4.3 Methodology

We used multiple analysis approaches to measure the influence of smoking on the clinical course of MS. Likelihood of developing progressive onset MS, differences in average severity score, time to EDSS score milestones 4 and 6 and also time to SP MS were examined between smoking groups using appropriate statistical models. The effects of age at smoking initiation and number of pack-years smoked ((number of cigarettes smoked per day $\times$ number of years smoked) $\div$ 20)) were also examined.

Descriptive statistics were used to summarise the baseline demographic characteristics and clinical outcomes. We examined the association between smoking and the type of MS at its onset (RR MS or PP MS) using logistic regression models. Linear regression models were used to determine the effects of pack-years of cigarettes smoked on the severity of the disease and level of disability measured by MSIS-29 and PDDS scores. The numbers of pack-years smoked were categorised into three categories of non-smokers, less than 10 pack-years and more than 10 as previously used by Koch and colleagues (Koch, van Harten et al. 2007). Detailed models were adjusted for onset age, sex, type of MS at the onset of the disease (RR vs. PP) and use of treatment. The disease phenotype was stratified into two binary groups of RR MS vs. PP MS. Although it may be interesting to look at differences between SP MS and PP MS in term of the influence of smoking on the progression of the disease, such analysis requires additional data including exact date of transition to SP MS which was not available for all the patients in our dataset. If the SP MS patients were going to be included in the analysis as a separate, bias might have arisen by using post-baseline values for modelling were data was missing

on the date of transition in many of the patients. If the most recent value before end of follow-up (2013) was used this could have introduced bias in estimating the hazard for PP MS and SP MS by lack of data on the date of transition to SP MS in many of the patients. Using the value at baseline was the approach that could eliminate this potential bias.

Although regression models of large sample size are robust to some degree of non-normality (Lumley, Diehr et al. 2002), all the linear models were controlled for homogeneity and distribution of residuals to avoid violation of underlying normality assumption.

Time to two EDSS milestone scores of 4 and 6 and to the onset of SP MS were estimated using the Kaplan-Meier method taking into account participation of those that has not yet reached the events. The smoking-specific rate ratios were calculated using Cox proportional hazard regression models (Cox and Oakes 1984) controlled for sex, onset age (continuous in years), use of DMTs (in a binary group of ≥1 year or <1 year) and initial clinical course of the disease (RR vs. PP). Cox regression models were also used to estimate the rate ratios of reaching MSSS score categories 5 or above (patient progress faster than half of the MS population) between smoking groups. Age is one of the most important factors in accumulation of disabilities in MS and the hazard will significantly change as a function of age. To account for this, patients in the cohort were followed from the date of birth, entered the study at the age at the onset of the disease (left truncation or late entry) and exited at their event/censoring age. This way the impact of age was controlled for more effectively (Korn, Graubard et al. 1997). The final Cox models were checked for proportionality assumption based on the Schoenfeld residuals and were

stratified by the factor violating the proportionality. A comparison of the effects of smoking on the likelihood of patients being in the upper quartiles (MSSS > 7.5) versus lower quartiles (MSSS < 2.5) of the MSSS spectrum was made using logistic regression model to ensure the robustness of the results when using MSSS as an outcome of interest. The use of extreme ends of the MSSS spectrum allows comparison of those with somehow an atypical disease clinical course compared with the majority of patients. Where possible (due to violation of normality assumptions) we also used MSSS in linear regression models. All statistical analyses were performed with Stata 11 (StataCorp. 2009. Stata Statistical Software: Release 11. College Station, TX: StataCorp LP).

4.4 Findings

Clinical and demographic data were available for 1245 patients. This included 681 patients with detailed smoking history.

4.4.1   Likelihood of progressive onset MS

We could not find any association between smoking and having progressive onset MS (PP MS). While controlling for sex and onset age, risk of developing PP MS was not associated with smoking. Pack-years smoked before the onset of MS was also not associated with the risk of progressive onset MS. As expected, male patients and those with older age at the onset of MS were more likely to develop PP MS (Table 4-3). As seen, each year increase in the age at

the onset of the disease was associated with 9% (95%CI: 7 to 11) increase in the risk of having progressive onset MS.

Table 4-3: Relation between cigarette smoking and progressive onset MS.

	n	Odds ratio (95%CI)	P-value
Smoking (ever vs. never)	1166	0.82 (0.54 to 1.24)	0.36
Smoking* (ever vs. never)	657	0.88 (0.49 to 1.59)	0.68
Pack-years smoked	615	1.00 (0.98 to 1.02)	0.90
Gender (female vs. male)	1166	0.34 (0.22 to 0.52)	< 0.001
Onset age	1166	1.09 (1.07 to 1.11)	< 0.001

* Patients are limited to those with returned questionnaire.

4.4.2 Disease severity

Average MSSS was 4.73 ($\pm$2.6) in our cohort. All the analyses in this section were conducted on data from those patients who returned the questionnaire and had detailed smoking data for calculating pack-years smoked and age at smoking initiation (n = 645). We used the linear regression model to measure the average MSSS differences between groups. Models were controlled for onset age, initial disease course, use of DMT and gender. Ever-smokers had an average 0.5 (95%CI: 0.11 to 0.87, P = 0.01) MSSS higher than never-smokers. Compared with never-smokers, the average MSSS was 0.8 (95%CI: 0.26 to 1.35, P = 0.004) and 0.35 (95%CI: -0.07 to 0.77, P = 0.1) higher in current and ex-smokers respectively. Age at smoking initiation did not influence the disease severity as average MSSS was not different between those who had

started smoking before or after the age of 15 years (Coef: -0.18, 95%CI: 0.79 to 0.41, P = 0.54). The number of pack-years smoked had a significant effect on the average MSSS. Those with pack-years smoked more than 10 had an average 0.62 (95%CI: 0.17 to 1.06, P = 0.006) MSSS higher than non-smokers (zero pack-years). Pack-years smoked from 1 to 10 was associated with 0.26 (95%CI: -0.28 to 0.81, P = 0.34) score increase in the severity of MS compared with non-smokers. Kaplan-Meier method was used to estimate the time to MSSS > 5.0 in different smoking groups. The estimated median time to MSSS > 5.0 from birth was 51 (95%CI: 47 to 55) years in current smokers, 57 (95%CI: 55 to 58) years in ex-smokers and 57 (95%CI: 55 to 59) in non-smokers. Log-rank test for equality of survival function showed significant difference between time to MSSS > 5.0 amongst these smoking groups (P < 0.001) (Figure 4-1).
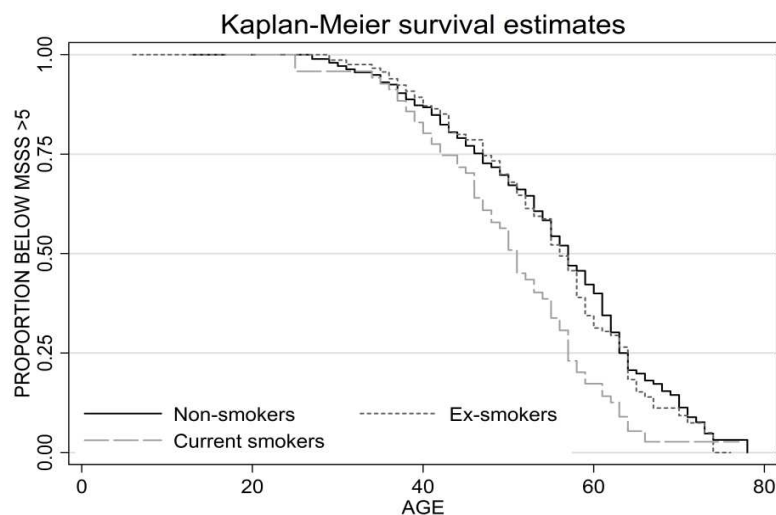
Figure 4-1: Kaplan-Meier estimates shows median time to MSSS > 5 from birth by smoking status

When stratified by smoking status, comparison of two upper and lower quartile MSSS showed significant difference in the proportion of patients in each quartile ($\chi^2 = 10.51$, P = 0.005). While 20% of patients in upper quartile MSSS were current smokers only 10% were current smokers in the lower quartile and 63% of patients in the lower quartile were non-smokers compared with 45% in the upper quartile MSSS. Likelihoods of being in the upper quartile MSSS were obtained from the logistic regression model when controlling for onset age, initial phenotype of the disease and gender (Table **4-4**). Each cigarette smoked per day was associated with an average 0.03 (95%CI: 0.1 to 0.4, P < 0.001) increase in MSSS.

Table 4-4: likelihoods of being in the upper quartile MSSS (MSSS > 7.5) compared with lower quartile (MSSS < 2.5)

	Odds ratio (95%CI)	P-value
Smoking status		
Non-smokers	---	---
Ex-smokers	1.3 (0.7 to 2.42)	0.39
Current smokers	2.88 (1.29 to 6.43)	0.01
Pack-years		
Non-smokers	---	---
1 to 10	1.06 (0.45 to 2.49)	0.88
More than 10	2.17 (1.17 to 4.02)	0.01

Odds ratios obtained from logistic regression models by current smoking status and pack-years of cigarettes smoked.

4.4.3 PDDS and MSIS-29 scores

Median PDDS score was 4 (range from 0 to 8) in our patients. Similar to MSSS, the PDDS score was significantly influenced by patients smoking status. We used multiple regression models to measure the differences between smoking groups in terms of PDDS score while controlling for the usual confounders including onset age, disease duration, sex, initial disease phenotype and use of treatment. Our analysis showed that the average PDDS was 0.41 (95%CI: 0.09 to 0.73, P = 0.01) score higher in ever-smokers compared with never-smokers. Smoking cessation appeared to have beneficial effects. The average PDDS score was 0.71 (95%CI: 0.25 to 1.17, P = 0.002) score and 0.27 (95%CI: -0.07 to 0.63, P = 0.12) score higher in current and ex-smokers compared with non-smokers respectively. Due to the non-normal distribution of PDDS score normality assumption behind the regression models was tested after each analysis (Figure 4-2).
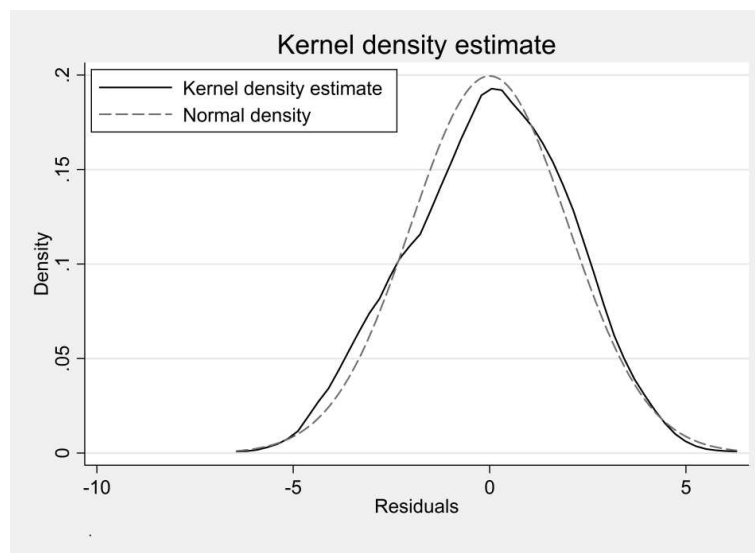
Figure 4-2: the Kolmogorov-Smirnov test for equality of distribution functions in PDDS

Using MSIS-29 as an outcome limited our analysis choices as no linear regression model could be used due to the violation of the underlying normality assumption. Hence, our analysis of MSIS-29 here is limited to a non-parametric test (Kruskal-Wallis one-way analysis of variance) of hypothesis to investigate the differences amongst smoking groups.

Table 4-5 presents median MSIS-29 (range from 29 to 145), MSIS psychological scale (range from 9 to 45) and MSIS physical scale (range from 20 to 100) scores stratified by current smoking status, gender and MS initial clinical course. Detailed analysis of MSIS-29 psychological scale showed higher level of impairments and disability for ever-smokers in all the questions asked (Table 4-6).

Table 4-5: median MSIS-29 scores by smoking status

	Non-smokers	Ex-smokers	Current smokers	P-value *
MSIS-29	77	92	90	< 0.001
MSIS (physical scale)	56	65	67	< 0.001
MSIS (psychological scale)	21	26	28	< 0.001

MSIS-29: multiple sclerosis impact scale
* P-value for the one-way analysis of variance using Kruskal-Wallis test.

Table 4-6: MSIS-29 psychological scale amongst smoking groups.

	Never-smoked		Ever-smoked		P-value *
	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	
Feeling unwell?	2.4 (±1.24)	2 (1 to 3)	2.74 (±1.3)	3 (2 to 4)	< 0.001
Problems sleeping?	2.45 (±1.4)	2 (1 to 4)	2.66 (±1.37)	3 (1 to 4)	0.02
Feeling mentally fatigued?	2.92 (±1.34)	3 (2 to 4)	3.36 (±1.31)	4 (2 to 4)	< 0.001
Worries related to your MS?	2.38 (±1.27)	2 (1 to 3)	2.73 (±1.39)	3 (2 to 4)	0.001
Feeling anxious or tense?	2.44 (±1.28)	2 (1 to 4)	2.78 (±1.34)	3 (2 to 4)	< 0.001
Feeling irritable, impatient, or short tempered?	2.43 (±1.26)	2 (1 to 3)	2.92 (±1.33)	3 (2 to 4)	< 0.001
Problems concentrating?	2.68 (±1.34)	2 (2 to 4)	3.06 (±1.3)	3 (2 to 4)	< 0.001
Lack of confidence?	2.48 (±1.39)	2 (1 to 4)	2.84 (±1.44)	3 (2 to 4)	< 0.001
Feeling depressed?	2.11 (±1.29)	2 (1 to 3)	2.61 (±1.4)	2 (1 to 4)	< 0.001

* P-value from Mann-Whitney test for differences in medians between ever- and never-smokers.

4.4.4 Time to EDSS score milestones 4 and 6

Data needed (including information regarding whether patient has reached the specific EDSS score milestone and if yes at what age) to estimate the time to EDSS score 4 was available in 1026 of 1246 patients (82.3%) and data to estimate the time to EDSS score 6 was available in 1090 (87%). Form 1026 patients, 628 patients had reached EDSS score 4 in median 14 (95%CI: 13 to 15) years after the disease onset and median age of 49 (95%CI: 48 to 50). For time to EDSS score 6, from 1090 patients with available data, 530 patients had reached EDSS score 6 in median 20 (95%CI: 18 to 21) years after the disease onset and median age of 53 (95%CI: 52 to 54).

Amongst those patients with returned questionnaire time to EDSS score 4 could be estimated in 83.3% and time to EDSS score 6 in 87.5% of patients. In this group of patients, 337 patients (of 577) had reached EDSS score 4 and 279 (of 607) had reached EDSS score 6. Table **4-7** summarises the median time to two EDSS score milestones 4 and 6 amongst smoking groups.

We estimated the smoothed hazard of reaching EDSS score 6 in males and females (Figure 4-3), relapsing and progressive onset MS (Figure 4-4) and ever- and never-smokers (Figure 4-5) in all the 1245 patients. Our estimated time to EDSS score 6 from the onset of the disease (20 years (95%CI: 18 to 21)) is comparable with the results reported from Lyon, France and Flemish MS register (Confavreux, Vukusic et al. 2000; D'Hooghe M, Haentjens et al. 2012) with reported median time to EDSS score 6 of 21 years, although it is shorter than the 27.9 years found in British Columbia cohort (Tremlett, Paty et al. 2006) and 28.6 years in Olmsted County, Minnesota (Pittock, Mayr et al. 2004).

Table 4-7: Median times to EDSS score milestones 4 and 6 from the birth and onset of MS by ever- and never-smoking status

	From the disease onset (95%CI)	P-value	From birth (95%CI)	P-value
Time to EDSS score 4				
Never-smoked	15 (14 to 18)		44 (41 to 46)	
Ever-smoked	13 (11 to 14)	< 0.001	38 (16 to 43)	< 0.001
Time to EDSS score 6				
Never-smoked	21 (18 to 25)		50 (48 to 52)	
Ever-smoked	19 (16 to 21)	0.001	43 (16 to 49)	0.01

EDSS: expanded disability scale status
CI: confidence intervals
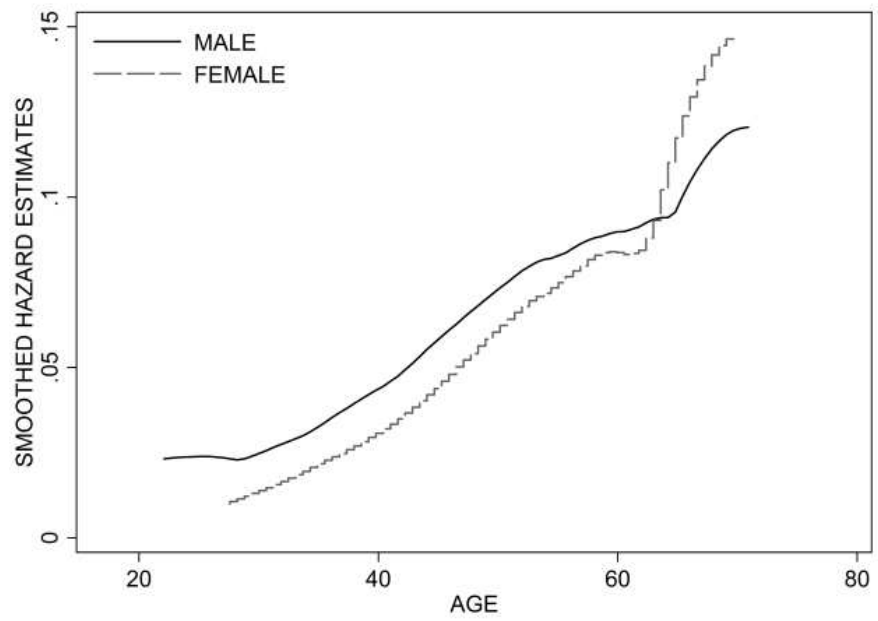
Figure 4-3: Smoothed hazard estimates of reaching EDSS score 6 by gender
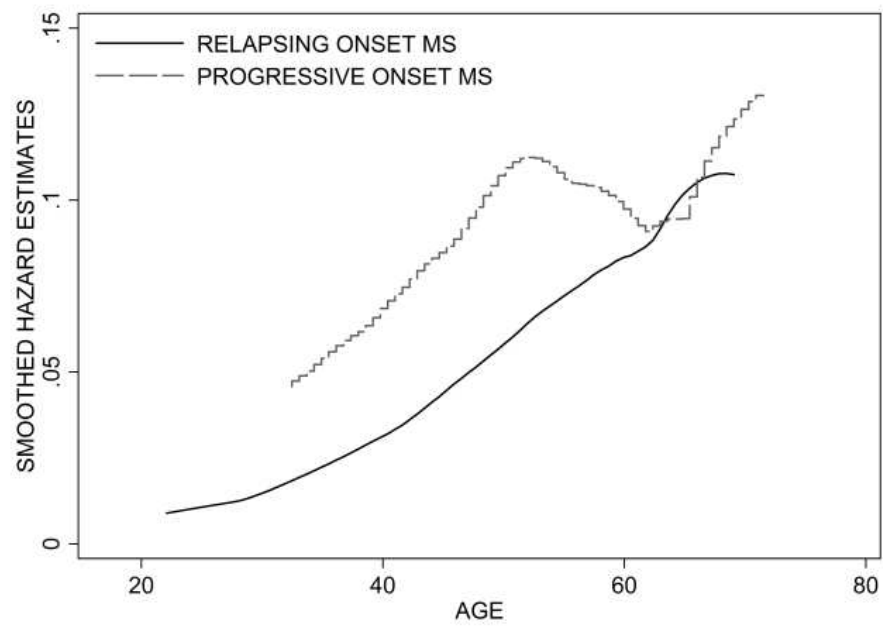
Figure 4-4: Smoothed hazard estimates of reaching EDSS score 6 by MS initial clinical course
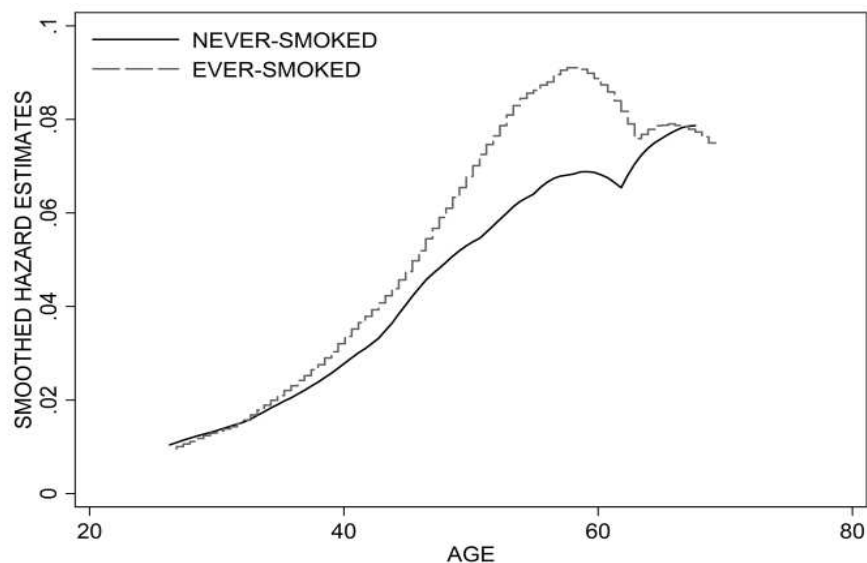
Figure 4-5: Smoothed hazard estimates of reaching EDSS score 6 by patients' life-long smoking status (regular smoking)

Cox proportional hazard regression models were used to estimate the risk of reaching EDSS scores 4 and 6. With the models controlled for onset age, DMT, MS onset phenotype and gender, ever-smoking was associated with 20% (95%CI: 1.00 to 1.42, P = 0.04) increased risk of reaching EDSS score 6 and 32% (95%CI: 1.12 to 1.55, P = 0.001) increased risk of reaching EDSS score 4. Our further analyses of the effects of current smoking status on the risk of reaching the two EDSS milestones were limited to the patients with the returned questionnaire. At the age of EDSS score 4, 20.75% and 30% of the patients were current and ex-smokers respectively. This was not changed significantly at the age of EDSS score 6. At the time of EDSS score 6, 20.45% and 30.3% were current and ex-smokers respectively. The Cox regression models showed that smoking cessation could be beneficial in reducing the risk of reaching EDSS score 4. In our cohort current smokers had 88% (95%CI: 1.43 to 2.48, P < 0.001) higher risk of reaching EDSS score 4 compared with non-smokers. Ex-smokers had no increased risk of reaching EDSS score 4

compared with non-smokers (HR: 0.93, 95%CI: 0.72 to 1.20, P = 0.6). When ex-smokers were stratified to those who gave up smoking before and after the disease onset, smoking cessation after the onset of MS was still beneficial. Risk of reaching EDSS 4 was 14% (95%CI: 0.84 to 1.54, P = 0.37) for patients who gave up smoking after the onset of MS and 12% (95%CI: 0.83 to 1.52, P = 0.44) for those who developed MS after smoking cessation compared with non-smokers. Risk of reaching EDSS score 6 was also influenced by patients' smoking status. Similar to the risk of reaching EDSS score 4, current smokers had 66% (95%CI: 1.17 to 2.35, P = 0.004) higher risk of reaching EDSS score 6. There was no increased risk of reaching EDSS score 6 in ex-smokers (HR: 0.81, 95%CI: 0.58 to 1.12, P = 0.21) whether they quit before or after MS onset (Figure 4-6).
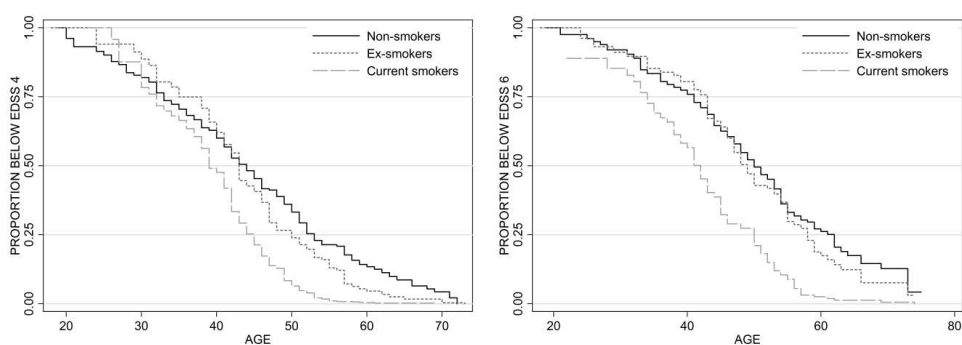
Figure 4-6: Left: Kaplan-Meier graph shows time to EDSS score 4 by patients' smoking status adjusted for durations and intensity of smoking. Right: Kaplan-Meier graph shows time to EDSS score 6 by patients' smoking status adjusted for durations and intensity of smoking.

We then investigated the influence of smoking intensity and time since smoking cessation on the risk of reaching EDSS score milestone 6 while dealing with the smoking duration as a time varying covariate. The intensity of smoking was significantly associated with the risk of reaching EDSS score 6. We found that each cigarette smoked per day was associated with 3% (95%CI:

1.01 to 1.05, P < 0.001) increased risk of reaching EDSS score 6. Each year increase in the time since cessation of smoking was associated with 5% (HR: 0.95, 95%CI: 0.93 to 0.97, P < 0.001) decreased risk of reaching EDSS score 6 (Figure 4-7).
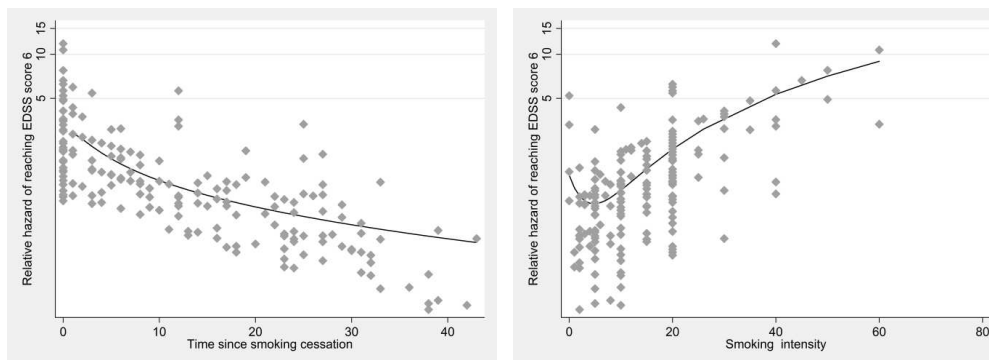
Figure 4-7: Relative hazard of reaching EDSS score 6 plotted against left, time since smoking cessation, right, intensity of smoking

4.4.5   Time to SP MS

Data needed to estimate the time to the onset of SP MS was available in 735 patients (including RR MS patients who had not transited to SP MS). In total 130 patients had transited to SP MS during the study period. Median age at the time of transition to SP MS was 61 (95%CI: 59 to 67) years. We could not find any differences in time to SP MS between ever- and never-smokers (P = 0.58). Also, no influence of ever- or never-smoking on the risk of SP MS was evident when using Cox hazard regression model. After controlling for onset age, treatment and gender, the risk of transition to SP MS was independent of patients' smoking status (HR: 1.16, 95%CI: 0.81 to 1.65, P = 0.39). Further stratification of ever-smokers into current and ex-smokers showed significant effect of current smoking on the risk of developing SP MS. We found that current smokers have 2.38% (95%CI: 1.39 to 4.08, P = 0.001) higher risk of

developing SP MS. The risk of transition to SP MS was not increased amongst ex-smokers compared with non-smokers (HR: 0.9, 95%CI: 0.54 to 1.51, P = 0.71) (Figure 4-8). Pack-years cigarettes smoked also showed a significant impact on the risk of transition to SP MS. In our Cox regression model, each unit increase in the pack-years smoking was associated with 1% (95%CI: 1.001 to 1.02, P = 0.03) increased risk of developing SP MS. Each year increase in time since smoking cessation was associated with 3% decreased risk of developing SP MS (HR: 0.97, 95%CI: 0.95 to 0.99, P = 0.04).
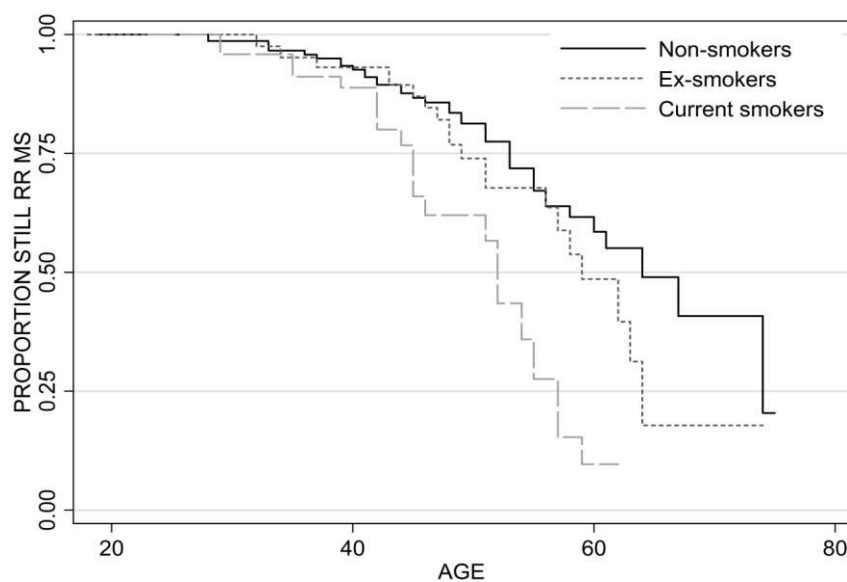
Figure 4-8: Kaplan-Meier graph showing time to SP MS from birth by patients' smoking status adjusted for intensity and duration of smoking

4.4.6 Effects of comorbidity

Comorbidity in MS is complex in terms of diagnosis and classification. In this part of the analysis we intended to only use the data from those patients with no concomitant comorbid condition. The comorbidity data used here was based on the data collected from the patients at the time of diagnosis and/or

their routine clinic follow-ups. In order to validate the comorbidity information in our database, data from 28 (10%) randomly selected patients in our subset analysis of comorbidity was independently rechecked for report of any concomitant comorbid condition using hospital electronic records. From the 28 patients, 21 had no comorbid condition reported, two were using treatment for depression, however, it was felt that depression is secondary to MS. One patient had cholecystectomy in the past, one had psoriasis in the leg which required 2 sessions of phototherapy, one patient had uterine fibroids and two patients had the diagnosis of osteoporosis. Overall, there was more than 80% agreement between the data recorded in the database and those rechecked later.

As expected the prevalence of concomitant comorbid diseases was significantly higher in ever-smokers (58% in ever-smokers vs. 42% in never-smokers, P = 0.002). In order to measure the influence of higher comorbidity prevalence on our outcomes, we compared the average MSSS amongst smoking groups and measured the risk of reaching EDSS score milestone 6 amongst those patients who had no reports of concomitant comorbid conditions which could have interfered with the disability scores.

When the analysis was limited to the patients with no concomitant medical condition, the average MSSS was still significantly higher in ever-smokers compared with never-smokers (Coef: 0.89, 95%CI: 0.46 to 1.32, P < 0.001). Average PDDS score was also significantly 0.77 (95%CI: 0.31 to 1.23, P = 0.001) score higher in ever-smokers compared with never-smokers. 527 patients with available data on time to EDSS score 6 and no concomitant comorbid condition were included in our survival analysis. After stratifying the model by sex and controlling for the disease initial clinical phenotype, onset

age and use of treatment, ever-smokers had 34% (95%CI: 1.02 to 1.75, P = 0.03) higher risk of reaching EDSS score 6.

4.4.7 Correlation between outcomes

Table **4-8** summarises the Spearman correlation coefficient between outcomes used in our study. The highest correlation was seen between EDSS score and MSSS and the lowest were between MSIS-29 and MSSS score. Nevertheless, the impact of smoking was evident on all the outcomes used in our research.

Table 4-8: correlation between outcomes used in our research

	PDDS	EDSS	MSSS	MSIS-29
PDDS	1			
EDSS	0.8616	1		
P-value	< 0.001			
MSSS	0.7054	0.9098	1	
P-value	< 0.001	< 0.001		
MSIS-29	0.7104	0.6262	0.5432	1
P-value	< 0.001	< 0.001	< 0.001	

P-values from Spearman correlation coefficient with Bonferroni adjustment.
EDSS: expanded disability scale status
MSIS-29: multiple sclerosis impact scale
MSSS: multiple sclerosis severity score
PDDS: Patient Determined Disease Steps
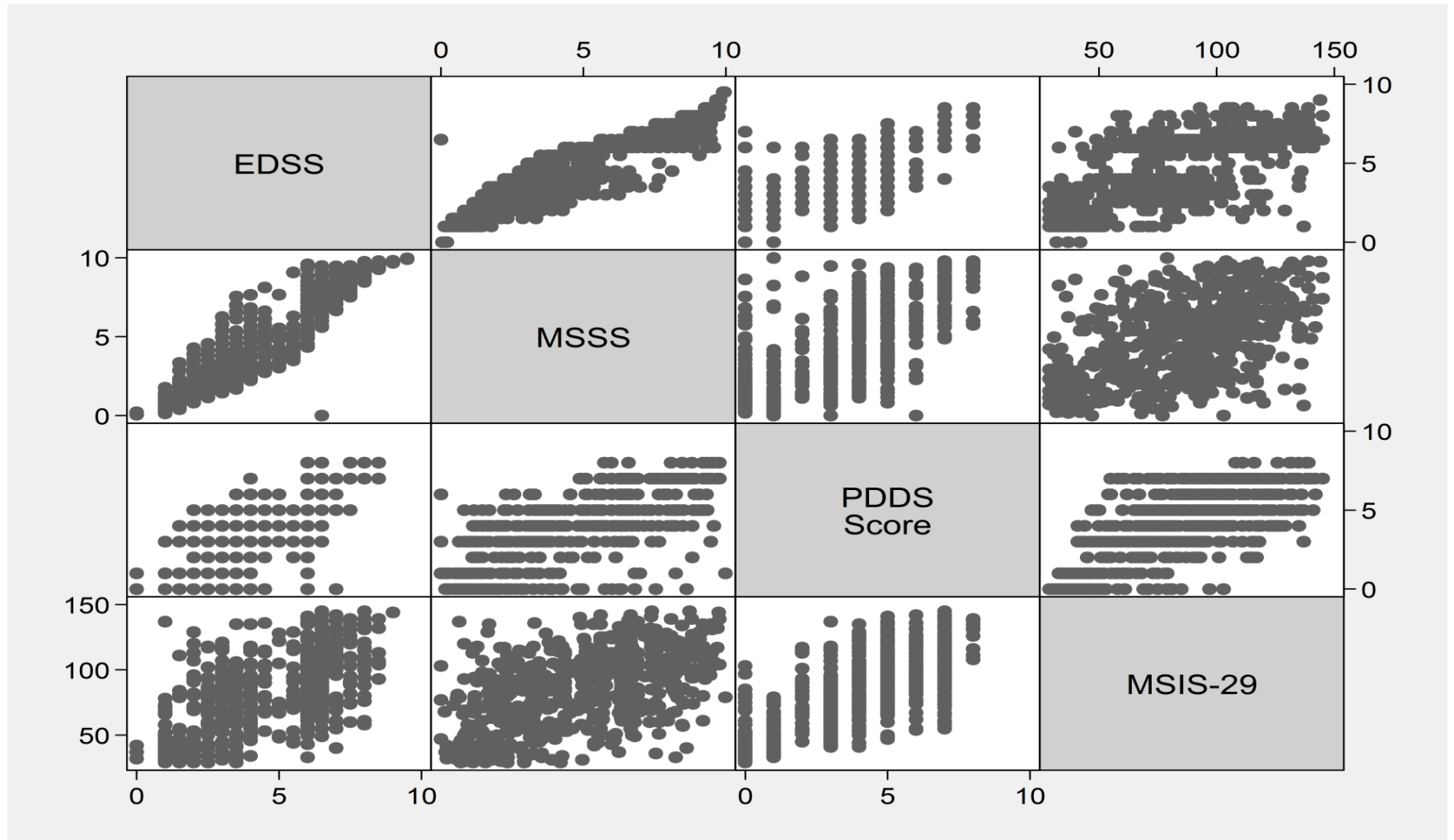
Figure 4-9: Matrix graph showing correlation between outcomes used in our research

4.5 Discussion

In this study, we found that disease progression is more rapid in ever-smokers. Current smoking in our cohort was associated with a significant increase in all of the clinical outcomes (PDDS, MSIS-29, time to SP MS and EDSS scores). Here we present, to our knowledge for the first time, evidence on the potential beneficial effects of smoking cessation on disability progression in MS patients. By using MSIS-29 psychological scale, we also showed that the effects of tobacco smoking on patients' quality of life are beyond physical impairments. Our ever-smokers had significantly higher levels of sleep problems, anxiety, fatigue, etc. We found that ex-smokers have a significantly reduced risk of reaching EDSS score milestones 4 and 6 as well as SP MS compared with current smokers, and that this risk reduction was similar between those who stopped smoking before or after the onset of MS and each outcome. Thus, there are positive effects of smoking cessation on disease progression even after MS onset. Our study provides new, important clinical findings on the influence of tobacco use in a cohort of patients with MS. We used a population of MS patients, all with clinically definite MS confirmed by MS specialist neurologists. Most importantly, our clinic and consequently our cohort, has a population based nature as it is estimated to cover majority of the MS patients in Nottinghamshire and defined parts of Lincolnshire and Derbyshire regions. Our findings are based on diagnosis, identification and classification of patients and like any other study may be subject to bias in ascertainment, recruitment, and misclassification of status. However, the fact that the confirmation of the diagnosis and the disability scores in our study were obtained from a clinical database of MS specialist neurologists increases

the homogeneity and integrity of our results. Thus, our findings are robust, and characteristics such as gender distribution, age of onset and type of MS distribution of our cohort are similar to those reported in most other MS cohorts. Thus our sample population appears to be representative of the MS population at large and our findings can be generalised and are reflective of routine clinical practice. Our study has some limitations that may have influenced our estimates. It is likely that our cohort missed some patients with very severe disease who died before attending our MS clinic.

We believe our study is one of the most comprehensive studies to examine the correlation of tobacco smoking and MS clinical outcomes, with particular emphasis on disability progression. In a study of a United Kingdom population, Hernan and colleagues found a risk ratio of 3.6 (95%CI: 1.3 to 9.9) for transition to SP MS in 179 cases of MS with median 5.3 years follow-up using the General Practice Research Database (Hernan, Jick et al. 2005). In our study we could not find any influence of ever-smoking on the risk of transition to SP MS. What we found was that current smoking was significantly associated with the risk of developing SP MS but not ever-smoking. We also estimated the risk of reaching EDSS score 6 which is a robust outcome measure and almost a surrogate of time to SP MS (Scalfari, Neuhaus et al. 2010) and also allows inclusion of PP MS. Using these outcomes we found a higher risk ratio of reaching EDSS score 6 in smokers, although the risk ratio was lower than the risk ratio reported by Hernan et al. for development of SP MS. While the risk ratios were in the same direction, differences in patient sample size (1245 in our study vs. 179 in the Hernan et al study), longer duration of follow-up (20 vs. 5.3), and number of patients reaching the

outcome (450 vs. 20) may account for the difference. Others have also used time to EDSS 4 and 6 however with no significant evidence of association between cigarette smoking and progression (Koch, van Harten et al. 2007). We believe that greater sample size and longer duration of follow-up makes our estimates more robust. Our results are in accordance with the results from an observation by D'hooghe et al. which showed higher risk of reaching EDSS score 6 amongst occasional and daily cigarette consumers (D'Hooghe M, Haentjens et al. 2012). D'hooghe and colleagues relied on questionnaires for obtaining data on disease onset and self-reported disability scores which may introduce some bias. The advantage of our study is its higher homogeneity in terms of clinical data used such as disease type and EDSS scores which were based on face to face patient examination and recorded by MS specialist neurologists. By comparing the two lower and upper MSSS quartiles we showed that smokers are more likely to have a severe disease course as shown previously (Gholipour, Healy et al. 2011). A higher probability of progressive onset amongst smokers has been observed previously (Sundstrom and Nystrom 2008; Healy, Ali et al. 2009); however, our estimate in a much larger sample showed no evidence that smoking favours a progressive onset of the disease.

Smoking is known to be a significant risk factor for the development and progression of several autoimmune diseases (Prummel and Wiersinga 1993; Saag, Cerhan et al. 1997; Hardy, Palmer et al. 1998; Hudson, Lo et al. 2011) and is a frequently studied health behaviour because of its well-known associations with chronic diseases. Since disease progression is more rapid in ever-smokers, preventing smoking may be important in reducing the progression of MS. Estimating the impact of smoking in terms of costs shows

its relevance. Costs of MS (direct and indirect) can increase by nearly twofold in patients with EDSS score 3.5 to 6 compared with those with EDSS score ≤3, from £7,273£ to £12,875 per patient per year (Kobelt, Berg et al. 2006). In the UK, it has been estimated that each quality adjusted life year gained in MS by means of DMTs costs from £18,700 to £25,500 (Gani, Giovannoni et al. 2008). Based on our results and compared to these figures, preventing or stopping smoking could be an economical strategy and an effective way to improve outcomes in MS which can be implemented along other MS therapeutic approaches.

It is not entirely clear whether this additional increase in impairment and disability in smokers is purely due to the biological influence of tobacco smoking on MS specifically, or is due to other underlying factors such as increase in comorbidities associated with smoking. It has been previously reported that smokers with MS are more likely to report comorbid autoimmune diseases (Marrie, Horwitz et al. 2011). Here we also showed that ever-smokers are significantly more likely to have concomitant comorbid diseases. Comorbidity has been hypothesised to be liable for parts of the progression seen in ever-smokers. We tested the hypothesis by limiting our analysis to the patients with no reported concomitant medical condition. The effects of smoking on the selected outcomes were still present even in the absence of any concomitant medical condition. This finding indicates that tobacco smoking may have direct biological impact on the clinical course of MS. There are lines of evidence that suggest a potential pathophysiological role of tobacco smoke on the progression of the disease in MS. Of note, findings from MRI studies, evidence on the negative impact of smoked tobacco but not moist snuff on risk

of MS as demonstrated by Hedstrom and colleagues (Hedstrom, Baarnhielm et al. 2009) as well as a significant association between smoking intensity and disease severity (dose-response rate) as demonstrated in our study may suggest a direct impact of tobacco smoke on MS progression. Nevertheless the possibility of an indirect impact of smoking on MS progression, or a combination of direct and indirect effects cannot be excluded. There are some likely biological explanations for a mechanistic pathway between smoking and disability accumulation in MS (Pryor, Stone et al. 1998; Bijl, Horst et al. 2001; Malkawi, Al-Ghananeem et al. 2009). Exposure to tobacco smoke has been shown to alter the innate and adaptive immune cells (Holt and Keast 1977). Increased risk of cancer, cardiovascular and other chronic diseases amongst smokers may possibly be related to smoking-induced changes in the immune system. Further work is needed to elucidate the mechanism by which smoking increases the risk of progression in MS.

4.6 Conclusion

In summary, we found that ever-smokers with MS accumulate more disability over a shorter period of time, reach progressive stage faster, have higher level of psychological and physical impairments and disabilities and suffer from more severe disease than never-smokers. Our findings point toward the beneficial effect of smoking cessation even after the disease onset in patients with MS. Measures to prevent and reduce smoking may lead to improved outcomes in MS. We observed that the longer the time since smoking cessation, the lower the risk of reaching disability scores milestones in older former smokers. This fact calls for effective smoking cessation programs.

Chapter five: smoking and MS. Effects on mortality and patients' life expectancy

5.1 Summary of the chapter

Mortality in patients with MS has been studied in several populations but not many studies have evaluated environmental factors associated with increased mortality risk. The current work was undertaken to determine whether patients' life style and in particular their smoking habits can describe some of the excess mortality reported in MS populations.

In the next section, **section 5.2**, we performed a systematic review and pooled meta-analysis of standardised mortality ratios obtained from the previous studies of MS mortality. We believe that this has been able to summarise the mortality in MS more effectively.

We then performed a survival analysis to investigate the influence of tobacco smoking on the risk of death due to all-cause mortality in MS patients. **Section 5.3** discussed our methodology.

The findings of our analyses were presented in **section 5.4**.

Section 5.5 discusses our findings and presents implication, generalisation and clinical relevance of our findings.

5.2 Background

MS is an unpredictable and disabling disease and individuals with MS are found to have a life expectancy shorter than the general population (Scalfari, Knappertz et al. 2013). It has been shown that patients with the diagnosis of MS usually live 7 to 14 years shorter than their counterparts in the general population (Scalfari, Knappertz et al. 2013). In MS it has been hypothesised that the excess mortality is mainly due to the enhanced susceptibility to concomitant comorbid diseases such as infection or complications raised from severe disability rather than direct impact of physiological changes in the brain and spinal cord. To date several studies which examined excess mortality in MS have suggested an increased risk of death in MS patients compared with the general population, but the results are not consistent in all the studies and long survivals in MS patients have also been reported frequently. In 2013, Scalfari and colleagues (Scalfari, Knappertz et al. 2013) reviewed the current literature of mortality in MS and accurately pointed out two very important issues in the way of reaching meaningful conclusions regarding mortality in MS patients. First, from the current literature it is not entirely clear whether the improved survival in MS patients is also seen when the survival rates are compared with the rates from the general population. Second, data with regard to the influence of gender on the mortality rates is contradicting.

Due to the ambiguity of the results we performed a pooled meta-analysis of studies with report of standardised mortality ratios (SMR). SMRs will enable us to investigate whether mortality rates in MS patients are different from those reported from the general population. In addition to this we measured the MS incidence mortality in the studies. Another issue that has been investigated

in the current work concerns the gender difference in the SMRs in MS patients as it is commonly believed that female patients have survival advantage over males. We investigated whether mortality in MS patients has changed during the past decades compared with the general population.

5.2.1   Design and Methodology

5.2.1.1 Data source

Medline, Embase and the Cochrane Library up to May 2013 were searched using the keywords "Multiple Sclerosis" and "standardised mortality" or "standardized mortality". The search resulted in a number of publications which were identified and screened.

5.2.2   Inclusion criteria

Inclusion criteria were availability of data on the number of deaths and mean or median patient follow-up, reports of SMR and being a longitudinal study. For multiple studies using the same cohort, the study with the longest duration of follow-up was used that met the study inclusion criteria.

5.2.3   Data extraction

Total number of patients, number of deaths, mean or median duration of follow-up, person-year, type of the study, study onset and publication date were extracted from the papers. Incidence mortality rates (IMR) with 95% confidence intervals were calculated. SMR with 95% confidence intervals were extracted for total population and each sex.

5.2.4 Data analysis

For each study, IMR was calculated as follows: number of deaths during the study follow-up period divided by the mean (or median if mean was not available) number of patients during the study follow-up multiplied by the mean or median patient follow-up or total person year follow-up time. The IMRs and SMRs then were pooled by the method of the inverse of the variance. Natural logarithm of the SMRs were used in our analyses as log-SMR has more normalised sampling distribution and it is preferred when the reference population is different between studies (Breslow and Day 1987). The pooled log-SMRs were then back-transformed for interpretation. The standard error of log-SMR was estimated by $1/\sqrt{number\,of\,deaths}$ (Breslow and Day 1987) in order to be used in a meta-regression model. We performed meta-regression to assess the trend in SMRs over the past 50 years. Random effects models were used and heterogeneity was measured by the I2. In case of high heterogeneity sub-analysis was repeated multiple times, each time with removal of a single study to estimates its effect on the heterogeneity. Begge's test was used to investigate whether any publication bias is present in the model with the least heterogeneity.

5.2.5 Results

Figure 5-1 shows the study identification procedure according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline (Moher, Liberati et al. 2009). From 16 full-text articles assessed for eligibility four were from the same cohort (Danish MS cohort), hence, three were excluded. One study had only reports of regional SMRs but no reports of

overall SMR (Tassinari, Parodi et al. 2001). No SMR was reported in one study (Llorca, Guerrero et al. 2005). One study was excluded due to reports of SMR for suicide only (Fredrikson, Cheng et al. 2003). One study reported SMR but not the confidence interval, therefore it was excluded (Wallin, Page et al. 2000). Overall nine studies were included (Bronnum-Hansen, Koch-Henriksen et al. 2004; Leray, Morrissey et al. 2007; Grytten Torkildsen, Lie et al. 2008; Hirst, Swingler et al. 2008; Smestad, Sandvik et al. 2009; Ragonese, Aridon et al. 2010; Sumelahti, Hakama et al. 2010; Kingwell, van der Kop et al. 2012; Lalmohamed, Bazelier et al. 2012). Table 5-2 summarises included studies. These studies represented 23,289 patients. In total 6,589 deaths occurred during 402,909 person-year follow-up time. The pooled IMR was 12.45/1000 person-years (95%CI: 8.97 to 17.28) ranging from 2.8/1000 person-years (95%CI: 2.2 to 3.5) in France to 38.8/1000 person-years (95%CI: 33.8 to 43.9) in Wales (Figure 5-4). The pooled SMR was calculated for both sexes and each sex separately from the reported SMRs extracted from the studies (Table 5-1). The pooled overall SMR was 2.65 (95%CI: 2.44 to 2.88, I2 = 81.0%) for both sexes, 2.25 (95%CI: 1.98 to 2.55, I2 = 78.3%) in males and 3.01 (95%CI: 2.76 to 3.28, I2 = 68.4%) in females. The sensitivity analysis of the model showed that a single study explained the high heterogeneity (Leray, Morrissey et al. 2007). Hence, the study was excluded in our sensitivity analysis and pooled SMR was calculated for the rest of the studies. This resulted in slightly higher pooled SMR but with almost no heterogeneity. The pooled overall SMR was 2.84 (95%CI: 2.74 to 2.94, I2 = 15.7%) for both sexes, 2.45 (95%CI: 2.25 to 2.66, I2 = 48.8%) in males and 3.12 (95%CI: 3.02 to 3.23, I2 = 0.0%) in females. Forest Plots of the sub-analyses are presented in

figures 5-4 to 5-7. No evidence of influence of cohorts' mid-year on overall SMR was found (P = 0.72) (Figure 5-3). We also could not find any evidence of publication bias (Figure 5-2).

| Records identified through database searching (n = 39) |
| Additional records identified through other sources (n = 2) |
| Records after duplicates removed (n = 25) 8 not MS related, 3 conference papers |
| Records screened (n = 16) 3 same cohort, 1 suicide, 1 no CI, 1 no overall SMR |
| Full-text articles assessed for eligibility (n = 10) |
| Studies included in qualitative synthesis (n = 9) |
| Studies included in quantitative synthesis (meta-analysis) (n = 9) |

Figure 5-1: diagram of the study identification procedure
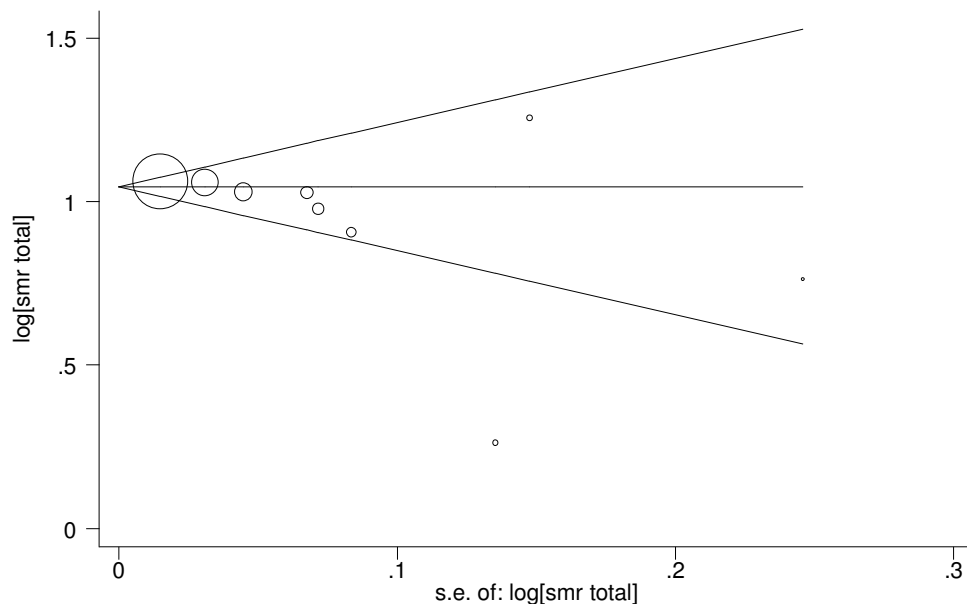
Figure 5-2: Begg's funnel plot with pseudo 95% confidence intervals

Figure 5-3: Impact of cohort mid-year on SMR. Results from meta-regression analysis
(P = 0.72)

Table 5-1: standardised mortality ratios with 95% upper and lower confidence
intervals

First author	SMR total	lower CI	upper CI	SMR male	lower CI	upper CI	SMR female	lower CI	upper CI
Hansen et al. (Denmark)	2.89	2.81	2.98	2.66	2.54	2.78	3.14	3.01	3.27
Torkildsen et al. (Norway)	2.66	2.31	3.06	2.23	1.81	2.76	3.11	2.58	3.74
Ragonese et al. (Italy) *	2.14	1.32	3.46	2	0.89	4.46	2.22	1.23	4
Sumelahti et al. (Finland)	2.8	2.6	3.1	2.2	1.9	2.6	3.4	3	3.9
Smestad et al.(Norway)	2.47	2.09	2.9	2.02	1.56	2.58	2.94	2.36	3.62
Leray et al. (France)	1.8	1.4	2.2	1.4	1	1.9	2.2	1.7	2.9
Kingwell et al. (Canada)	2.88	2.71	3.06	2.68	2.43	2.96	3.01	2.79	3.25
Hirst et al. (Wales)	2.79	2.44	3.18	2.26	1.79	2.85	3.14	2.67	3.69
Lalmohamed et al. (UK)	3.51	2.63	4.69	2.96	1.84	4.77	3.94	2.73	5.68

SMR: standardised mortality ratios
CI: confidence intervals
* SMRs from unadjusted relative risk

Table 5-2: Characteristics of studies reporting mortality ratios in MS. IMR per 1000 person-year

First author	study onset	publication date	study type	Patients (n)	Deaths (n)	follow-up time	person-year	Study period	IMR	lower CI	upper CI
Hansen et al. (Denmark)	1949	2004	Cohort	9881	4254	21.03	207862	1949-1996	20.466	19.857	21.074
Torkildsen et al. (Norway)	1953	2008	Population based survey	878	198	40	35120	1953-2003	5.638	4.855	6.421
Ragonese et al. (Italy)	1960	2010	Population based survey	193	30	21.7	4188.1	1960-2007	7.163	4.609	9.717
Sumelahti et al. (Finland)	1971	2010	Survey	1595	464	36	32165	1971-2006	14.426	13.123	15.729
Smestad et al.(Norway)	1972	2009	Cohort	386	263	40	12172	1972-2005	21.607	19.024	24.190
Leray et al. (France)	1976	2007	MS Clinic	1879	68	12.7	23906	1976-2004	2.844	2.169	3.520
Kingwell et al. (Canada)	1980	2011	Cohort	6841	1025	47.5	77950	1980-2004	13.149	12.350	13.949
Hirst et al. (Wales)	1985	2006	Population based survey	366	218	18.5	5609	1985-2005	38.866	33.808	43.924
Lalmohamed et al. (UK)	2001	2012	Population based survey	1270	69	3.1	3937	2001-2008	17.526	13.427	21.625

IMR: incidence mortality rate
CI: confidence intervals
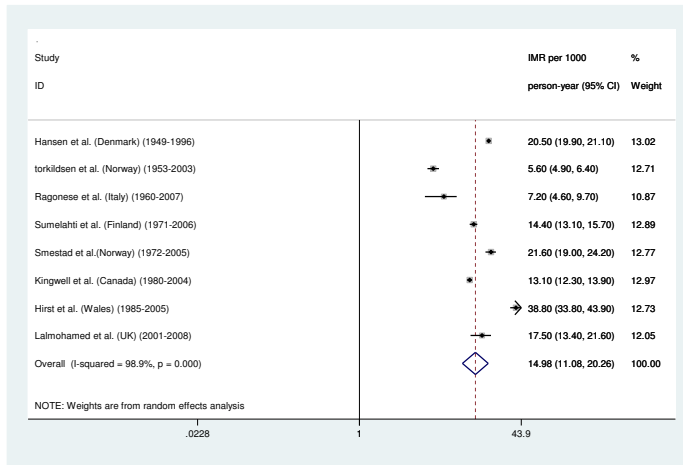
Figure 5-4: pooled incidence mortality rate per 1000 person-year sorted by study onset
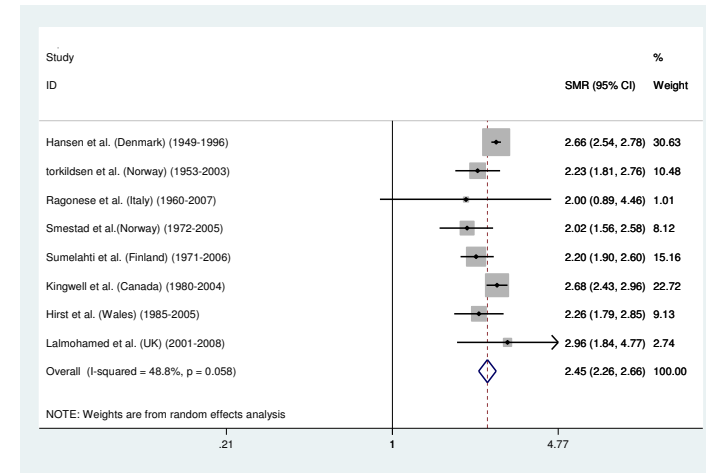
Figure 5-6: pooled standardised mortality ratios in males sorted by study onset
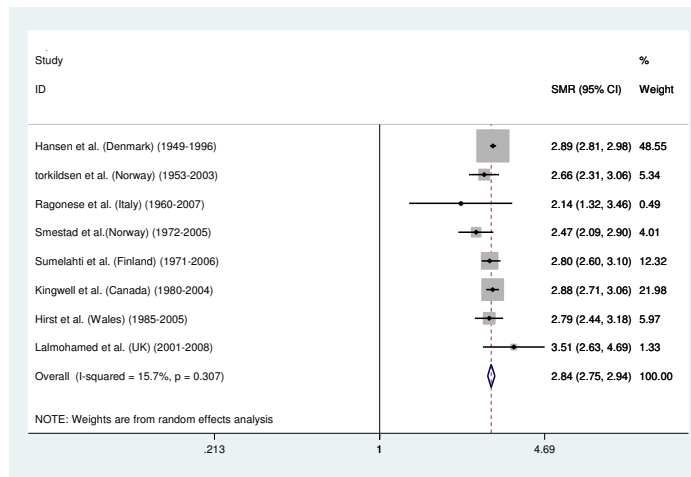
Figure 5-5: pooled standardised mortality ratios for both sexes sorted by study onset
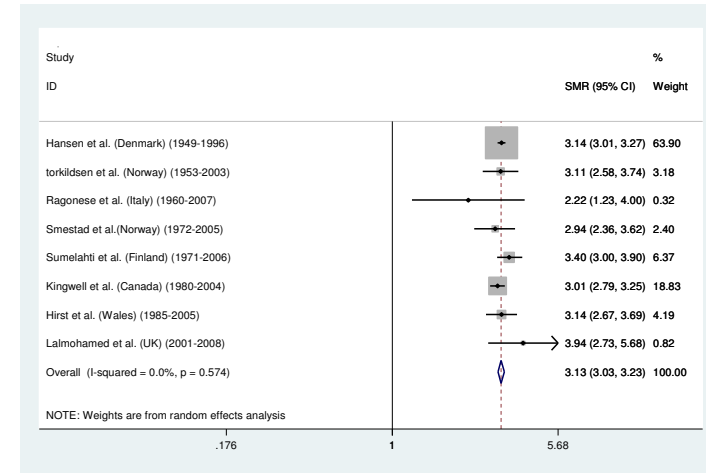
Figure 5-7: pooled standardised mortality ratios in females sorted by study onset

5.2.6 Comment

This pooled analysis of SMRs suggests that both male and female patients with MS are at higher risk of death compared to their matched controls in general population. However, contrary the general concept that female patients have better prognosis, they had higher overall SMR than males. The analysis also shows that the reported increase in MS survival rates may be explained by increased life expectancy in general population as no change in the SMR was observed over time. The calculation of IMR enables us to make a direct comparison between the IMR of each cohort and SMR. Heterogeneity can offer valuable insights if explained. The removed study by Larey et al. (Leray, Morrissey et al. 2007) in our analysis explained much of the heterogeneity (almost 65%) hence it was excluded. However, exclusion of this study made essentially no difference to the pooled SMR in our analysis. The study is based on the data acquired from MS clinics. It is possible that patients with severe disease have died before the data collection (selection bias) in their study. It seems that short duration of follow-up and consequently low number of deaths may explain the low SMR reported in this study. Nevertheless, the relatively high number of patients (57%) who had at least 6 months exposure to treatment should also be considered as a potential factor in altering mortality rates in this study. Our study of pooled SMRs has some limitations. Pooled SMRs from different population settings and observational studies can be controversial because of the biases which can arise from observational studies and various methodological approaches undertaken by these studies. In this case presenting a single pooled estimate of SMR without additional details may give a simple statistic that could be misleading (Egger, Schneider et al.

1998). Nevertheless, a single summary statistics of SMR could be highly appealing for clinicians and health professionals working with MS patients. In addition, our pooled meta-analysis showed relatively little heterogeneity particularly when the study by Leray and colleagues were excluded. The implication of our study is that the SMR in these studies was essentially the same over almost 50 years.

There is ample epidemiologic evidence that, regardless of gender, MS patients still have significantly higher mortality rates compared with the general population. Like many diseases, there are risk factors associated with increased mortality rates in MS patients. Some of these factors are demographic such as gender. As shown here and previously (Poser, Kurtzke et al. 1989; Wallin, Page et al. 2000; Grytten Torkildsen, Lie et al. 2008), female patients with MS have higher mortality rates than males, relative to the general population.

In addition to the influence of demographics like gender, environmental risk factors as well as healthcare interventions can also influence the mortality rates. For example, a hypothetical cohort of MS patients with a significantly higher number of smokers than the general population is expected to report more deaths due to cardiovascular disease or cancer and hence have higher SMRs. Thorough investigation of the role of environmental factors in MS can provide valuable explanations for some of the still unsettled and controversial questions. For example, the proportion of smokers in the studied cohorts can explain some of the discrepancy seen in the studies comparing cancer-related mortality rates in MS patients to those of the general population (Bronnum-Hansen, Koch-Henriksen et al. 2004; Grytten Torkildsen, Lie et al. 2008). Amongst the environmental risk factors, tobacco smoking is the biggest

preventable cause of premature death accounting for nearly 6 million deaths worldwide (WHO 2011). Tobacco smoking will continue to be the biggest cause of premature death during the 21st century with approximately 1 billion smoking-related deaths (Jha 2009). Tobacco smoking has previously been linked to more severe disease in MS patients (Bailey 1922; Hernan, Jick et al. 2005; Hawkes 2007; Di Pauli, Reindl et al. 2008; Sundstrom and Nystrom 2008; Healy, Ali et al. 2009) and has recently been reported to be associated with a significant decrease in smokers' life span (Huxley and Woodward 2012; Sakata, McGale et al. 2012). Despite the relatively large number of studies investigating mortality in MS, the influence of life-style factors, mainly cigarette smoking, on mortality rates in MS patients remained to be examined. A recent survey of MS patients in the UK has shown a significant influence of cigarette smoking on the risk of death in MS patients compared with the reference subjects (Lalmohamed, Bazelier et al. 2012). On the basis of our previous findings with regard to the influence of tobacco smoking on the progression of disability and severity of the disease we raised the question of such an influence on the risk of MS- and non-MS-related mortality in patients with MS. In this study we investigated the impact of tobacco smoking on life span and mortality rates in a large cohort of MS patients and tried to find whether can patients' smoking habits describe some of the increased mortality seen in MS populations.

5.3 Design and Methodology

5.3.1 Settings and study design

We studied participants enrolled in the Nottingham University Hospitals MS Clinic data base. These clinics are a major regional referral centre for MS in the East Midlands Counties of England. Our methods and cohort characteristics have been described in detail elsewhere (Manouchehrinia, Tench et al. 2013). Briefly, the centre covers more than 3000 MS patients. Among all the patients who are regularly seen in these MS specialised clinics, 1,032 patients were routinely followed-up and details of the disease clinical course, disability scores, date of diagnosis and disease onset, treatment, comorbid conditions, results of medical investigations, etc. were systematically documented. Our final study population consisted of patients with clinically definite MS according to the McDonald and/or Poser criteria (Poser, Paty et al. 1983; McDonald, Compston et al. 2001) made by an MS specialist neurologist.

5.3.2 Measurements

5.3.2.1 Clinical data and smoking history

Smoking history was obtained during the patients' first clinic visit at the time of disease onset and/or diagnosis and patients were grouped as non-smokers, ex-smokers or current smokers. In the majority of the cases smoking history was updated and recorded more than once after the disease onset during regular clinic follow-ups. For this study we used the latest smoking status recorded in the database. Date of the first manifestation of the disease, date of diagnosis, duration of exposure to disease modifying treatments (DMTs), latest Expanded Disability Status Scale (EDSS) score recorded in clinic, sex and

initial clinical course of the disease (relapsing-remitting (RR) vs. Primary-progressive (PP)) were used in this study. We also calculated a global MS severity score (MSSS) which integrates EDSS score and disease duration according to the guidelines published by Roxburgh et al. (Roxburgh, Seaman et al. 2005).

5.3.2.2 Vital Status and cause of death

Vital status of patients was monitored and the exact date of death was obtained through linkage of the MS cohort to the National Health Service (NHS) vital statistics as of December 2012 (index date). For the cause of death, both medical records and death certificates were used. In England the death certificate is issued upon death and divided in to two parts. Part l shows the immediate cause of death and is further subdivided into section a, b and c which are used to highlight any underlying cause or causes. Part II is used for any significant condition or disease not leading directly to death but contributing to the death. Causes of death were categorised into MS-related (death due to MS disability such as bronchopneumonia, pulmonary embolism, sepsis) and non-MS-related deaths (e.g. cancers, suicides, cardiovascular diseases) (Hirst, Swingler et al. 2008).

5.3.2.3 Statistical analysis

Patient data were included from the time of entry into the database until either death or the index date (December 2012), whichever occurred first. Time at risk and death rates were calculated per 1000 person-years. Confidence intervals for median survival age could not be calculated as the survival function did not reach 0.45. Since the distribution was approximately normal

we used parametric measures and calculated mean survival from birth and after MS onset using the Kaplan-Meier method. Smoking-specific rates were calculated using Cox proportional hazard regression models controlled for sex, onset age, use of DMTs (in a binary group of ≥1 year or <1 year) and initial clinical course of MS (RR vs. PP). The final model was checked for the proportionality assumption based on the Schoenfeld residuals and stratification was made if necessary to hold the proportionality assumption. We also calculated SMRs and performed external comparison of our mortality to the England general population data to investigate whether mortality in our cohort differs from that of the general population of England. SMR was calculated by dividing the observed number of deaths in the cohort by the number of deaths expected from the general population for each sex and age band stratum (20-24, 25-29, … up to >85 years). Since all the deaths (n=80) in our cohort occurred in the 2001 to 2012 calendar period we used 2006 England mortality rates as the corresponding reference rates, obtained from the UK Office for National Statistics for each sex and age group (2012). All statistical analyses were performed with Stata 11 (StataCorp. 2009. Stata Statistical Software: Release 11. College Station, TX: StataCorp LP). The study was approved by the National Research Ethics Service East Midlands Ethics Committee Derby-1.

5.4 Findings

From the original 1032 subjects with records in the database we excluded 45 subjects with missing smoking data, 3 with missing date of onset, 30 not fulfilling criteria for clinically definite MS (12 clinically isolated syndrome and 18 suspected MS only) and 30 patients with only one visit to the clinic. Our final cohort consisted of 923 (89% of 1032 subjects) individuals with clinically definite MS and full data for analysis.

70% of the subjects were female, 11% had PP MS at disease onset compared with 89% RR MS. Of those RR MS subjects 40% had transited to secondary progressive (SP) MS. In general, deceased patients in our cohort had significantly higher onset age, longer disease duration and higher MSSS and EDSS scores compared with survivors. 58% of the deceased patients were male while 30% of survivors were male. 45% of the survivors had received DMTs for one year or longer compared with only 16% of deceased subjects.

No survival advantage was found for relapsing onset MS patients compared with progressive onset (P = 0.93). Despite the decrease in the prevalence of ever-smoking in the UK general population, there was an increase of approximately 12% in the proportion of ever-smokers in our cohort in 2000-2010 compared with of the proportion before 1990 (Figure 5-8 and Table 5-4) in the MS cohort. Table 5-3 shows the baseline demographic and MS-specific characteristics of our cohort stratified by vital status at our index date.

Table 5-3: general demographic characteristics of the MS cohort stratified by vital status at December 2012

	Deceased (n = 80)	Alive (n = 843)	P
Sex (female)	34 (42%)	610 (72%)	< 0.001
Last recorded EDSS (median(IQR))	7.5 (±1.5)	5.5 (±3.5)	< 0.001
MSSS (mean)	7.9 (±2.23)	5.34 (±2.59)	< 0.001
Age at the onset (mean)	35 (±10.67)	32(±9.74)	0.02
Disease duration (median)	20 (±17)	15 (±14)	< 0.001
Type of MS			< 0.001
Relapsing-remitting	9 (11%)	445 (52%)	
Primary-progressive	16 (20%)	87 (10%)	
Secondary-progressive	55 (69%)	311(37%)	
DMT ≥1 year	13 (16%)	384 (45%)	< 0.001

DMT: disease modifying treatment
EDSS: expanded disability status scale
IQR: interquartile range
MSSS: multiple sclerosis severity score

Figure 5-8: Standardised prevalence ratio (SPR) of ever-smoking in the MS cohort the past three decades

Table 5-4: age and sex standardised prevalence ratios of smoking in the MS cohort during the past three decades.

Decade	sex	observed	expected	SPR	95% CI	P-Value
1981-	male	37	40	0.92	0.62 to 1.22	0.66
1990	female	73	79.6	0.91	0.70 to 1.13	0.80
1991-	male	65	57.08	1.13	0.87 to 1.45	0.16
2000	female	109	108.83	1.00	0.82 to 1.20	0.50
2001-	male	37	24.47	1.51	1.06 to 2.08	0.01*
2010	female	56	47.65	1.17	0.88 to 1.52	0.12

SPR: standardised prevalence ratios
CI: confidence intervals
* Note that SPR has reached significance level in the last decade in male patients

5.4.1 Length of follow-up and smoking status

The 923 patients contributed a total of 18,717 person-years of data. There were 80 deaths recorded in our cohort, representing a mortality rate of 4.35 (95%CI: 3.43 to 5.34) per 1000 person-years. This rate was higher in males (8.68, 95%CI: 6.52 to 11.56) than in females (2.47, 95%CI: 1.76 to 3.48). Compared to the frequency of 50% in the whole cohort, 54 (67%) of the deceased subjects were ever-smokers. All nine patients with RR MS who died were ever-smokers, compared with 44% of PP MS and 68% of SP MS. Age at onset was slightly but significantly higher in ever-smokers (30.4 vs. 31.2; P = 0.003). The crude mortality rates were 6.66 (95% CI: 4.71 to 9.43) per 1000 person-years among current smokers, 5.06 (95% CI: 3.3 to 7.77) for ex-smokers and 2.76 (95%CI: 1.89 to 4.02) for non-smokers.

5.4.2 Survival rates and impact of smoking

Mean survival was 76.8 years (95% CI: 74.6 to 79) for the whole cohort, but compared with ever-smokers, never-smokers lived almost 6 years longer. Mean survival was 81 (95%CI: 78 to 83.6) years in non-smokers, 78.4 (95% CI: 75.2 to 81.5) years in ex-smokers and 71.5 (95% CI: 68.8 to 74.2) in current smokers (Figure 5-9).

Current smokers and ex-smokers were at higher risk of death, with a hazard ratio relative to never smokers of 2.70 (95% CI: 1.59 to 4.58; P < 0.001) and 1.30 (95% CI: 0.72 to 2.32; P = 0.37) respectively. Limiting our survival analysis to those who died from MS-related causes, current smokers were still at higher risk for death, with a hazard ratio of 2.93 (95% CI: 1.48 to 5.76; P < 0.001) relative to non-smokers. Among ex-smokers, the hazard ratio was 1.18 (95% CI: 0.53 to 2.61; P = 0.67), not statistically significant.
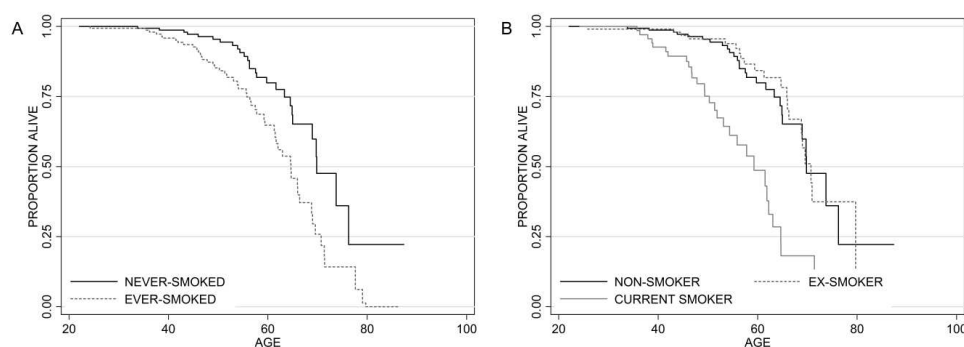
Figure 5-9: A, Kaplan-Meier graph showing survival age of MS patients by lifelong smoking status, B, Kaplan-Meier graph showing survival from birth by smoking status

5.4.3 Disability status and cause of death

As shown in Table 5-3, disability status immediately preceding death was significantly higher in deceased subjects relative to survivors as measured by

EDSS score and MSSS. Time to EDSS score milestone 6 (requires assistance to walk for 100 meters) was available in 72 of 80 deceased patients. Never-smokers reached an EDSS score 6 almost 11 (95%CI: 6 to 12) years after the disease onset whereas ever-smokers reached same disability score in 7 years (95%CI: 5 to 12), though this difference was not statistically significant. We could not obtain causes of death in two of the decedents. Cause of death was MS-related in 60% of cases (Table **5-5**), with bronchopneumonia accounting for the majority of MS-related deaths. Cancer (17.5%) was the commonest non-MS-related cause, and lung cancer the dominant cancer (6 deaths). Deaths from cardiovascular diseases (11 cases), suicide (one case), motor neuron disease (one case), kidney failure (two cases), liver cirrhosis, and intestinal infarction were also recorded.

Table 5-5: summaries of causes of death, onset age, disability score and disease severity stratified by smoking status

Cause of death	Ever-smoked				Never-smoked			
	n	Onset age (median)	Last EDSS (median)	Mean MSSS	n	Onset age (median)	Last EDSS (median)	Mean MSSS
MS related cause	32	36	8	8.63	15	33	8.5	9.32
• **Pneumonia**	21		8		9		8.5	
• **Sepsis**	4		8		2		8	
• **Other or not specified**	7		8		4		8	
Cancer	10	33	6	5.65	4	27	7	7.12
Cardiovascular	7	38	6	6.35	4	36.5	7	6.60
Other	2	27.5	7	8.19	4	38	8	7.98

EDSS: expanded disability status scale
MSSS: multiple sclerosis severity score

5.4.4 SMR

All-cause mortality in MS patients was significantly higher than expected in age and sex matched general population, the SMR being 2.41 (95%CI: 1.95 to 2.96; P < 0.001) for males, 1.80 (95%CI: 1.40 to 2.30; P < 0.001) for females, and 1.99 (95% CI: 1.70 to 2.33; P < 0.001) for both sexes combined. Among current smokers, the all-cause SMR was increased to 3.83 (95%CI: 2.71 to 5.42; P < 0.001) and in ex-smokers by 1.96 (95%CI: 1.27 to 3.0; P < 0.001); there was no significant increase among never-smokers, SMR was 1.27 (95%CI: 0.87 to 1.86; P > 0.05).

5.5 Discussion

In this MS population current smoking was associated with more than 2.5-fold increased risk of death. Current smokers with MS had a reduction of about 10 years in their life expectancy relative to non-smokers with MS. Compared with the general population, increased mortality in this cohort as measured by SMR was seen in current and ex-smokers but not non-smokers. However, the risk of death in ex-smokers was considerably lower than in current smokers. Our data suggest that much of the excess mortality seen in MS populations can be explained by patients' smoking habits. This data is consistent with our recent findings of the negative impact of smoking on disability progression and severity of the disease in MS (Manouchehrinia, Tench et al. 2013).

The survival age of 77 for our MS population in the present study is comparable to the findings from a recent study in British Columbia (Kingwell, van der Kop et al. 2012). The observed number of deaths in our cohort was significantly higher than would have been expected if our MS population had

the mortality rates of the English general population. Others have reported higher mortality rates for MS patients (Poser, Kurtzke et al. 1989; Midgard, Albrektsen et al. 1995; Wallin, Page et al. 2000; Sumelahti, Tienari et al. 2002; Grytten Torkildsen, Lie et al. 2008; Ragonese, Aridon et al. 2010; Lalmohamed, Bazelier et al. 2012), relative to the general population, which was also evident in our results. Our patients were 2 times more likely to die prematurely compared with their age- and sex-matched counterparts in the general population. Although SMR cannot be compared due to differences in the distribution of the standardisation variable (age and sex) across the study populations, our presented SMR here is slightly lower, though still comparable with those reported previously (Sumelahti, Tienari et al. 2002; Bronnum-Hansen, Koch-Henriksen et al. 2004; Hirst, Swingler et al. 2008; Smestad, Sandvik et al. 2009; Kingwell, van der Kop et al. 2012). The reported SMR here is similar to the reported SMR in an Italian MS cohort (1.81) (Ragonese, Aridon et al. 2010). Apart from methodological variations, treatment and access to health care resources may further explain the lower SMR in the cohort presented here. Long term follow-up of patients participating in the original interferon β clinical trial has shown that exposure to treatment may decrease risk of premature death in MS patients (Goodin, Reder et al. 2012). This may explain some of the differences seen between our reported SMR and those reported previously. While 57% of the patients in the French study (Leray, Morrissey et al. 2007) and 42% of our cohort have been exposed to treatment, the majority of the patients in British Columbia (Kingwell, van der Kop et al. 2012) were never exposed to treatment and the follow-up time in the Danish study (Bronnum-Hansen, Koch-Henriksen et al. 2004) predated

treatment. Nevertheless, the potential influence of treatment intervention in MS on mortality deserves further investigation. In the UK, it has been suggested that current smokers with MS have 6.7-fold increased mortality rate compared with the sex- and age-matched counterparts without MS (Lalmohamed, Bazelier et al. 2012). Our results confirm a higher mortality rate in smokers than non-smokers with MS, but with a lower hazard rate of 2.7. Short patient follow-up time and the use of the General Practice Research Database and Hospital Episode Statistics may explain the higher hazard rate in the study by Lalmohamed and colleagues by capturing the more severe cases of MS. Our study offers longer duration of follow-up in a homogeneous cohort of MS patients with detailed clinical data. All the patients in this report have the diagnosis of clinically definite MS made by MS specialist neurologists. In addition our cohort has a population based characteristics and wide spectrum in terms of factors such as disease type and disability score. Our study also had some limitations. It is possible that our study has missed some of the patients with severe disease who have died before the data collection. Despite these limitations our overall cohort characteristics such as gender, MS type ratio (RR vs. PP), onset age, etc. were similar to those reported from other MS cohorts (Weinshenker, Bass et al. 1989; Confavreux, Vukusic et al. 2003).

During the past fifty years prevalence of tobacco smoking has decreased in the UK general population. This along with advances in medical sciences was associated with an increase in general life expectancy and a significant decrease in smoking attributable deaths (Thun and Jemal 2006). Despite this, we observed a steady increase in the proportion of ever-smokers in our MS population specifically in men, who also had higher mortality rates compared

with women. To the best of our knowledge, this is the first study investigating the impact of smoking on life expectancy in patients with MS. Here we have presented data from a cohort of MS patients in the UK, but the relevance of this study is worldwide. As shown here smoking is a significant risk factor for all cause and MS-related death, causing almost 10 years loss of life expectancy in patients with MS. In contrast, mortality among non-smokers was only modestly and not statistically significantly increased relative to the general population.

On the basis of our findings there is a strong association between tobacco smoking and premature mortality in patients with MS. Reduced risk of death amongst former smokers calls for effective cessation strategies. Our data suggest that reduction in prevalence of tobacco smoking in people with MS can potentially eliminate or decrease the excess mortality rates seen in MS patients. Tools and information to help smokers with MS stop smoking and non-smokers to never start smoking should be routinely provided to all patients with MS.

5.6 Conclusion

Smoking is an important factor for death due to all cause and MS-related death in patients with MS. The higher mortality rates in our cohort could be attributed to patients' smoking habits. Patients who gave up smoking had a considerably lower risk of death compared with those who continued to smoke. We propose that premature death due to MS disability can be considered as smoking-attributable death which requires effective cessation interventions.

Chapter six: Comparison of different approaches for modelling smoking in MS

6.1   Summary of the chapter

Tobacco smoking is a complex multi-dimensional environmental factor implicated in a large number of diseases. Measuring the precise influence of tobacco smoking on any outcome requires careful modelling of smoking specific to the characteristics of that disease. In this chapter we explored some simple strategies for modelling smoking in MS. We would try to investigate the performance of different modelling approaches and possibly recommend the most comprehensive model for use in the future studies.

The next **section 6.2** will offer some key ideas and assumptions on some of the most commonly used and new approaches in modelling of smoking.

Section 6.3 will cover our methodology.

Our results are presented in **Section 6.4** and **Section 6.5** discusses our findings, limitations, and strengths of our study as well as the implications of our findings.

6.2   Background

Epidemiologic studies of the impact of tobacco smoking on many diseases encounter several difficulties in terms of proper and appropriate modelling of smoking as a time varying multi-dimensional variable. Among the dimensions of smoking are intensity or the number of cigarette smoked, duration of smoking, age at smoking initiation and time since smoking cessation in ex-smokers. Although widely used, multivariable modelling of separate effects of several smoking factors such as intensity, duration and time since cessation is not favourable and can reduce statistical power because of the increase in variance inflation and multicollinearity of the statistical model. The majority of the studies evaluating the impact of smoking on MS has focused on a simplified analysis and one aspect of smoking only or were at best limited to calculating pack years smoked. However, we believe that ignoring any of these factors may result in over or under estimation of the influence of smoking and can potentially include confounding, especially in a disease as chronic as MS in which duration and intensity of exposure can change overtime.

The main objective of this chapter was to make a comparison of different approaches to modelling of smoking in MS. Of the most commonly used smoking indexes in MS are conventional non- ex- and current smoking categories and pack-years smoked. Here we compared the performances of these conventional approaches together. We also investigated the performance of these models to those of more advance indexes to identify a relatively easy, widespread approach to model smoking in MS.

Comprehensive indexes such as body mass index (BMI) have been widely used in epidemiologic studies for evaluating the overall burden of various dimensions of one risk factor on individuals' health. Several smoking related indexes have been proposed for modelling of smoking in epidemiologic studies. A Comprehensive Smoking Index (CSI) (Equation 1) was first used for modelling smoking history in the context of periodontal diseases by Dietrich and Hoffmann (Dietrich and Hoffmann 2004). Since then, the developed version of the CSI has been used in several diseases such as lung cancer and systemic sclerosis with some promising results (Leffondre, Abrahamowicz et al. 2006; Dietrich, Garcia et al. 2007; Hudson, Lo et al. 2011). CSI is a multi-dimensional mathematical representation of individuals' smoking history and works as a single aggregate measure of smoking exposure that integrates three the main factors of smoking history; intensity, duration and time since cessation based on a series of assumptions. The original CSI was developed based on the assumption of an exponential decline of the effect of past smoking over time.

$$\left(1 - 0.5^{\left(\frac{dur}{\tau}\right)} \right)\left(0.5^{\left(\frac{tsc}{\tau}\right)} \right) int$$

Equation 1: dur; duration of smoking, tsc: time since cessation, int: average number of cigarette smoked per day, τ: the half-life parameter

In 2006, Leffondre and colleagues (Leffondre, Abrahamowicz et al. 2006) developed a new version of the CSI (Equation 2) to account for a lag between 'causal action' and disease detection (δ) and given the fact that smoking

intensity has some non-linear effect which showed better results in the context of lung cancer. The assumption of non-linear effects of smoking intensity fits our data as well (Figure 4-7 in chapter 4).The new CSI by Leffordre was developed based on the removal of the exponential decline and suggested that the impact of smoking on individual's health gradually increases over time which seems more appropriate for using in studies of MS.

$$\left(1 - 0.5^{\left(\frac{dur*}{\tau}\right)}\right)\left(0.5^{\left(\frac{tsc*}{\tau}\right)}\right)\ln(int + 1)$$

$$\boldsymbol{tsc} * = \max\,(tsc - \delta, 0)\ \text{and}\ \boldsymbol{dur} * = \max\,(dur + tsc - \delta, 0) - tsc *$$

Equation 2: dur; duration of smoking, tsc: time since cessation, int: average number of cigarette smoked per day, τ: the half-life parameter, δ: the lag-time (the length of delay between initiation of smoking and the first neurological MS related symptom).

The implementation of the new CSI requires the estimation of two parameters: δ; the length of delay between the initiation of smoking and the first symptom and τ; the rate at which the health impact of smoking decays over time. This can be fixed prior to the analysis if known (e.g. from biological experiments) or can be estimated by maximising the goodness-of-fit statistics from the data at hand. The estimation of these parameters is also of interest in itself since they may give insights into the form of the dose–response rate between the outcome and smoking. Better understanding of the formal property of the new CSI requires some levels of visualisation which are presented here. Figure 6-1 shows the estimated CSI in hypothetical ex-smokers (tsc = 20 years & int=30 / day) for selected combinations of δ and τ. The estimated CSI in current

smokers for selected combinations of δ and τ are graphically presented in Figure 6-2. Figure 6-3 demonstrates how the CSI behaves in ex-smokers following smoking cessation for the selected combinations of δ and τ. As shown in the figures, a shorter τ yields lower CSI due to faster levelling-off of the impact of smoking and the longer τ would keep the risk of that particular outcome high for a longer time as it will take longer for the effects of smoking to level off. In the improved CSI δ would represents the lag-time after smoking cessation and also after smoking initiation.
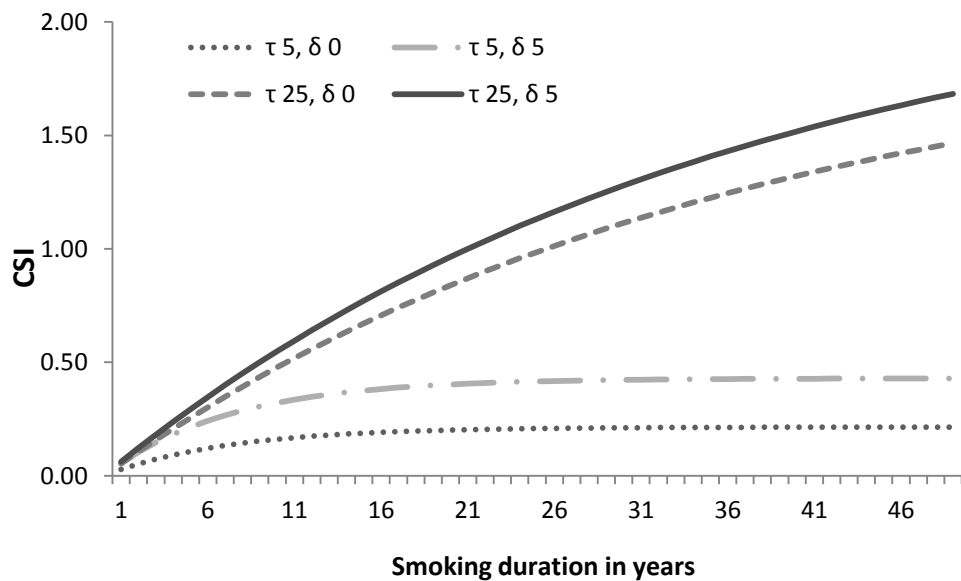
Figure 6-1: The CSI of hypothetical Ex-smokers by duration of smoking for selected combinations of τ and δ (tsc = 20 years & int=30 / day).
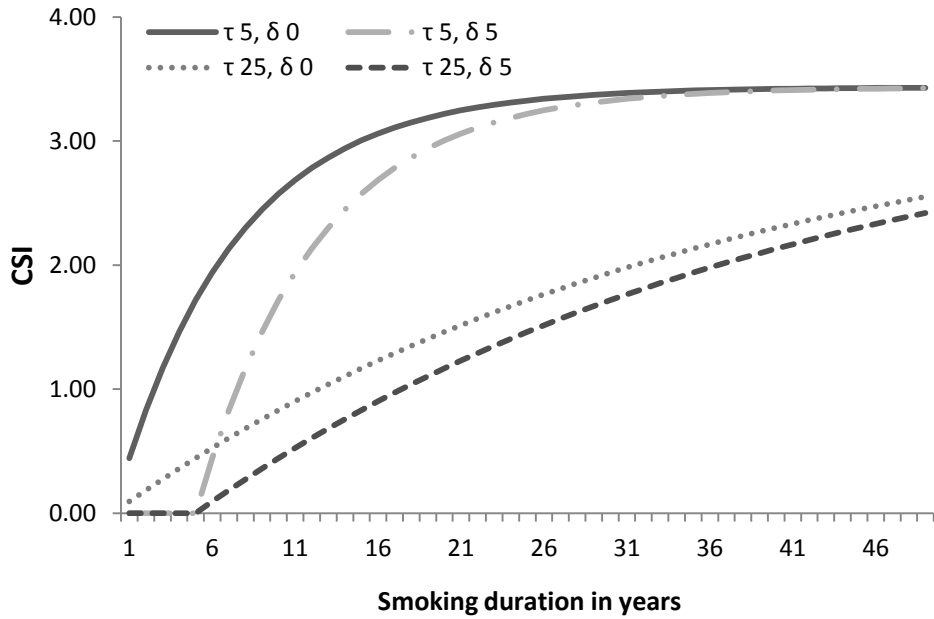
Figure 6-2: The CSI of current smokers (tsc=0 & int=30/day) by duration of smoking for selected combinations of τ and δ.
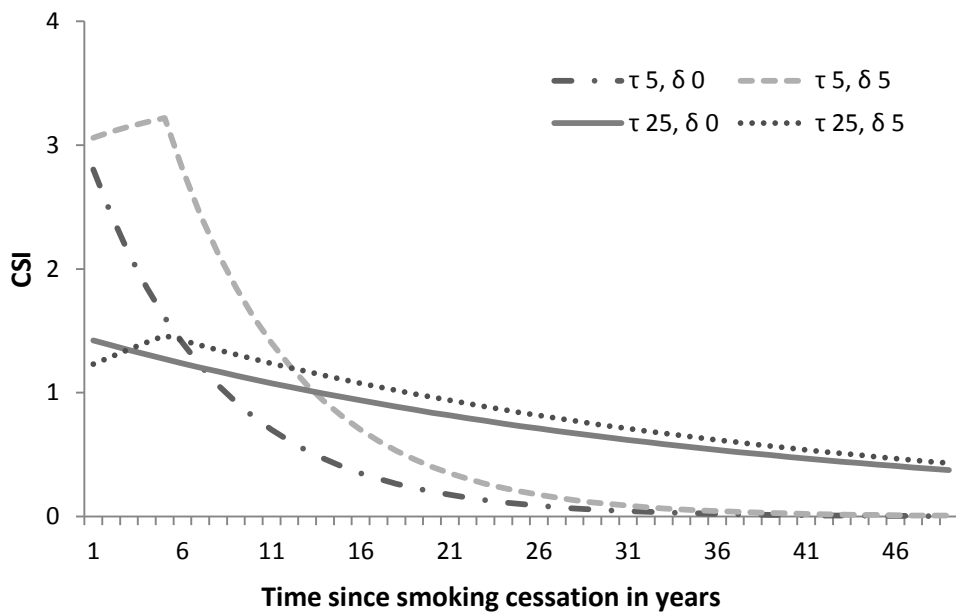
Figure 6-3: The CSI of ex-smokers (dur=20 & int=30/day) by time since smoking cessation for selected combinations of τ and δ.

Alongside all the advantages of using indexes for measuring the influence of smoking on individuals' health, the implementation of indexes is somehow

restricting in terms of interpretation of the effects of smoking. Hence, the objective of this study is to present an index to measure the cumulative burden of smoking while covering its various dimensions. This would come handy when one needs to evaluate the cumulative effects of smoking on an outcome such as risk of developing MS or risk of having progressive MS at the disease onset or just need to control for smoking in a study such as a clinical trial.

6.3   Methodology

One of the difficulties in the implementation of CSI is the estimations of δ and τ. In our study, δ and τ were estimated using nested loops search algorithm from the data at hand as no previous study had looked at the biology of smoking in MS. In the present study, τ is allowed to range from 1 to 50 years with 1-year increments, while δ is allowed to range from 0 to 20 years in 0.1 increments. Then, the CSI was computed (for all patients), for each possible combination of values of τ and δ in the range specified, and the Akaike information criterion (AIC, measure of goodness of fit statistics) of the regression model in the presence of the CSI and all the confounders including onset age, sex, initial disease phenotype, use of treatment and disease duration was determined. AIC is a statistical method of selecting the best fitted model from a series of models. The chosen model will be the model that has a good fit to the truth but few parameters.

The CSI equals zero for never-smokers but would not necessary be higher for current smokers compared with ex-smokers as both duration and time since cessation are taken into account. With the range specified above we fitted 51 $\times$

210 = 10710 models for each outcome. The 'optimal' values of τ and δ were identified as that corresponding to the minimum regression model AIC value which consequently would be that corresponding to the best fitted model. We also implemented the models by maximising the R-squared to define the goodness of fit statistics when obtaining AIC was more time consuming. When the optimum values of δ and τ were obtained from the search algorithm, they were fixed for all individuals and overall burden of the smoking on the risk of progressive onset MS, risk of reaching EDSS score milestone 6, average MSSS and PDDS score were estimated. Values of δ and τ were estimated separately for each outcome. We then compared the AIC, Bayesian information criterion (BIC) and degree of multicollinearity of models with conventional smoking modelling and the CSI to find the best method of modelling smoking in MS. AIC and BIC are both penalised-likelihood criteria used for choosing best predictor subsets in regression models. A lower AIC means a model is considered to be closer to the truth and a lower BIC means that a model is considered to be more likely to be the true model. Their only difference is the size of the penalty for model complexity which weighs more heavily in BIC. For the comparison of the performance of the models we employed the models listed in **Table 6-1**.

Table 6-1: list of the models with different modelling of smoking which were compared in our analyses

Model 1	Ever vs. never smoked
Model 2	Non / Ex / Current
Model 3 (pack-years)	dur * (int / 20)
Model 4	Int & dur & tsc
Model 5	Log (int + 1) * dur & tsc
Model 6	The CSI

6.4   Results

Table **6-2** shows the estimated values of δ and τ from the regression models for outcomes used in our study.   When the optimum values of δ and τ were obtained, the CSI was calculated for each outcome and all patients. Regression analyses were performed in the presence of CSI which integrated smoking intensity, duration and time since cessation.

Table 6-2: estimated values of δ and τ obtained from the regression models for each outcome used.

	Statistical model	τ_{org}	τ	δ
Risk of PP MS at onset	Logistic regression	50	2	19.8
PDDS	Linear regression	28	1	18.8
MSSS	Linear regression	50	50	0
Hazard ratio of reaching EDSS score 6	Cox regression	3	1	3

EDSS: expanded disability scale status
PDDS: patient determined disease steps
MSSS: multiple sclerosis severity score

6.4.1 Risk of progressive onset MS

The first outcome used was the risk of progressive onset MS. Our logistic regression model in the presence of the CSI, sex and onset age, failed to show any evidence of an influence of tobacco smoking before the onset of the disease on the risk of progressive onset MS. Similar to the model with the conventional smoking modelling of ever- and never-smoked, in the model older age at the onset of the disease and being male were significantly associated with progressive onset MS. The two logistic regression models here (with and without the CSI) yielded similar results, however, the CSI integrated some important features of smoking which were missing when conventional modelling was used. Table 6-3 shows the results from conventional modelling and the model with CSI with reports of AIC and BIC of each model. No evidence of any influence of any smoking factors on the risk of progressive onset was seen in any of the models. As seen in the table, the model with the pack-years smoked has the lowest BIC and AIC which indicate that it fits our data best relative to the other methods used. The model with the original CSI showed a better fit to our data than the improved CSI.

Table 6-3: Comparison of the fitness of Models for the risk of progressive onset MS

	OR (95%CI)	P-value	AIC	BIC
Ever vs. never smoked	0.88 (0.49 to 1.59)	0.68	327.85	345.81
Non /	----	---	329.68	352.12
Ex /	0.84 (0.44 to 1.61)	0.61		
Current	0.98 (0.41 to 2.33)	0.97		
Pack-years smoked	0.99 (0.98 to 1.01)	1.00	275.21	292.70
Int	0.99 (0.96 to 1.03)	0.87	306.03	332.55
& dur	1.00 (0.96 to 1.04)	0.81		
& tsc	0.99 (0.94 to 1.03)	0.66		
Log (int + 1) * dur	1.0 (0.99 to 1.00)	0.91	304.08	326.18
& tsc	0.99 (0.95 to 1.03)	0.69		
Original CSI	0.99 (0.94 to 1.05)	0.88	303.84	321.57
The CSI	1.16 (0.88 to 1.51)	0.8	314.36	332.19

All models were controlled for onset age and gender.
AIC: Akaike's information criterion with lower AIC indicating the best fit to data.
BIC: Bayesian information criterion with lower BIC indicating the best fit to data.
dur: duration of smoking before MS onset.
Int: intensity of smoking.
OR: odds ratio
tsc: time since smoking cessation if gave up smoking before onset of MS.

6.4.2 Time to EDSS score milestone 6

Summaries of the findings from different approaches for modelling of smoking and the risk of reaching EDSS score milestone 6 using Cox proportional hazard regression models is presented in Table 6-4. As seen, the binary modelling of smoking, model 1, has the highest AIC and no influence of smoking has been detected using this approach. Model 2, which contains

smoking variables categorised as non- ex- and current smokers showed the second highest AIC and the highest BIC and revealed the influence of current smoking status on the risk of reaching EDSS score 6 but no influence of smoking in former smokers. Although the model dismisses some important features of smoking such as duration and intensity, it is relatively informative when the effects of smoking cessation and current smoking are of interest and data at hand is limited to simple categorical status. The next model containing the pack year smoked showed relatively high AIC and BIC compared to the rest of the models and surprisingly was not sensitive enough to detect any influence of smoking on the risk of reaching EDSS score 6. In addition the model was not proportional thus its result is not reliable. The best model with the lowest AIC and BIC was the model which included the time since cessation, intensity and duration of smoking. Treating duration of smoking as a time varying covariate increased the AIC and BIC of the model very marginally but the model was still very informative. The product of the log transformation of intensity and duration of smoking together with time since cessation showed relatively good AIC and BIC but could not detect any influence of intensity and duration of smoking on the risk of reaching EDSS score 6. The CSI showed an average performance when used with the Cox regression model and was able to detect the influence of smoking on the risk of reaching EDSS score 6.

Table 6-4: Comparison of the fitness of Models for the hazard ratio of reaching EDSS score 6.

	HR (95%CI)	P-value	AIC	BIC
Ever vs. never smoked	1.16 (0.91 to 1.48)	0.21	2763	2785
Non /	----	---	2753	2779
Ex /	0.9 (0.68 to 1.20)	0.51		
Current	1.67 (1.23 to 2.26)	0.001		
Pack-years smoked †	1.00 (0.99 to 1.01)	0.29	2685	2707
Int	1.02 (1.01 to 1.04)	< 0.001	2497	2527
& dur	0.98 (0.97 to 1.00)	0.059		
& tsc	0.97 (0.95 to 0.98)	< 0.001		
Int	1.02 (1.01 to 1.04)	< 0.001	2503	2529
& tsc	0.97 (0.96 to 0.98)	< 0.001		
dur §	0.99 (0.96 to 0.99)	0.18		
Log (int + 1) * dur	1.00 (0.99 to 1.00)	0.29	2507	2533
& tsc	0.97 (0.96 to 0.99)	0.001		
Original CSI	1.02 (1.01 to 1.03)	< 0.001	2506	2528
The CSI †	1.27 (1.16 to 1.40)	< 0.001	2701	2723

§ dur modelled as time varying covariate.
† model was not proportional.
All models controlled for onset age, gender, duration of treatment and the disease initial phenotype. AIC: Akaike's information criterion with lower AIC indicating the best fit to data.
BIC: Bayesian information criterion with lower BIC indicating the best fit to data.
dur: duration of smoking before EDSS score 6.
HR: hazard ratio
int: intensity of smoking.
tsc: time since smoking cessation if gave up smoking before EDSS score 6.

6.4.3 Effects on PDDS score and MSSS

Linear regression models were used with PDDS and MSSS as outcomes. Thus, the reported coefficient here will be adjusted for onset age, initial clinical course of the disease, use of treatment, gender and disease duration (in PDDS only). The reported regression coefficients in Table 6-5 were obtained from various approaches for modelling smoking. Like the findings from the previous outcomes, presented above, the binary categorisation of smoking

yielded the highest AIC for both outcomes and was the least informative approach. Despite having the second highest AIC, model 2 was relatively informative as it showed beneficial effects of smoking cessation in former smokers. Pack-years smoked showed average AIC in both outcomes. The original CSI showed the lowest AIC and BIC, however, like the pack-years smoked and model 5 the interpretations of the coefficients were somehow difficult and restricted as the effects of smoking cessation could not be evaluated. The model with the separate variables of smoking showed the highest variance inflation factor (VIF) and no effects of time since smoking cessation and duration were evident.

Table 6-5: Comparison of the fitness of the Models for MSSS and PDDS score

	Regression coefficient		AIC		Mean variance inflation factor (VIF)	
	MSSS (P)	PDDS (P)	MSSS	PDDS	MSSS	PDDS
Ever- and never-smoked	0.49 (0.01)	0.41 (0.01)	2957	2683	1.12	1.22
Non/	---	---				
ex /	0.35 (0.1)	0.27 (0.12)	2956	2682	1.14	1.23
current	0.80 (0.004)	0.7 (0.003)				
Pack-years smoked	0.01 (< 0.001)	0.01 (< 0.001)	2803	2526	1.12	1.22
int	0.02 (0.03)	0.02 (0.004)				
& dur	0.004 (0.7)	< -0.001 (0.96)	2807	2568	1.51	1.57
& tsc	-0.004 (0.6)	-0.01 (0.15)				
Log (int + 1) * dur	0.007 (0.001)	0.006 (0.001)	2807	2512	1.11	1.23
& tsc	< 0.001 (0.99)	-0.003 (0.5)				
Original CSI	0.09 (0.004)	0.05 (< 0.001)	2499	2526	1.11	1.22
The CSI	0.64 (0.001)	0.23 (< 0.001)	2863	2530	1.12	1.23

All models are controlled for onset age, gender, duration of treatment and the disease initial phenotype
AIC: Akaike's information criterion with lower AIC indicating the best fit to data.
IVF: Variance inflation factor.
tsc: time since smoking cessation if gave up smoking before the study date. dur: duration of smoking . int: intensity of smoking.
MSSS: multiple sclerosis severity score. PDDS: patient determined disease steps.

6.5   Discussion

Here we evaluated the performance of different approaches for modelling smoking in MS. We examined a range of approaches from a simple binary to more complex models with interesting promises. Modelling smoking in MS is complex and requires clear definition of the level of information required in the study. For example, if the main purpose of the study is just to control the cumulative effect of various dimensions of smoking on the risk of a particular outcome, the CSI used here showed a good sensitivity to detect any effects of smoking and offered an interesting approach. The only inconvenience of the CSI is the estimation of τ and δ. However, the interpretation of τ and δ can be interesting in itself. Except in MSSS, our estimated values of τ were relatively short.

The short τ means that the effect of smoking decays relatively rapidly in MS. However, in MS, the interpretation of τ is ambiguous since each component of tobacco smoke may have different rates of decay and subsequently different half-lives. The very long δ in PDDS and risk of progressive onset MS (almost 20 years) means that there is a relatively long delay between the biological effects of smoking and its clinical presentation. Hence, there may be a significant delay of up to 20 years between initiation of smoking and its manifestation on PDDS score and risk of progressive onset MS.

In addition, this long δ would cause the effects of smoking to remain high even after smoking cessation in former smokers. In contrast, the short δ of risk of reaching EDSS score 6 indicates that the effects of smoking on this outcome can be observed shortly after smoking initiation and can also disappear shortly after smoking cessation.

For illustration purposes the CSIs for all the four outcomes with their estimated values of δ and τ (Table **6-2**) for a hypothetical patient with smoking component values of 30 years duration of smoking, one year since smoking cessation and 20 cigarettes per day are plotted in Figure 6-4, Figure 6-5, Figure 6-6 and Figure 6-7.
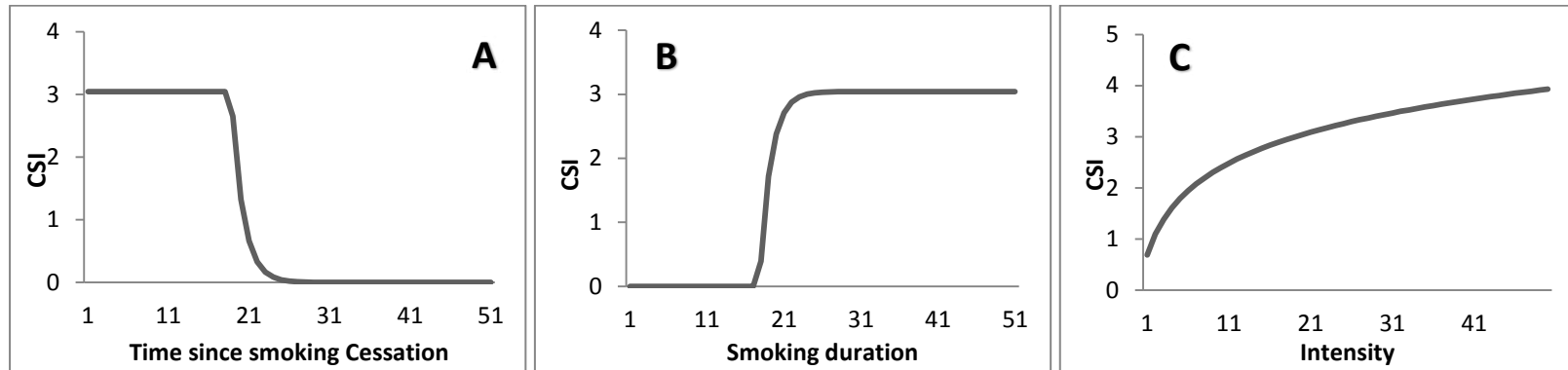
Figure 6-4: the CSI in PDDS plotted against A, time since cessation of smoking, B, duration of smoking, and C, intensity of smoking
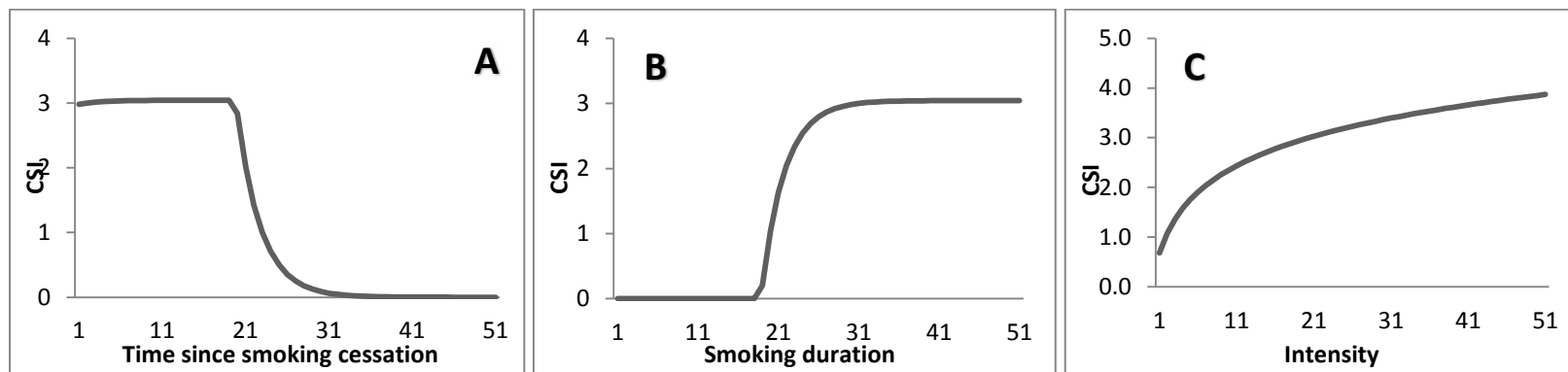
Figure 6-5: the CSI in risk of progressive onset MS plotted against A, time since cessation of smoking, B, duration of smoking, and C, intensity of smoking
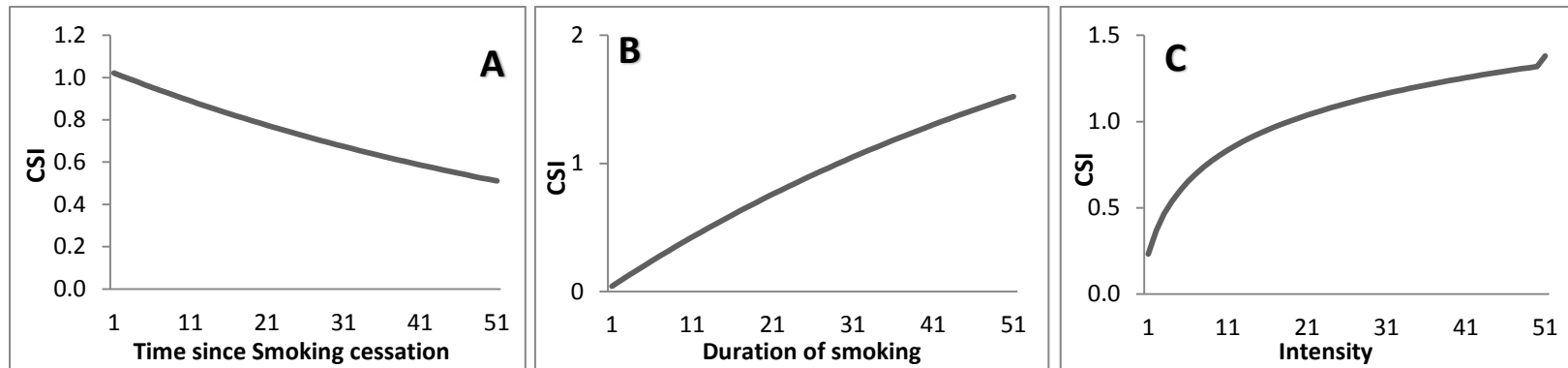
Figure 6-6: the CSI in MSSS plotted against A, time since cessation of smoking, B, duration of smoking, and C, intensity of smoking
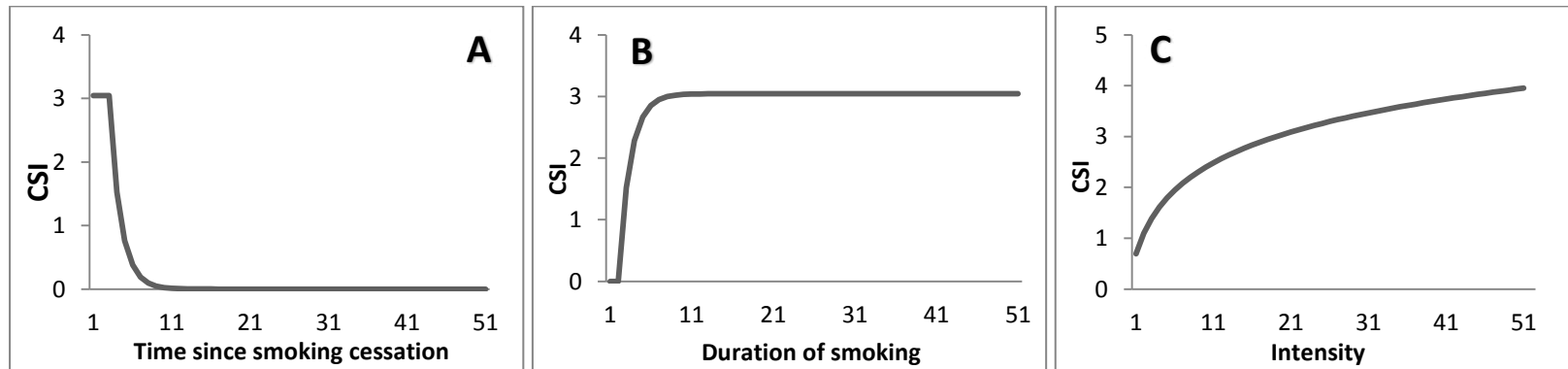
Figure 6-7: the CSI in the risk of reaching EDSS score 6 plotted against A, time since cessation of smoking, B, duration of smoking, and C, intensity of smoking

Time to EDSS score 6 is an example of outcome with a relatively short δ and τ time. As stated before the notation of δ and τ can be interesting in themselves. As seen in Figure 6-7 A, about 10 years after smoking cessation the effects of smoking on the risk of reaching EDSS score 6 disappears. However, three years after smoking cessation the impact of smoking on the risk of reaching EDSS score 6 still remains at a high level. Figure 6-7 B, plots CSI against duration of smoking. As seen, the effect of smoking on the risk of reaching EDSS score 6 increases very rapidly after smoking initiation and after about 8 years of continuous smoking the CSI reaches a plateau with no further increase in the risk of reaching EDSS score 6 upon continuation of smoking. This might be the reason for the poor performance of pack-years smoked in our analysis.

Our study has some limitations. One of the limitations of our work is that we compared a limited number of modelling approaches. However, the selected approaches here were the ones that have been commonly used in the studies of smoking and MS. The other limitation is that we ignored some aspects of smoking history such as depth of inhalation. Nevertheless, our results offer new insights to the effects of smoking on MS but require further investigations possibly in different populations and study designs.

Overall, both versions of the CSI which were used in the current work showed average performance with some very interesting information on the magnitude of various effects of smoking. The final decision on the best model to be used in the studies of MS depends very much on the availability of data and more importantly the properties of the outcome used in the study.

Chapter seven: Conclusion and recommendations

7.1   Summary of the thesis

Each of the chapters of this thesis was dedicated to investigating the impact of tobacco smoking on a specific stage of MS clinical course. The introduction at the beginning of this thesis briefly summarised our current understanding of the epidemiology and natural history of MS. This was followed by series of specified introductions and systematic review of the effect of tobacco smoking on the occurrence of MS, risk of progression of disability and risk of premature death at the beginning of each chapter. Chapter 2 described our study procedure and gives a descriptive summary of the general and clinical features of our sample population. Chapters 3 to 5 were dedicated to describing the findings from a matched case-control study of the effects of tobacco smoking on the occurrence of MS (chapter 3), a cohort study of the influence of tobacco smoking on the risk of progression of disability (chapter 4) and another cohort study of the impact of tobacco smoking on the risk of premature death and life expectancy (chapter 5). A comparison of the different approaches for modelling smoking in MS was made in chapter 6.

In summary, this study found that tobacco smoking is associated with a significantly higher risk of occurrence of MS, higher levels of disease severity and disability and higher risk of mortality. Here we performed the most comprehensive study of the impact of tobacco smoking on MS to date. Our study gives a new insight on the magnitude of the effects of different smoking factors. On the basis of our findings there is a strong association between tobacco smoking and inverse clinical outcomes in patients with MS.

Reduced risk of disability progression and death amongst former smokers with MS observed in our study, point toward the beneficial effect of smoking

cessation. Our data suggest that reduction in prevalence of tobacco smoking can potentially decrease the risk of occurrence of MS, risk of disability progression and excess mortality rates seen in patients with MS.

7.2 Strengths and limitations of the study

Our study has several strengths. Firstly, unlike previous studies examining the influence of smoking on one aspect of the disease only, our study employed a wide variety of outcomes. This enabled us to comment on the magnitude of the effects of tobacco smoking not only on one aspect of the disease but on its whole natural history, from development to mortality.

Secondly, we used prospectively collected clinical data from a large population based cohort with a very long duration of follow-up. Our methodology and the statistical methods employed were robust and were adjusted for important prognostic variables. An example of that is time to EDSS score milestones 4 and 6, which is a robust outcome with many advantages. This has reduced the potential for confounding.

Finally, when appropriate, we used relative rates with age and sex matched controls to minimise the risk of any misinterpretation of the results.

Our study also had some limitations. First, our research had a response rate of almost 50%. A potential bias could rise from the half of the patients who did not respond to our questionnaire. Generally, the low response rate bias will occur when the non-respondents are not a random subset of the whole population with respect to the variable being measured in the research. So if the two populations are similar in the variable in question, increasing response

rate will only increases the cost of a survey without increasing the data quality. In order to measure the effect of non-respondents on our results, we made a demographic and clinical comparison between respondents and non-respondents, the result of which is presented in the second chapter. With regard to the gender, age distribution and disease phenotype the two groups were identical. However, disease duration, percentage of patients who received treatment, economic status and disability score were significantly different between the two groups. We do not think that the differences in the clinical features will have a significant influence on the prevalence of smoking in the two groups as smoking is more closely related to demographics than clinical features. However, the non-respondents were from more deprived areas which are often associated with higher smoking prevalence. Nevertheless, it is unlikely that this has influenced our results very dramatically. Even, in theory, this should increase the risk estimate of occurrence of MS as the number of smokers is going to be increased in MS cases.

Secondly, despite our efforts to cover a wide spectrum of disabilities caused by the disease, still some aspects of the disease such as cognitive impairments were not assessed in our research. We acknowledge the importance of this but it was practically beyond the scope and ability of this PhD research to conduct a project of that size.

7.3 Generalisability of the results

I think this research has made a considerable contribution to knowledge in the field and has identified important factors to be considered for increasing patients' quality of life. Here we presented data from a population based cohort

of patients with MS in the UK but I believe the relevance of our findings is worldwide. Our research was carried out in the UK with a predominantly white Caucasian population but we see no reason that the results are not applicable in other populations as the demographic and clinical characteristics of our sample population were similar to those reported from other MS cohorts.

7.4   Practical implications and applicability

This study found that smoking cessation is associated with a significantly lower risk of progression of disability. In our findings each year since cessation of smoking was associated with 3% to 5% reduction in the risk of disability progression. This represents the first positive results with regard to the beneficial effect of smoking cessation in patients with MS.

The study also found a significantly reduced risk of premature mortality due to MS-related and non-MS-related deaths amongst ex-smokers compared with those who continued smoking. In our sample population life expectancy was decreased by 3 and 10 years in former and current smokers compared with never-smokers. The other significant finding was the contributory role of smoking to the excess mortality rates in MS patients. We observed that never-smokers with MS in our cohort could live as long as their age and sex matched counterparts in the England general population.

Our findings, presented here, have some significant clinical importance to the clinicians and health professionals involved in MS care and management. We believe that patients' quality of life and life expectancy can be significantly improved in a very cost-effective manner if advice on smoking prevention and

cessation has been extensively available at the time of the disease onset and/or diagnosis. Tools and information to help smokers with MS to stop smoking and non-smokers to never start smoking should be routinely provided to all patients with MS.

Assessing applicability and external validity is difficult but it is essential for developing guidelines and it is extremely helpful to grade the strength of recommendations. Applicability of smoking cessation strategies has been extensively discussed before but not in the context of MS. Although smoking cessation help kits are readily available to all patients under NHS cessation programs for free, no data is available with respect to the extent to which patients with MS benefit from these programs. Many of patients with MS live with permanent and restricting disabilities. This potentially limit their access to the NHS smoking cessation services. Hence, cessation interventions in patients with MS require careful planning, shaped according to patients' physical and psychological restrictions, especially at the time of the diagnosis when the cessation will have its highest influence. This should be feasible if types of cessation interventions and their effectiveness are known.

7.5   Future direction

Our findings from this large cohort study call for a multi-centre randomised control trial of smoking cessation in patients with MS. Unfortunately, this was not feasible during my PhD due to our limited time and lack of pilot data on the clinical effectiveness of different types of smoking cessation strategies in patients with MS. Until then, raising patients' awareness on the adverse health impact of tobacco smoking should be the priority of health professionals.

Appendices

Appendix 1: Invitation letter

INVITATION LETTER

(Final version 2.0: 16/05/2011)

Title of Study: EXPOSURE TO TOBACCO SMOKE IN PATIENTS WITH MULTIPLE SCLEROSIS, AN EPIDEMIOLOGICAL EVALUATION

Dear ………………………………

You are being invited to take part in the above research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the enclosed information sheet carefully. Talk to others about the study if you wish. This study will be investigating the effects of smoking on Multiple Sclerosis (MS). MS is a disease caused by misdirected immune system. Some research has shown that the smoking manipulates the immune system. The study has been approved by Nottingham Ethics Committee and is being carried out in the Division of Clinical Neurology, C Floor, South Block, Queen's Medical Centre, Nottingham, NG7 2UH. We are looking to recruit 1500 patients with MS who are over 18 years old. You will be asked to complete a questionnaire booklet and sign the consent form and return it back to us if you choose to take part in this study.

If you wish to know more about the study, please take time to read the enclosed information sheet. Alternatively you can contact us should you require more information or you have any concern about the study.

Contact Details;

Professor Cris Constantinescu or Mr. Ali Manouchehrinia,
Division of Clinical Neurology, C Floor, South Block, Queen's Medical Centre, Nottingham, NG7 2UH
Phone: +44 (0)115 823 1441, +44 (0)115 823 1038
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Yours sincerely,
Professor Cris S Constantinescu
Chair of Neurology
University of Nottingham

Appendix 2; Patient information sheet

Patient Information Sheet

Study: EXPOSURE TO TOBACCO SMOKE IN PATIENTS WITH MULTIPLE

SCLEROSIS, AN EPIDEMIOLOGICAL EVALUATION

Chief Investigator: **Professor Cris S Constantinescu;**
Sponsor: **University of Nottingham**

You are being invited to take part in a research study. Before you decide whether or not to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish. Ask us if anything is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

We are interested in the clinical consequences (disease progression and worsening of symptoms) of tobacco smoke and respiratory diseases such as asthma on Multiple Sclerosis (MS). Although the increasing importance of MS has prompted a surge in research into the disease, there are still many gaps in

our knowledge and understanding of MS. Little is known about its physiopathology, epidemiology, genetic and environmental factors including the effect on stress levels, smoking status, quality of life and level of disability. Since the frequency of adverse behaviors is high among many chronic disorders (such as cancer, heart diseases, diabetes and etc), environmental factors may be identified via the frequency of abnormal or adverse behaviors in MS patients. Smoking shares many of the pathological consequences of chronic inflammatory conditions. For the first time we may be able to identify an environmental factor which can contribute to the development and progression of vascular complications in MS.

This study will provide important information to the health professionals aiming to develop effective interventions to prevent further progression and treat MS by advancing our understanding of the cause and care of MS. We believe that there is a need to determine the smoking levels in our patient population in order to develop and target future interventions appropriately to improve MS management as well as MS prevention in at risk populations.

We aim first to identify the frequency of smoking amongst MS population and second, to identify a link between disease progression and patients' smoking status. We intend to examine if smoking can be recognized as a factor, which can speed up the process of transition to Secondary Progressive MS over shorter period. This will allow for the development of a broad understanding of the disease and its co-morbidities across a large sample of individuals. We aim to identify target areas where new preventative interventions and treatment interventions could be developed and implemented to significantly improve patient quality of life.

We need to collect data from our MS population to allow us to compare the results with healthy population in order to identify the scale of the problem in MS patients and develop effective awareness and screening strategies.

Why have I been chosen?

We are looking to recruit 1500 people with MS. Participants will be recruited from Nottingham University Hospital MS clinic those enrolled in the Queen's Medical Centre MS clinic registry. You must have a diagnosis of MS made by the referring neurologist and be over 18 years of age.

Do I have to take part?

No. It is up to you to decide whether to take part. If you do, you will keep this information sheet and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason, without this affecting your future medical care.

What will happen to me if I take part?

This research just involves completing selected questions from four standardized questionnaires assessing your lifetime smoking history, asthma, allergy and levels of your disability due to the disease. You also need to sign the consent form to allow us to review your medical notes in order to estimate your level of disability based on neurological examination by neurologist. We also need to collect some MS related information about the results of your medical examinations such as your MRI report, Lumbar Puncture (if you had),

immunological tests and to see whether if you have ever received any treatment for MS.

What do I have to do?

Should you wish to take part in the study you will need to sign the consent form and complete the questionnaires and return them to the research team by the prepaid envelop provided.

What are the possible benefits of taking part?

The information we get might help improve the treatment of people with MS. More information on the relationship between smoking and the development and progression of MS, particularly in the UK setting, is vital to better understand the area for the development of preventative and treatment interventions. Because of the severe consequences of disease progression for individuals and the relatively high prevalence of disease transition from RRMS to SPMS in our MS population, it is important for us to characterize this condition in our patients and use this information to develop interventions specific to our population to improve their MS care and quality of life.

What happens after the research study stops?

After analysing the data, we will let you know the major findings if you wish.

What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed.

Complaints

If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your questions (Mr Ali Manouchehrinia or Professor Cris Constantinescu on 0115 87 54597). If you wish to complain formally, you can do this through the normal NHS complaints procedure. Details can be obtained from the hospital.

Harm

In the event that something does go wrong and you are harmed during the study, there are no special compensation arrangements. If you are harmed and this is due to someone's negligence you may have grounds for legal action for compensation but you may still have to pay legal costs. The normal NHS complaints mechanisms will still be available to you.

Will my taking part in the study be kept confidential?

Yes. We will follow ethical and legal practice and all information about you will be handled in confidence. If you join the study, some parts of the data collected for the study will be looked at by authorised persons from the University of Nottingham who is organising the research. They may also be looked at by authorised people to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and we will do our best to meet this duty.

During the study your data will be assigned a study identity code number, for use on study documents and the electronic database. Your data will be anonymized after data collection. Data will contain minimum identifying details sufficient to trace participants for audit purposes only.

All information that is collected about you during the course of the research will be kept **strictly confidential**, stored in a secure and locked office, and on a password protected database. All research data will be kept securely for 7 years. After this time your data will be disposed of securely. During this time all precautions will be taken by all those involved to maintain your confidentiality, only members of the research team will have access to your personal data.

What happens if I don't want to carry on with the study?

You can withdraw from the study at any time if you wish. Information collected up to the point of your withdrawal cannot be erased and with your consent may still be used. Any paper or electronic data that can still be identified as yours will be destroyed.

What will happen to the results of the research study?

Data will be analysed, written up and sent for publication in medical journals or international conferences. Some part of this study will be written up as part of a PhD thesis. We will also inform you by letter of any significant findings if you wish, and we can send you copies of the published papers at your request.

Who is organising and funding the research?

The Division of Clinical Neurology, C Floor, Medical School, University of Nottingham, Queen's Medical Centre, Nottingham, NG7 2UH.

Who has reviewed the study?

All research in the NHS is looked at by independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by the Derby 1 Research Ethics Committee.

Contact details

If you have any queries about the study, please contact Mr Ali Manouchehrinia or Prof Cris Constantinescu, Division of Clinical Neurology, C Floor, Medical School, Queen's Medical Centre, Nottingham, NG7 2UH. Phone: 0115 87 54597

Email: msxam3@nottingham.ac.uk

Thank you for taking the time to read this leaflet and considering taking part in this study.

Appendix 3; Questionnaire booklet

The University of
Nottingham

Questionnaire Booklet

WHAT DO I NEED TO DO?

➤ Please answer **ALL** the questions on the following pages simply by putting an X or filling the box which you think most nearly applies to you.

➤ Once you have completed the questionnaire, please sent it to the Division of Clinical Neurology by the prepaid envelop provided. (**Please do not forget to include your signed and dated consent forms (retain a copy for your records)**)

➤ If you have any questions regarding the completion of this questionnaire please contact the research team. You can find our contact details in patient information sheet.

➤ Date of Birth: .... Sex:.................... Initials: ....

PART 1; Asthma and Allergy

1.   Have you ever had asthma?   YES | NO
IF 'NO' GO TO QUESTION 1.11, IF 'YES':

1.1   Was this confirmed by a doctor?   YES | NO
1.2   How old were you when you had your first attack of asthma?
1.3   How old were you when you had your most recent attack of asthma?

1.4   Did your father ever have asthma?   YES | NO
1.5   Did your mother ever have asthma?   YES | NO

1.6.   Which months of the year do you usually have attacks of asthma

1.6.1 January / February
1.6.2 March / April
1.6.3 May / June
1.6.4 July / August
1.6.5 September / October
1.6.6 November / December

1.7   Have you had an attack of asthma in the last 12 months?   YES | NO
1.8   How many attacks of asthma have you had in the last 12 months?
1.9   How many attacks of asthma have you had in the last 3 months?
1.10   Are you currently taking any medicines including inhalers,   YES | NO
aerosols or tablets for asthma?

1.11   What term best describes the place you lived most of the time
when you were under the age of **five** years?

a) farm   a
b) village in a rural area   b
c) small town   c
d) suburb of a city   d
e) inner city   e

2.   Do you have any nasal allergies, including hay fever?   YES | NO
IF 'NO' GO TO QUESTION 3, IF 'YES':
2.1 How old were you when you first had hay fever or nasal allergy?

3.   Have you **ever** had eczema or any kind of skin allergy?   YES | NO
4.   Have you had Appendectomy?   (the surgical removal of the appendix)   YES | NO
      Have you had Tonsillectomy?   (the surgical removal of the tonsils)   YES | NO
5.   Have you **ever** had an itchy rash that was coming and going for   YES | NO
at **least 6 months**?

PART 2; SMOKING

6. Have you ever smoked for as long as a year?   `YES | NO`
['YES' means at least 20 packs of cigarettes or 12 oz (360 grams) of tobacco in a lifetime, or at least one cigarette per day or one cigar a week for one year]
IF 'NO' GO TO QUESTION 6.6, IF 'YES':

6.1 How old were you when you started smoking?
6.2 Do you now smoke, as of one month ago?   `YES | NO`
IF 'NO' GO TO QUESTION 6.4, IF 'YES':

6.3 How much do you *now* smoke on average?

   6.3.1 Number of cigarettes per day
   6.3.2 Number of cigarillos per day
   6.3.3 Number of cigars a week
   6.3.4 Pipe tobacco in a) ounces / week
                          b) grams / week

6.4 Have you stopped or cut down smoking?   `YES | NO`
IF 'NO' GO TO QUESTION 6.5, IF 'YES':

6.4.1 How old were you when you stopped or cut down smoking?

6.5 *On average* of the entire time you smoked, before you stopped or cut down, how much did you smoke?

   6.5.1 Number of cigarettes per day
   6.5.2 Number of cigarillos per day
   6.5.3 Number of cigars a week
   6.5.4 Pipe tobacco in a) ounces/week
                          b) grams/week

6.6 **Have you ever smoked a cigarette, a cigar, or a pipe?**   `YES | NO`
   IF 'NO' GO TO QUESTION 7, IF 'YES':
6.7 Do you smoke cigarette at all nowadays?   `YES | NO`
6.8 How old were you when you started to smoke cigarette regularly
6.9 Do you smoke at least one cigar of any kind per month nowadays?   `YES | NO`
6.10 Have you ever regularly smoked at least one cigar of any kind per month?   `YES | NO`
6.11 Have you ever smoked a pipe regularly?   `YES | NO`
6.12 Do you smoke a pipe at all nowadays?   `YES | NO`

7. Have you been **regularly** exposed to tobacco smoke in the last **12 months**? ['Regularly' means on most days or nights]

YES	NO

7.1 Not counting yourself, how many people in your household smoke regularly?

7.2 Do people smoke regularly in the room where you work?

YES	NO

7.3 How many hours per day are you exposed to **other people's** tobacco smoke?

7.4 Please provide more information;
How many hours per day, are you exposed to other peoples tobacco smoke in the following locations?

       a) At home
       b) At workplace
       c)In bars, restaurants or similar social settings
       d) Elsewhere

7.5 Did your father smoke regularly during your childhood?

YES	NO

7.6 Did your mother smoke regularly during your childhood?

YES	NO

Now please go to part 3

PART 3; Multiple Sclerosis Impact Scale (MSIS-29)

- The following questions ask for your views about the impact of MS on your day-to-day life **during the past two weeks**

- For each statement, please **circle** the **one** number that **best** describes your situation

Please answer **all** questions

In the <u>past two weeks</u>, how much has your MS limited your ability to...	Not at all	A little	Moderately	Quite a bit	Extremely
1. Do physically demanding tasks?	1	2	3	4	5
2. Grip things tightly (e.g. turning on taps)?	1	2	3	4	5
3. Carry things?	1	2	3	4	5

In the <u>past two weeks</u>, how much have you been bothered by...	Not at all	A little	Moderately	Quite a bit	Extremely
4. Problems with your balance?	1	2	3	4	5
5. Difficulties moving about indoors?	1	2	3	4	5
6. Being clumsy?	1	2	3	4	5
7. Stiffness?	1	2	3	4	5
8. Heavy arms and/or legs?	1	2	3	4	5
9. Tremor of your arms or legs?	1	2	3	4	5
10. Spasms in your limbs?	1	2	3	4	5
11. Your body not doing what you want it to do?	1	2	3	4	5
12. Having to depend on others to do things for you?	1	2	3	4	5

Please check that you have answered all the questions before going on to the next page

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In the past two weeks, how much have you been bothered by...	Not at all	A little	Moderately	Quite a bit	Extremely
13. Limitations in your social and leisure activities at home?	1	2	3	4	5
14. Being stuck at home more than you would like to be?	1	2	3	4	5
15. Difficulties using your hands in everyday tasks?	1	2	3	4	5
16. Having to cut down the amount of time you spent on work or other daily activities?	1	2	3	4	5
17. Problems using transport (e.g. car, bus, train, taxi, etc.)?	1	2	3	4	5
18. Taking longer to do things?	1	2	3	4	5
19. Difficulty doing things spontaneously (e.g. going out on the spur of the moment)?	1	2	3	4	5
20. Needing to go to the toilet urgently?	1	2	3	4	5
21. Feeling unwell?	1	2	3	4	5
22. Problems sleeping?	1	2	3	4	5
23. Feeling mentally fatigued?	1	2	3	4	5
24. Worries related to your MS?	1	2	3	4	5
25. Feeling anxious or tense?	1	2	3	4	5
26. Feeling irritable, impatient, or short tempered?	1	2	3	4	5
27. Problems concentrating?	1	2	3	4	5
28 Lack of confidence?	1	2	3	4	5
29. Feeling depressed?	1	2	3	4	5

Please check that you have circled ONE number for EACH question

PART 4; Patient-determined Disease Steps

Please read the choices listed below and choose the one that best describes your own situation. **This scale focuses mainly on how well you walk.** You might not find a description that reflects your condition exactly, but please mark the **one** category that describes your situation the closest.

☐ 0 **Normal:** I may have some mild symptoms, mostly sensory due to MS but they do not limit my activity. If I do have an attack, I return to normal when the attack has passed.

☐ 1 **Mild Disability**: I have some noticeable symptoms from my MS but they are minor and have only a small effect on my lifestyle.

☐ 2 **Moderate Disability:** I don't have any limitations in my walking ability. However, I do have significant problems due to MS that limit daily activities in other ways.

☐ 3 **Gait Disability:** MS does interfere with my activities, especially my walking. I can work a full day, but athletic or physically demanding activities are more difficult than they used to be. I usually don't need a cane or other assistance to walk, but I might need some assistance during an attack.

☐ 4 **Early Cane**: I use a cane or a single crutch or some other form of support (such as touching a wall or leaning on someone's arm) for walking all the time or part of the time, especially when walking outside. I think I can walk 25 feet in 20 seconds without a cane or crutch. I always need some assistance (cane or crutch) if I want to walk as far as 3 blocks.

☐ 5 **Late Cane:** To be able to walk 25 feet, I have to have a cane, crutch or someone to hold onto. I can get around the house or other buildings by holding onto furniture or touching the walls for support. I may use a scooter or wheelchair if I want to go greater distances.

☐ 6 **Bilateral Support:** To be able to walk as far as 25 feet I must have 2 canes or crutches or a walker. I may use a scooter or wheelchair for longer distances.

☐ 7 **Wheelchair / Scooter:** My main form of mobility is a wheelchair. I may be able to stand and/or take one or two steps, but I can't walk 25 feet, even with crutches or a walker.

☐ 8 **Bedridden:** Unable to sit in a wheelchair for more than one hour.

Thank you very much for your cooperation

Appendix 4; Consent form

CONSENT FORM
(Final version 1.0: 15/04/2011)

Title of Study: EXPOSURE TO TOBACCO SMOKE IN PATIENTS WITH MULTIPLE SCLEROSIS, AN EPIDEMIOLOGICAL EVALUATION

REC ref: 11/EM/0158

Name of Researcher: Professor Cris Constantinescu
Name of Participant:

Please initial box

1. I confirm that I have read and understand the information sheet version number …………dated................... for the above study and have had the opportunity to ask questions.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without my medical care or legal rights being affected. I understand that should I withdraw then the information collected so far cannot be erased and that this information may still be used in the project analysis.

3. I understand that relevant sections of my medical notes and data collected in the study may be looked at by authorised individuals from the University of Nottingham, the research group and regulatory authorities where it is relevant to my taking part in this study. I give permission for these individuals to have access to these records and to collect, store, analyse and publish information obtained from my participation in this study. I understand that my personal details will be kept confidential.

4. I agree to take part in the above study.

_____   _____   _____
Name of Participant   Date   Signature

_____   _____   _____
Name of Person taking consent   Date   Signature
(if different from Principal Investigator)

_____   _____   _____
Name of Principal Investigator   Date   Signature

3 copies: 1 for participant, 1 for the project notes and 1 for the medical notes

Appendix 5; Reminder letter

REMINDER LETTER

(Final version 2.0: 16/05/2011)

Title of Study: EXPOSURE TO TOBACCO SMOKE IN PATIENTS WITH   MULTIPLE SCLEROSIS, AN EPIDEMIOLOGICAL EVALUATION

Dear Madam or Sir

We would like to remind you of our invitation regarding your participation in the above study. An invitation was mailed to you several weeks ago and so far we have heard nothing from you.

If you have already sent the package back to us, please disregard this notice. If you have not received our invitation and you wish to take part in the study please contact us. If you have received our invitation and you have not had time to go through it please take time and read the information sheet carefully. If you wish to take part in the study please sign the consent from, fill in the questionnaire and return them by the prepaid envelope provided.

Thank you in advance for your cooperation in this matter.
Contact Details;

Professor Cris Constantinescu or Mr. Ali Manouchehrinia,
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Centre, Nottingham, NG7 2UH
Phone: +44 (0)115 823 1441, +44(0)1158231038
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Yours sincerely,

Professor Cris S Constantinescu

Chair of Neurology

University of Nottingham

References