Insights from comorbidity into multiple sclerosis aetiology and outcomes

Homayoun Roshanisefat
Scientific environment

This study was conducted at the Multiple Sclerosis Centre, Department of Neurology, Karolinska University Hospital and at the Department of Clinical Neuroscience, and the Clinical Epidemiology Unit in the Department of Medicine at Karolinska Institutet from 2007 to 2014.

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INSIGHTS FROM COMORBIDITY INTO MULTIPLE SCLEROSIS AETIOLOGY AND OUTCOMES

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To whom:
- let me think
- let me work
- let me write
- let me learn
- let me shape
- let me wish
- let me believe that “Knowing is not enough; we must apply. Willing is not enough; we must do.”
- 
- 
- To my lovely family
ABSTRACT

Aim: The overall aim of this research was to investigate the nature of the comorbidities in multiple sclerosis (MS) in four large population-based studies. The first study assessed whether the diagnosis underlying appendicectomy is a useful marker of MS risk. The second study identified whether an inherited risk of other immune-mediated diseases (IMD) contributes to the likelihood of developing MS. In the third study, we investigated if MS is associated with a raised risk of cardiovascular disease (CVD) and whether this varies by MS disease course, while in the final study we determined if cancer is under-diagnosed in MS patients by assessing mortality following cancer as a marker of delayed cancer diagnosis.

Methods: This thesis uses Swedish register data to identify patterns of comorbid disease among subjects with a diagnosis of MS compared with subjects without MS. We have identified subjects with MS through the Patient Register (PR) and the MS Register (SMSreg) and subjects without MS through the Total Population Register (TPR). By using personal identity numbers and ICD codes, comorbidities among MS patients and diseases in those without MS were identified. These included appendicectomy, other immune-mediated disease, cardiovascular disease and all-cause mortality following cancer diagnosis. The study designs included in this thesis are a nested case-control study and three cohort studies.

Results: There was a lower risk of MS in the group with perforated appendicitis but it was not statistically significant.
Patients with MS had an elevated higher risk of other immune-mediated diseases while their parents had no increased risk.
MS patients were more at risk of CVD and in particular, more likely to suffer from deep venous thrombosis than subjects without MS. This finding was observed in all MS courses.
There was a lower magnitude raised risk of all-cause mortality after a cancer diagnosis in MS patients compared with the risk following cancer in the general population cohort.

Conclusions: We found equivocal evidence that association of acute appendicitis with MS risk may vary depending on the diagnosis underlying the appendicectomy. We found no convincing evidence of a raised risk of other immune-mediated diseases in the parents of patients with MS. MS sufferers themselves appear to have an increased risk of a diagnosis of several immune-mediated diseases. There appears to be a statistically significant increased relative risk of CVD in MS patients and it is of a notably high magnitude for venous thromboembolic disorders in progressive MS. We found a lower magnitude raised risk of all-cause mortality following a cancer diagnosis in MS compared with the mortality risk following a cancer diagnosis in a general population sample.

Keywords: Multiple Sclerosis, epidemiology, appendicectomy, comorbidity, cardiovascular, cancer, mortality,
List of Publications


IV. Roshanisefat H, Bahmanyar S, Hillert J, Olsson T and Montgomery S. "All-cause mortality following a cancer diagnosis amongst multiple sclerosis patients". (Submitted).
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# 1 LIST OF ABBREVIATIONS

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<th>Description</th>
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<tbody>
<tr>
<td>AID</td>
<td>Autoimmune diseases</td>
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<tr>
<td>AF</td>
<td>Atrial Fibrillation</td>
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<td>AP</td>
<td>Angina Pectoris</td>
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<td>BMI</td>
<td>Body Mass Index</td>
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<td>CR</td>
<td>Cancer Register</td>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<tr>
<td>CIS</td>
<td>Clinically isolated syndrome</td>
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<td>COPD</td>
<td>Chronic obstructive pulmonary disease</td>
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<td>CVD</td>
<td>Cardiovascular disease</td>
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<td>DMTs</td>
<td>Drug modifying therapies</td>
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<td>EDSS</td>
<td>Expanded Disability Status Scale</td>
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<tr>
<td>EBV</td>
<td>Epstein–Barr virus</td>
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<tr>
<td>Gad+</td>
<td>Gadolinium enhancement</td>
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<tr>
<td>GWAS</td>
<td>Genome-wide association study</td>
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<td>HLA</td>
<td>Human leukocyte antigens</td>
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<td>HF</td>
<td>Heart Failure</td>
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<tr>
<td>ICD</td>
<td>International Classification of Diseases</td>
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<tr>
<td>IMD</td>
<td>Immune-mediated disease</td>
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<td>IMT</td>
<td>Immunomodulation therapies</td>
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<tr>
<td>IRR</td>
<td>Incidence rate ratio</td>
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<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<td>MS</td>
<td>Multiple sclerosis</td>
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<tr>
<td>MHC</td>
<td>Major histocompatibility complex</td>
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<td>OCB</td>
<td>Oligoclonal bands</td>
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<td>OR</td>
<td>Odds ratio</td>
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<tr>
<td>PwMS</td>
<td>Patient with MS</td>
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<tr>
<td>PIN</td>
<td>Personal identity number</td>
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<tr>
<td>PP-MS</td>
<td>Primary progressive multiple sclerosis</td>
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<tr>
<td>PR</td>
<td>Patient Register</td>
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<tr>
<td>SMSreg</td>
<td>The National Swedish MS Register</td>
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<tr>
<td>SP-MS</td>
<td>Secondary Progressive Multiple Sclerosis</td>
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<tr>
<td>RR</td>
<td>Relative Risk</td>
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<tr>
<td>TNF</td>
<td>Tumour Necrotic Factor</td>
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<td>VEP</td>
<td>Visually evoked potential</td>
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A short foreword:

I am a neurologist doing clinical work on Multiple Sclerosis (MS). The experience and expertise that I have gained over the years through my professional work in the field of MS have taught me that MS is a complex disorder. When I began to collect and study cases with accidental discoveries of MS in early 2003, I became aware of a different aspect of MS, namely, patients without clinical symptoms that were being investigated by a specialist other than a neurologist. In another series of MS cases, I observed that an MS diagnosis was given to patients with other immune-mediated diseases after they had been exposed to anti-TNF-alpha therapies. These observations, my professional experience and my current research have made me curious about MS aetiology and related comorbidity.
2 INTRODUCTION

Multiple Sclerosis (MS) is a disease of the central nervous system (CNS) with heterogeneity in clinical course and neurological appearance. However, there is an incomplete understanding of its aetiology (1). Diagnosis remains difficult as no single clinical feature or confirmatory test is sufficient. MS has a variable onset and is described clinically by a mixture of symptoms and signs. MS is pathologically characterized by multifocal damage of myelin and axonal loss in the CNS, resulting in various physical disabilities cognitive or psychological impairments (2-4). MS tends to have its onset in young adulthood (5) with increasing disability over time.

As the disease progresses and destruction of the myelin sheaths protecting the nerves continues, continuously brain atrophy occurs with an associated decline in more physical and mental abilities (6, 7) (Fig. 1). Measures of disease activity worsen and severity increases with progression (8). The issue of comorbidity between MS and other diseases has gained increasing prominence in neurology internationally during the last few years (9, 10). There have been a number of studies of Swedish populations that include the presence of comorbid conditions in patients with MS. Some have reported a higher risk of congestive heart failure (11) and chronic respiratory diseases such as chronic obstructive pulmonary disease (COPD) (12), pulmonary embolism (13), IMD (14) and cancer (15), as well as some cardiovascular diseases (CVD) (16).

This thesis is concerned with using comorbidity in MS to elucidate aspects of the aetiology as well as prognosis following a diagnosis of MS. This has been achieved through epidemiological research that has made use of Sweden’s extensive national registers. Appendicectomy has been examined as a marker of early immunological characteristics, while risk of other IMD in parents was examined to identify possible inherited risks. The thesis is also concerned with outcomes following an MS diagnosis. One study assessed whether there is a raised risk of CVD in MS patients and another investigated and whether survival following cancer is shorter in MS patients than among the general population.
3 BACKGROUND

3.1 Worldwide distribution of MS
In Western countries generally, there is an increased incidence of MS over time (17), although with regional variations (18). The estimated regional risk in Scandinavia is almost the same for Sweden, Norway and Finland (4.1 - 5.3/100,000) (18-20). However, despite geographical similarity, in contrast to what was recently reported from Sweden (21), the Norwegian study group could not confirm a clear association between latitude and MS risk (22). The review of studies of association between latitude and MS suggests that environmental factors that vary by latitude – most prominently ultraviolet light (23) and vitamin D (24) – do play a more significant role.

3.2 Epidemiology of MS in Sweden
Epidemiological studies of MS rank Sweden as one of the high-risk areas for MS in Europe (25-29).

Incidence and prevalence of MS in Sweden:
The nationwide risk of MS in Sweden has increased in recent years. The regional incidence rate of 5.2 (4.4–6.2) per 100,000 per year was estimated for Västerbotten County for the period 1988–1997 (19, 30). At present, the suggested estimated incidence is 10/100,000/year (31). The prevalence in 2010 was estimated roughly to be around 190/100,000 (21).

Sex Ratio in MS:
An interesting feature of MS is that it disproportionately affects women more than men, as in many other immune-mediated diseases (32). Females are affected approximately more than twice as often as males (33). However, the female-to-male ratio (sex ratio) of MS appears to have been changing over time: 1.70 for birthdates in the 1930s to around 2.6 for subjects born in recent years (33). Data from two population-based studies are consistent in the finding that the increased frequency of MS in females arises from an increase in female relapsing-remitting MS (RR-MS) cases (29). However, RR-MS is the most common form of the disease and can partly explain the sex ratio differences in the initiating phase of the disease (34).

3.3 Clinical features of MS
The clinical symptoms of MS can vary, often coming and going without any specific pattern, which can in some cases make MS difficult to diagnose during the early phase. The most common clinical signs and symptoms, occurring in isolation or in combination, include sensory disturbance in the limbs, optic neuritis, acute and sub-acute motor dysfunction of the limbs, balance, and gait dysfunction (35). Symptoms may involve the entire ascending or descending axons and can sometimes predominantly affect the spinal cord and optic nerves (36, 37).

The rating of neurologic impairment in MS is mostly achieved by use of the expanded disability status scale (EDSS) (38). This scale only gives a rough picture of the disease
progression (39), but its repetitive measuring with progression can also be associated with CSF findings (40).

**Clinical course**
On the basis of symptoms and exacerbations, MS courses can be described in brief as described in some international reports (41, 42).

**Relapsing-Remitting course (RR-MS)**
Patients experience clearly defined relapses with episodes of acute deterioration of neurological function followed by remission after attacks and regain of function. This course of MS is the most frequent and the risk of disability is moderate. The RR-MS course is diagnosed in individuals who have at least one clinical attacks (each lasting ≥24 hours and separated by ≥1 month) or a slow, progressive course for at least six months (40). The new diagnostic criteria for MS from 2010 are based to a greater extent than previously on MRI finding and allows that sometimes even after the first relapse determine an MS diagnosis, if MR images can show that at least two inflammatory foci in time arises at least in two separate occasions (41, 43).

**Secondary-Progressive course (SP-MS)**
Patients in this phase of the disease experience a gradual worsening of disability, with or without occasional relapses or minor remissions (44). Diagnosis of SP-MS is usually straightforward except during the conversion stage when the two courses RR-MS and SP-MS merge (44).

Figure (1):
The figure depicts the natural course of MS. The preclinical phase of the disease can be identified as radiologically isolated syndrome (RIS), clinically isolated syndrome (CIS) and then courses of MS described as follows. The best established knowledge of MRI comes from studies done on these courses and shows chronological changes. Alongside disease progression, the volume of brain diminished and MRI series showed more atrophy. The corpus callosum damage is the major cause of most of the brain atrophy and thereby the cognitive impairment over time (6, 45).
Primary-Progressive course (PP-MS)
Diagnostic criteria for PP-MS include a minimum period of clinical progression of at least 12 months and onset between the age of 25 and 65 years. This course is not associated with distinct relapses or remissions. However, there are variations in rate of progression over time, sporadic plateaus, and temporary minor improvements, although with a persistent steady reduction of mobility over time (Fig. 1) (46).

Progressive-Relapsing course
Patients with this type of MS experience a steady deterioration of symptoms from the onset but also have clear acute relapses, with or without recovery. In contrast to the RR course, the periods between relapses are defined by ongoing disease progression (47). This progression often entails more intensive uninterrupted treatment (47).

3.4 Pathology and pathological subtypes
The MS plaque is the hallmark of its pathology. The cores of the plaques are filled with a central vein that is surrounded with inflammatory components and demyelination which, on gross examination, appear as greyish foci within otherwise dense white matter (48). Histologically, plaques contain constituents of cellular and humoral immune components (e.g. lymphocytes, macrophages, natural killer cells and antibodies) and may demonstrate marked heterogeneity (49). Early on in the disease, remission of symptoms is likely to be due to resolution of inflammation (49), channel redistribution, and re-myelination following initial neurodegeneration (50); however, after frequent attacks, axonal damage is more likely to occur and axonal loss accumulates. Hence the balance between injury and repair probably determines the progression of MS (36) with progressive neurodegeneration (51). Within active inflammatory MS lesions, the transected axons are a consistent feature of the MS lesions (Fig. 2) (48, 52).

Figure (2):
The figure below illustrates the pathological subtype of MS in different courses of MS. Subtypes I and II show ongoing inflammatory attacks while the other shows a slower degeneration of myelin.

Pathological evidence indicates that subtyping based on clinical courses alone does not reflect the actual course of MS and the heterogeneity of MS courses. Analyses of biopsy and autopsy specimens have identified at least four histopathological
subtypes with respect to oligodendrocyte/myelin pathology and immunopathology. The most commonly observed lesion in RR-MS is the type II plaque. This lesion contains T-cells, B-cells, antibody and complement deposition, and has the typical look of inflammation in the surrounding veins. Other types of plaque include damage to oligodendrocyte. These subtypes of MS plaque are associated with heavy macrophage activation, T-cell infiltration and expression of inflammatory mediator molecules (35, 48).

The neuroimmunological involvement in each subtype of T- or B-cells is critical. Regulatory T-cells downregulate the attacks in MS while the Th1 CD4+ subset is widely recognized as a critical lymphocyte population for initiating inflammation. Despite the critical role of T-cells in MS pathogenesis, macrophages are the final vectors of tissue destruction. Upon infiltrating the CNS, activated T-cells secrete cytokines that in turn recruit activated macrophages, resulting in tissue destruction (49, 53) (Fig. 2).

3.5 Diagnosis
MS is primarily a clinical diagnosis. However, there are a number of laboratory and radiological examinations that are used to confirm the diagnosis. More recent criteria specifically integrate MRI findings in parallel with clinical and paraclinical observations (36). MS criteria can be divided roughly to the time before MRI use in diagnosis of MS (the Schumacher Committee developed the first official criteria for the diagnosis of MS in 1965, from which all subsequent criteria have been derived) and subsequent to the introduction of MRI (the Poser Committee generated the Poser criteria in 1983). The most recent updated criteria of MS were reviewed in 2013 (41).

Criteria for MS diagnosis
MRI has been used to define the Poser diagnostic criteria for MS, and, most recently, the McDonald Criteria, which have undergone three further revisions since their introduction in 2001. These criteria are used for the diagnosis of the majority of our MS subjects.

(a) The Poser criteria described the following:
a.1 Definite MS, subdivided into clinically definite MS or laboratory-supported definite MS.
a.2 Probable MS, subdivided into clinically probable MS or laboratory-supported probable MS (54).

(b) The McDonald criteria
The new revised McDonald criteria were published in 2011 and they cover clinical and imaging features in both adults and children who have clinical symptoms suggestive of a first episode of MS (55, 56). The McDonald criteria describe MS as:
b.1 Definite MS
The diagnosis of MS is constructed on the finding of MS-typical CNS lesions disseminated in space and time based upon clinical findings alone or a combination of clinical and MRI findings.

b.2 Possible MS (42, 57).

MRI
MRI was a diagnostic breakthrough in MS and was introduced in Swedish hospitals in the early 1980s. Pathological findings in MRI, e.g. Dawson fingers, are now internationally considered in the evaluation of MS disease burden. The medullary veins in MS lesions can confirm relatively specific signs for MS when investigation is performed by the newer generation of MRI (callososeptal location) (56, 58, 59).

Worldwide, MRI has improved the definition of what constitutes a relapse and what signifies inflammation. This has helped in the identification of novel imaging features that are likely to have an important role in the diagnostic work-up of patients with suspected MS and its differentiation from other diseases and progression (36, 56, 60).

Cerebrospinal fluid
The investigation of cerebrospinal fluid (CSF) was a necessary part of MS diagnosis long before the launch of MRI in Sweden. Nowadays, this procedure is still an important diagnostic tool (61), especially in some patients where MRI scanning may not be entirely conclusive due to the dissemination of lesions in space/time and because T2 white-matter lesions may be somewhat uncharacteristic. Particularly in PP-MS, the spinal cord MRI and the brain MRI may not conclusively indicate dissemination in space. It is in this group of patients with few or somewhat atypical brain white-matter lesions that examination of the CSF immunopathy findings is of helpful diagnostic value. Information on the oligoclonal bands (OCB) in CSF is useful in making an MS diagnosis (62-64).

Additional novel newer biomarkers for MS include osteopontin, TNF-alpha, various cytokines and chemokines and \( \alpha \)-crystalline. However some variation in sensitivity and specificity is reported (36, 65).

Evoked potentials
An evoked potential test measures the time it takes for nerves to respond to stimulation. Visual evoked response or potential (VER or VEP), Somatosensory evoked response or potential (SSER or SSEP), and Auditory brain stem evoked response or potential (ABER or ABEP) can also be used in differential diagnosis of MS (36, 55). Some suspected cases of MS, especially PP-MS, are referred for some of these neurophysiological investigations to exclude other differential diagnoses (55).
4 RISK FACTORS FOR MS

Genetic characteristics have been studied to identify potential risk factors for the disease. Leading environmental risk factor candidates at present include infections (especially early-life viral infection) (66), ultraviolet light exposure and vitamin D status (67), and inconsistent patterns of association with smoking (68, 69), psychosocial stress (70) and socioeconomic circumstances (71). There is thought to be an interaction of environmental risks with genetic factors, thus influencing the risk of developing MS (72, 73).

4.1 Genetic risk factors

4.1.1 Risk genes for MS

Available data suggest that MS is inherited as a complex multifactorial disorder that results from the interaction of genetic and environmental factors (74). The genetic component was originally only mapped to the human leukocyte antigen (HLA) region (75). However, genetic studies of large samples of patients and controls obtained through the International Multiple Sclerosis Genetics Consortium (I.M.S.G.C.) have identified a list of more than 100 MS risk-gene regions in addition to HLA-DRB1 (76). Today, many risk alleles have been discovered and the risk associated with each of these loci is low (77-80). In other words, it is likely that the risk genes contribute or indirectly influence interactions with the currently known environmental risk factors vitamin D, Epstein-Barr virus, and smoking (73).

**HLA and Non-HLA genes**

The HLA region on chromosome 6p21, also known as major histocompatibility complex class II (MHC II) genes, encodes cell surface α/β heterodimeric glycoproteins that present foreign antigens to T lymphocytes. There are three MHC II isotypes: HLA-DR, -DQ, and -DP encoded within the MHC II locus (81-83). The strongest association with MS has been observed in the DR2 serotype, which is refined with DNA-based typing methods to the DRB1*1501 allele (84). However, it has been hard to establish precisely whether the functionally relevant effect derives from the DRB1*1501, DQA1*0102-DQB1*0602, or DRB5*0101 loci of HLA-DR15 haplotype, their combinations, or their epistatic interactions. Nevertheless, most genetic studies have indicated DRB1*1501 as an important risk factor in MS (80, 85-87) (OR, 8.3, 95%CI: 4.8–14.5) (88). The HLA significant risk determinants in MS have been indicated to be less strong in Chinese meta-analysis than in Western MS populations (89). Conversely, non-HLA genes, e.g. IL7R gene, are also reported to have a critical role in MS neuroimmunology (90).
Single nucleotide polymorphisms and genome-wide association studies
A linkage study using a high-density single nucleotide polymorphism (SNP) map in 730 multiplex families resulted in overwhelming evidence for the involvement of the HLA region in influencing MS risk. The large number (typically 500,000 to 1,000,000) of tests covered most (>80%) of the common variation throughout the genome in genome-wide association studies (GWAS). The risk in non-HLA alleles had modest effect sizes, 1.1-1.2, and the major signal from the HLA region with an odds ratio of 3. The established variants of non-HLA together with the HLA variants are estimated to explain 25% of the sibling recurrence risk (91-94).

4.2 Environmental risk factors
Some environmental risk factors have aroused much interest among researchers in MS (68, 95-97), but in this thesis I will only describe the most frequently reported factors which have been attracting the greatest current interest for their potential role in MS pathogenesis.

Sunlight and vitamin D
Several genetic and environmental risk factors for MS may operate through the influence of vitamin D (98, 99). The research results are not consistent, when investigating the association between MS and vitamin D.

Figure (3):
The figure depicts a simplified pathway for sunlight-generated vitamin D in the skin to its target receptors in the nucleus of the cell (Open source: Sage publication Ltd 2013). VDR=vitamin D response, RXR=retinoid X receptor, VDRE= vitamin D response element.

Some studies have found that a low 25-hydroxyvitamin D level is associated with increased MS risk (100-102). Other studies suggested that the MS risk was lower among women whose mothers, while pregnant, had increased vitamin D intake. The risk was lower among women who had received vitamin D supplements in adolescence (103) but another recent study determined this result to be due to bias (104). A newly published Swedish study did not show any association between low vitamin D at birth and risk of MS later in life (105). With focus on MS course,
some longitudinal studies have shown an association between high 25-hydroxyvitamin D levels and lower relapse rates in patients with MS (101). A meta-analysis has found no significant association between high-dose vitamin D treatment and risk of MS relapse (OR, 0.98, 95% CI: 0.45–2.16) (106). Experimental findings revealed that administration of calcitriol could prevent the progression of experimental allergic encephalomyelitis (EAE). Vitamin D3 showed a beneficial effect on EAE severity in female mice. In contrast, low vitamin D could reduce EAE severity in mice whose mothers were vitamin D deficient (107). To summarise, the causal role of vitamin D in MS needs to be established through more population-based studies (105, 106).

**Smoking**

Case-control, cohort and meta-analysis studies cumulatively suggest that cigarette smoking is associated with increased MS risk (68, 69, 108, 109). Higher consumption of cigarettes (15 cigarettes per day for 15 years) results in greater risk (OR: 1.6, 95% CI: 1.4–2.0) (68) compared with people who have never smoked (never-smokers). There are probably important interactions between smoking and other environmental and genetic risk factors for MS development (110).

A meta-analysis combining data on 3,052 MS patients and 457,619 controls showed that smoking is associated with increased MS susceptibility (RR: 1.48, 95% CI: 1.35–1.63) (69). Interestingly, another study has also found a modest increased risk in never-smokers who had never been exposed to passive smoking (OR: 1.3, 95% CI: 1.1–1.6) (111) compared with smokers. The association of smoking and MS courses has also been studied and the findings are conflicting. However, in general, smoking is understood to be a potential modifier of the progression of MS (68, 109, 112).

**Infections in early life**

The hypothesis that MS could be the result from an aberrant immune response possibly triggered by delayed exposure to a common childhood infection (113) is in part based on indirect evidence from migration studies (114). Several studies have related the risk of MS to age of childhood infections such as measles, rubella, varicella, mumps, pertussis and scarlet fever (66, 115-117) and EBV (17). However, results have been inconclusive, whereas meta-analysis of the results found increased risk of EBV seropositivity (OR: 4.5, 95% CI: 2.8 - 7.2). This risk of MS suggested by funnel plot examination (sometimes referred to as a test for small study effects) has shown asymmetry and the risk of seropositivity was no longer significant (OR:1.4, 95% CI: 0.9 - 2.1) (118). It suggested publication bias for the reporting of the increased antibodies against EBV. A new Scandinavian study has reported evidence that the EBV antibodies in MS patients follow the effect of INF treatment (119).

The initial EBV infection occurs at an early age and is either asymptomatic or it produces some symptoms masked with many other childhood illnesses. However, in highly developed regions such as Northern Europe, the initial infection is
probably delayed until a later age. This viral infection seems specifically to target the epithelial cells of the oropharynx and the B cells and latently produce the antibodies which can attack CNS. However, this happens only in 1/900 of those subjects with previous mononucleosis (17). Several other hypotheses, like genetic (120) or transmitted (121) pathways, have been proposed to explain the links between EBV and MS risk. However, the evidence is inconsistent and these gaps require further research (122).

**Stress factors**

It is suggested that psychosocial stress can affect the risk of MS onset and its clinical course (123, 124). There are also review papers and meta-analysis reports (125, 126) that suggest a modest association, that stressful life events are associated with an increased risk of MS exacerbations in weeks or months after exposure (126). But it is not clarified whether life with higher stress may increase the risk of developing MS (70). Information about the biological mechanisms that mediate stress-MS relationship comes primarily from animal studies of experimental autoimmune encephalomyelitis (EAE) model (127, 128).

**Socioeconomic circumstances**

In general, reports on socioeconomic position are not consistent in risk association with MS across America or Europe. But regionally, in California, the groups with higher socioeconomic status, as measured by levels of education, occupation, income, profession, migration rate or dependency on social welfare support, have an increased overall risk of MS (71). This association in Europe is somewhat different as a Danish cohort study determined a slightly reduced risk of MS in children of mothers who had higher education (RR: 0.86; 95%CI: 0.76 - 0.97) (129). Conversely, a Swedish study showed parental age to be more important than the parents’ socioeconomic position (130). A study from France with a focus on socioeconomic status and social stress confirmed that MS appears to be more aggressive in North Africans dependent on the social welfare system than in European patients living in stressful regions in France without economic welfare support (131).
5 IMMUNOMODULATION THERAPIES (IMT):

5.1 Conventional immune modulating therapies
All current treatments and those that are about to enter the market act on systemic immunity. There is compelling evidence that treatment strategies targeting systemic immune activation or migration of immune cells to the brain and spinal cord prevent relapses in MS and has recently been reviewed by Olsson and Bridel (132, 133). There are currently a large number of treatments with well-documented effects on disease progression in MS. Common to all of these treatments is that they have shown effects on what is often called "the inflammatory component" in MS and new enhancement and changes on MRI. For all currently approved drugs for MS, it has been documented that these treatments reduce the progressive development of disability and they are categorised as first line or second line IMT (57, 132, 133).

However, it is important not to see this as evidence that these treatments are effective in progressive MS in the way we talk about progression when discussing PP-MS and SP-MS, but rather see it as if they reduce the accumulated disability arising from discrete inflammatory processes in form of relapses and well-demarcated lesions on T2-weighted images on MRI (134). In the reported studies of this thesis, we focused on those IMT which were approved in Sweden prior to 2006.

A. First-line immunomodulation therapies
Interferon beta and glatiramer acetate have been the mainstays of treatment in relapsing-remitting MS for around two decades. Interferon-β has been known since interferons (IFN) were first described in 1957 (135). Interferon-β preparations have a similar mechanism of action, although initial testing of these agents was based on the notion that MS was caused by a virus. Since it was believed that the natural course of MS could be altered, glatiramer acetate (Copaxone) was also added to first line therapy of MS; however with effect and side-effect (136, 137). These MS treatments have been available for many years but they are only partially effective in reducing annualised relapse rates (132, 133). On the other hand, with no major long-term safety concerns, despite the disturbing flu-like side-effects on injections and skin reactions, they are recommended as first-line therapies (135).

Corticosteroids
The use of corticosteroids in MS has primarily focused on delay of exacerbations, but there is evidence to support the use of steroids as a preventative therapy. Data from the Optic Neuritis Treatment Trial (ONTT) suggest that the use of high-dose steroids reduces the risk of development of MS at 2 years following the initial optic neuritis event (138). Furthermore, a randomized, controlled phase II trial utilizing pulsed high-dose methylprednisolone over the course of 5 years showed a reduction in brain atrophy (139).
B. Second-line immunomodulation therapies (IMT)

Natalizumab:
The first monoclonal antibody therapy for MS was approved in Sweden in 2004. Natalizumab blocks migration of leukocytes from the vasculature into the parenchyma of the brain, resulting in reduced inflammation (140). The latest Cochrane report considers this drug as the most effective IMT compared with INFs and GA (141).

Fingolimod
Fingolimod is an oral sphingosine-1 phosphate (S1P) receptor modulator that also reduces the risk of disability progression with a probability of disability progression (confirmed after 3 months) of 17.7% at the 0.5 mg dose. Almost 90% of patients receiving Fingolimod, at either dose, were free of enhancing lesions over the course of 2 years and approximately 50% were free of new or enlarging T2 lesions (142).

5.2 Non-conventional immune modulating therapies
This small group of drugs are containing a variety of treatment for MS and they are not considered in our studies as they were not licensed as IMT for MS.
6 Comorbidity as studied in this thesis

Study I: Appendicectomy and multiple sclerosis risk.

An apparent protective effect of acute appendicectomy for the risk of other IMD has been examined in previous studies, such as for ulcerative colitis (143, 144). Acute appendicitis, particularly at a younger age, is reported to be inversely associated with ulcerative colitis (UC) (OR=0.58; 95%CI: 0.38 - 0.87) (143, 145-147), but this risk reduction was not evident in subjects with appendicectomy due to non-specific abdominal pain (143, 145). It seems that it is not appendicectomy per se that reduces the risk of UC; it is rather the exposures or characteristics associated with appendicitis. A similar but somewhat weaker association has also been reported for coeliac disease (CD) (144) with lowered risk for appendicitis (identified through a perforated appendix) but not appendicectomy for other underlying causes.

The association between appendicectomy and MS has been investigated previously with inconsistent results. Poskanzer et al and Lamoureux et al have shown that patients with MS have an increased (148) or identical (149) likelihood of having an appendicectomy when compared with individuals without MS. Both studies were based on a small number of patients and did not consider the diagnosis underlying appendicectomy in their estimations (148-150). This may account for the variation in results. Without other information, it has been suggested that a perforated appendix is the most reliable indicator of acute appendicitis, while appendicitis without perforation and mesenteric lymphadenitis may be somewhat more heterogeneous.

Study II: Shared genetic factors may not explain the raised risk of comorbid inflammatory diseases in multiple sclerosis.

An excess occurrence of inflammatory diseases has been reported in MS patients (14, 151), suggesting that these diseases share inherited risks with MS. Such risks could include genes of the HLA complex, and the class II genes in particular, which are associated with many inflammatory diseases (152). There are also non-HLA genes that could be shared between MS and other inflammatory diseases (153). Different HLA alleles tend to predispose for different organ-specific inflammatory diseases (154, 155), so it is possible that the clinical manifestation of some of the other diseases may be altered in MS patients. For example, the HLA haplotype HLADRB1*15:01 predisposes for MS, while this allele is protective against T1D (14, 156), even though there may be other shared inherited risks (156). Furthermore, the epitope alleles within the HLA-DRB1 gene also share a common feature for rheumatoid arteritis (157). By assessing the manifestation of IMD in parents of subjects with MS, it may be possible to further illuminate inherited shared risks for MS and IMD. As a consequence of a complex model of inheritance, there will be a greater variation of HLA genotypes among first-


degree relatives of MS patients than among MS patients themselves (158). Disease associations in relatives could potentially be less influenced by MS HLA genotypes and provide more information on other hereditary factors (14, 151, 158-160).

Previous studies have reported an increased risk of immune-mediated disorders such as ulcerative colitis, and Crohn’s Disease, (14, 151, 161) psoriasis, (14, 102, 162) and T1D in parents of patients with MS (14, 163). However, some of these studies may have had methodological limitations leading to reporting bias or they have investigated a relatively large number of diseases and their sub-diagnoses, and are therefore subject to the risk of reporting chance associations (151).

**Study III: Multiple sclerosis clinical course and cardiovascular disease risk - Swedish cohort study**

There are some recent reports that suggest an increased risk of cardiovascular events in patients with MS (16, 164). These cardiovascular events have been linked with the recent introduction of some forms of IMT (165) for patients with MS. Another study has connected a higher risk of CVD with immobility, which is more common as disability progresses in MS (166).

Previous studies have reported an overall increased risk of CVD in MS (16, 164, 167-170); however in the latest review of CVD risk in MS, none of the included studies have examined the risk of CVD by MS course (171). The risks accumulated for CVD (171) may vary by MS course as it characterises the pattern of disease progression (172). Characteristics and exposures associated with MS progression (173) and potentially with CVD risk could include reduced physical activity, immobility, raised infection risk, inflammation and other sequelae of worsening chronic disease (170, 174, 175).

Here, with the focus on risk of CVD in each respective MS course, we use data from the National Swedish Multiple Sclerosis Register, as information on MS course is not usually available from hospital inpatient discharge summaries. The follow-up period for this study was at the time before the introduction of some MS therapies linked with CVD risk; thus it focused on other MS-associated factors that may influence CVD risk.

**Study IV: All-cause mortality following a cancer diagnosis amongst multiple sclerosis patients: a Swedish population-based cohort study**

The risk of cancer appears to be reduced in MS patients (15). It is not well understood whether the lower risk in subjects with MS is because of MS-related characteristics, or if it is influenced by ‘diagnostic neglect’, where cancer symptoms and signs are overlooked and attributed to MS. If cancer diagnoses are more often delayed in MS patients, then the mortality risk could probably be adversely influenced (176). As MS is associated with excess mortality from a variety of causes (177), cancer-specific mortality may be subject to problems with competing risks. Therefore, we used all-cause mortality as the outcome following a cancer diagnosis in people with and
without multiple sclerosis. It was necessary to consider the statistical interaction of a cancer diagnosis together with whether an individual had MS to assess whether the risk of excess mortality following cancer was of a higher magnitude among MS patients. This interaction testing helps to take into account differences in longevity among those with and without MS.
7 SPECIFIC OBJECTIVES OF EACH STUDY

The specific objectives of the four studies described in this thesis were as follows:

7.1.1.1 Study I
To test the hypothesis that acute appendicitis, but not appendicectomy with other underlying diagnoses, is a marker of immune function that may be relevant to the risk of MS later in life.

7.1.1.2 Study II
To test the hypothesis that the occurrence of immune-mediated diseases (other than MS) is increased in parents of MS patients. By examining these diseases in the patients themselves, it will be possible to assess if the pattern of risk is different. This could help to identify possible inherited risks of MS.

7.1.1.3 Study III
To test the hypothesis that the accumulation of CVD risks may vary by MS course as it signals a pattern of disease progression, such that the association with CVD risk differs by course.

7.1.1.4 Study IV
To test the hypothesis that the reported reduced risk of cancer in MS patients is influenced by diagnostic neglect: we hypothesise that if diagnostic neglect is common, cancer diagnoses will be delayed and thus the mortality risk will be increased. This involves testing whether survival following a cancer diagnosis is lower in subjects with MS than among those without MS.
8 STUDY POPULATIONS AND METHODS

8.1 General information about materials and methods

All of the studies presented here used information from Swedish population registers. All patients who received a diagnosis of MS in Sweden between 1964 and 2005 in the Patient Register (PR) were identified. MS patients registered in the national Swedish Multiple Sclerosis Register (SMSreg) were also identified. Parents of those with and without MS were identified through the Multi-Generation Register. Information on dates of death, immigration and emigration was provided by registers held by Statistics Sweden. The PR provided data on other diseases and procedures of interest: appendicectomy, cardiovascular diseases, immune-mediated diseases and cancer.

Matching:
Subjects with MS were individually matched with up to 12 (fewer in a small minority) individuals without the disease identified among the general Swedish population by Statistics Sweden. The matching criteria were date of birth, sex, vital status, region of residence and age at the time of diagnosis in the matched population. This matching was used in all four studies to increase precision or limit substantial bias or confounding.

8.2 Details of the registers

8.2.1.1 Total Population Register
Since 1947, every resident in Sweden has been registered in the Total Population Register (TPR) (Swedish: folkbokföring) and administered by the Swedish Tax Agency (Skatteverket). Each person is provided with a 10-digit personal identity number (PIN). This unique PIN has been used in research to link information from the registers. The TPR has provided us with information about emigration, immigration, socio-economic index (SEI), and region of residence.

Socio-economic Index drawn from the Population and Housing Census.
Population and housing censuses were conducted periodically between the years 1960 and 1990, (in 1960, 1970, 1980, 1985 and 1990). The socio-economic index (SEI) was based on occupation and used in all adjusted estimations in studies I-IV. SEI was categorized into manual workers, non-manual workers, professionals, self-employed, farmers, and others in Papers I-IV (15).

Studies I-III comprise data from 24 areas of Sweden that approximate to Sweden’s counties. Study IV categorises geographical places according to the six health centres of Sweden including the medical regions Umeå, Uppsala, Stockholm, Linköping, Göteborg and Lund-Malmö that are relevant to the provision of cancer care (173).
8.2.1.2 Swedish Multiple Sclerosis Register

The first Interactive Database for Multiple Sclerosis (IDMS), for several local Swedish databases, was developed at Huddinge University Hospital (now integrated into the Karolinska University Hospital) in Stockholm in 1995 (178). The National Swedish MS Register, SMSreg, was established in 1996 and then used a web-based interface from 2004 covering most of the geographical area of all the hospitals in Sweden. This register is now part of the Swedish neuroregister and more than 60 healthcare units from all counties across Sweden participate in the daily updating (178). Now with an estimated complete national coverage, and contains information on close to 14,600 Swedes diagnosed with MS (33). A previous study has confirmed over 90% diagnostic accuracy for patients included in the SMSreg (15).

The Swedish MS register (SMSreg) was established:
- To collect selected information about all subjects with MS living in Sweden
- To provide information about the distribution of MS care in Sweden
- To ensure treatment compliance and assess appropriateness of treatment indications
- To evaluate the effect of short-term effects of selected treatments
- To evaluate the long-term efficacy of modern immune modulation medications
- To create a base for epidemiological studies on a national level
- To enable collaboration for planned and future international projects (178).

8.2.1.3 The National Patient Register

This register is held by the National Board of Health and Welfare (179). Statistics on diseases and surgical treatments in Sweden have been publically published for more than 100 years (180).

In the 1960s, the National Board of Health and Welfare started to collect information on inpatients at public hospitals, the National Patient Register (PR).

Since 1987, the NPR has included all in-patient care in Sweden. PR includes 50 million discharges for the period 1964-2006. Since 2001, it has also contained information on outpatient visits including day surgery and psychiatry from both private and public care-givers. At present, the PR is updated once a year.

The register contains the following information:
- Patient data (personal identity number, sex, age), geographical data (county council, hospital/ clinic, department), administrative data (inpatients or outpatient; date of admission, date of discharge, length of stay, acute/planned admission, admitted from, discharged to), medical data (main diagnosis, secondary diagnosis, external cause of injury and poisoning, procedures). The PR has used several ICD classifications, starting with ICD-7 (1964-1968), ICD-8 (1969-1986) then ICD-9 (1987-1996). ICD-10 was introduced in 1997, with the exception of the county of Skåne where ICD-9 was still in
use throughout 1997. The accuracy of diagnoses recorded in the Patient Register is generally between 85-95%, but this can vary by disease (180).

8.2.1.4 *Multi-generation Register*
The Swedish Multi-generation Register (MGR) allows linkage between first-degree relatives. Information mainly exists for subjects born after 1931 and who were alive in 1961 when the register was established.
In 1961, the first census was conducted which was computerized and later used to serve as a basis for the MGR. The database has been used extensively in epidemiological research to assess familial disease risks (14, 15, 181).

8.2.1.5 *Cancer Register (CR)*
The cancer register has collected information since 1958 on all cancer diagnoses in Sweden. Additionally, site and histopathology of tumours has been coded in the ICD-O/3 systems of topography and morphology since January 1, 2005. In the present publication, ICD-O/3 figures for coding site have been translated to comply with earlier ICD-7, so that this coding system can be used consistently over time.
It is required that physicians, pathologists and cytologists separately report every tumour diagnosed from surgically removed tissues, biopsies, cytological specimens, bone marrow aspirates and autopsies. Thus the majority of diagnoses are notified twice, in separate reports sent to one of six regional cancer registers. Only persons that have an official personal identity number with residency in Sweden are included in the CR. Included in CR are patient data including personal identity number, sex, age, and, place of residence, medical data including site of tumour (the codes are available as ICD-7 codes for the whole period from 1958), histological type, stage (has been included since 2004), basis of diagnosis, date of diagnosis, reporting hospital and department, reporting pathology/cytology department, and identification number for the tissue specimen, follow-up data, including date and cause of death, as well as date of migration (15, 182, 183).

8.3 *Ethical considerations*
Whenever register-based data based on the PIN is to be used, the medical project is reviewed by an ethical review board (184). The principles of the declaration of Helsinki were applied for all data handling. Therefore all studies in this thesis were approved by the Regional Ethical Review Board in Stockholm attached to Karolinska-University hospital and Karolinska Institutet, Dnr: 2006/546-31/3.
9 DESIGN AND METHODS

9.1.1 Study I
In a nested case-control study, cases with MS diagnosis, \((n=20542)\), were identified and matched with controls \((n=204157)\) from the original cohorts. Appendicectomy was identified using the Swedish Classification of Operations and Major Procedures using data obtained from the PR. The underlying diagnosis of appendicectomy was characterized as perforated (most likely to indicate acute appendicitis), non-perforated and other appendicitis (least likely to indicate acute appendicitis).

9.1.2 Study II
A cohort study with a total of \((n=20543)\) patients who received a diagnosis of MS in Sweden between 1964 and 2005 were identified using the PR and SMSreg, and a comparison cohort without MS \((n=204163)\) was also identified. In total, 267 MS patients and 212 individuals without MS were excluded due to missing information. Using the Multi-Generation Register, data on 11,284 fathers and 12,006 mothers of patients with MS, and 123,158 fathers and 129,409 mothers for members of the comparison cohort members without MS, were obtained. If parents died or emigrated prior to the study period, then they were not included in the study.

Figure (4):
The subjects with and without MS (Index cohort) were linked to their parents through the MGR.

9.1.3 Study III
This is a cohort study, limited to patients with MS identified through the MS Register and their matched comparators. Through the SMSreg, a total of \((n=7,958)\) individuals with a diagnosis of MS were identified up until December 2005. The main clinical courses of MS recorded are relapsing–remitting (RR), secondary progressive (SP), primary progressive (PP) and progressive relapsing (PR) forms. The course is assessed during clinic visits and the SMSreg is updated if the disease course has changed. The most recently recorded course is used here.
A total of \((n=78,903)\) individuals were included in the matched comparison cohort. Some 291 patients with MS and 2,858 matched comparators who had a record of CVD before study entry were excluded. These were the matched comparison individuals from the larger cohorts.

CVD diagnoses were identified using the international classification of diseases (ICD) codes between 1969 and 2005 using the PR. The ICD codes for cardiovascular diseases used in the analysis included rheumatic heart diseases, hypertensive diseases, ischaemic heart diseases, pulmonary heart disease and diseases of pulmonary circulation, cerebrovascular diseases, and diseases of arteries, arterioles and capillaries, and diseases of veins, lymphatic vessels and lymph nodes, not classified elsewhere.

**9.1.4 Study IV**

The cohorts of subjects with MS \((n=20,543)\) and without MS \((n=204,163)\) were utilised. Those who did not have a cancer diagnosis prior to study entry were included. A total of 1,768 MS patients with cancer and 24,965 cancer patients without MS had a primary cancer diagnosis between 1969 and 2005.

The benign tumours were not considered in risk estimations of mortality after cancer (333 with multiple sclerosis and 3,739 without multiple sclerosis). Cancer diagnoses after death or based on autopsy findings were excluded from the analysis: 98 subjects from the multiple sclerosis cohort and 878 from the general population cohort.
10 STATISTICAL ANALYSIS

Shoenfeld’s partial residuals were used to test violation of the proportional hazard assumption in Studies II-IV and there was no indication of violation in Study II. All tests were two-sided and P<0.05 was considered to be statistically significant.

10.1 CONDITIONAL LOGISTIC REGRESSION IN STUDY I
The relative risk was estimated using Odds ratios (OR) with 95% confidence intervals (95%CI). Conditional logistic regression (using the risk-set structure), with adjustment for socio-economic index, assessed the association of diagnosis underlying appendicitis with MS risk. Further analyses were stratified by age at MS diagnosis, year of diagnosis and sex.

10.2 COX REGRESSION IN STUDY II
The relative risks were expressed as hazard ratios (HR) with 95% confidence intervals (CI 95%) produced by Cox regression. The outcome variables were dates of IMD diagnoses. The risk of IMD among fathers and mothers of patients with MS were investigated separately and combined. Follow-up time was the underlying time scale and the models were stratified based on period (in 10-year intervals). The models were adjusted for age, sex of the index person, region of residence and SEI. Follow-up was from 1964 or from birth or immigration if this occurred subsequently. Follow-up time ended on the date of first discharge diagnosis of immune-mediated diseases, emigration, death, or study period end date, 31 December 2005.

10.3 POISSON REGRESSION IN STUDIES III AND IV
We estimated relative risks with 95% confidence intervals using Poisson regression models in Studies III and IV. Both studies had attained age (age at follow-up) as time-scale, where subjects enter the analysis at their baseline age (left-truncation) and exit at their event/censoring age. All analyses in these two studies were adjusted for follow-up duration, with further adjustment for year at entry, age at entry, sex, SEI and area of residence or regional cancer centre.

10.3.1 Study III
Analysis includes risk comparison between the MS cohort, and the non-MS cohort with follow-up time beginning at MS diagnosis (and the same time-point for the matched comparators). The follow-up ended at diagnosis of first event of CVD, emigration, death, or study end in 31 December 2005, whichever occurred first. CVD diagnoses were examined as outcomes (using separate models) as one of the following: all CVD combined, grouped CVD diagnoses, or by specific diagnoses (Appendix 1).
Similar analyses were repeated separately to estimate the associations of CVD with MS courses (relapsing-remitting (RR-MS), secondary progressive (SP-MS), primary progressive (PP-MS) and progressive relapsing (PR-MS)) compared with the non-MS cohort. Then the MS courses were compared with each other. In total, 5,955 PwMS had a registered course defined as RR-MS, SP-MS, PP-MS or PR-MS. Due to small numbers of observations for PR-MS, only overall risk of CVD was estimated. The analyses were also stratified by sex, age at study entry, age at exit and by duration from study entry.

To estimate the potential influence of surveillance bias, sub-analysis excluded events during the first year of follow-up. To further minimise potential surveillance bias, we also performed analyses in which the outcome was CVD and recorded as the primary diagnosis at hospital discharge.

Where specific CVD diagnoses generated fewer than 50 events, the results for such rare outcomes were not presented. The diagnoses were: rheumatic heart disease, post-thrombotic syndrome, sexual organ varicose veins, oesophageal varicose veins, varicose veins of lower extremities, portal vein thrombosis, truncal vein thrombosis and some other less common diagnoses.

10.3.2 Study IV
Poisson regression was used to estimate relative risks (RR) and 95% confidence intervals (CI) for mortality after diagnosis of cancer. The RRs were reported as either unadjusted or adjusted. The adjustment was for follow-up duration, year at entry, sex, regional cancer centre (six cancer centres) and socioeconomic index. The logarithm of accumulated person-years served as the offset variable. Attained age was the underlying time scale.

Follow-up time for subjects with a diagnosis of cancer was split as they were moved to the cohort with cancer risk from the date of their first cancer diagnosis and followed until the date of second cancer, emigration, death or 31 December 2005, whichever occurred first. The association of cancer with all-cause mortality was first assessed in the multiple sclerosis and general population cohorts separately. Then the cohorts were combined for multiplicative interaction testing. The interaction of multiple sclerosis with cancer was adjusted for main effects (multiple sclerosis and cancer) as well as the other measures.

The analyses were for all cancer types combined and also separately for more specific diagnoses (index 2). Brain tumour diagnoses were only included in overall risk estimation, as a recent study using the same material to examine mortality risk for brain tumour was published in 2013 (185).

Sensitivity analysis
To examine whether the results may be influenced by changes in treatment, including immunomodulatory therapy (IT), the follow-up period was truncated to before 1996.
and from this time onwards, as the first of the more recent therapies, interferon-beta, was introduced in Sweden in 1996 (178).
An analysis was performed, limiting subjects with multiple sclerosis to those included in the SMSreg, to ensure the high diagnostic accuracy for multiple sclerosis (15).
11 RESULTS

11.1 Study I

Some characteristics of cases and controls in Study I are described in Table 1. There was no overall statistically significant association for appendicectomy (in any category) with MS. Although not statistically significant, there was some evidence of variation in association by diagnosis underlying appendicectomy and MS risk. The odds ratios with MS for perforated appendix (most likely to be due to acute appendicitis) are (OR: 0.86; 95% CI: 0.70-1.04); with (OR: 1.04; 95% CI: 0.94-1.16) for non-perforated appendicitis; and (OR: 1.14; 95% CI: 0.98-1.33) for all other appendectomies (least likely to be due to acute appendicitis). Adjustment for socioeconomic index did not change the interpretation of the results (Fig. 5). Assessment of associations with appendicectomy prior to age 20 years was limited by the small numbers.

Table (1): Characteristics of the cases and controls:

<table>
<thead>
<tr>
<th></th>
<th>Individuals with MS</th>
<th>Individuals without MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number (224,699)</td>
<td>20,542</td>
<td>204,157</td>
</tr>
<tr>
<td>Female (%)</td>
<td>13,400 (65.3%)</td>
<td>132,780 (65.1%)</td>
</tr>
<tr>
<td>Male (%)</td>
<td>7,142 (34.7%)</td>
<td>71,377 (34.9%)</td>
</tr>
<tr>
<td>Mean age (SD)</td>
<td>46.3 (15.5)</td>
<td>46.3 (15.5)</td>
</tr>
</tbody>
</table>

Figure (5): Associations between underlying diagnosis of appendicectomy and MS.

Risk of MS after acute appendicectomy

OR (95%CI), odds ratio (95% confidence interval).
The analysis was stratified by sex and subjects born prior to 1964 were excluded. No significant temporal variations in the associations were detected for the entire study period, 1964-2005. The associations were also largely unchanged when the analysis was restricted to cases identified through the MS register (data not shown).

11.2 Study II

a. Risk of immune-mediated disease among fathers and mothers of patients with MS

Overall, there was no statistically significant association with immune-mediated diseases among parents of patients with MS, compared with parents of index subjects without MS, with an exception for T1D (hazard ratio 1.08, 95% confidence interval 1.01–1.16) among fathers.

b. Risk of immune-mediated diseases among patients with MS

Statistically significant unadjusted and adjusted increased risks in the index cohort with MS were identified for UC, CD, T1D, psoriasis, PN and pemphigoid. The magnitude of these associations became greater when limited to diagnoses following the MS diagnosis (Table 2).

Table 2: Hazard Ratio (HR) 95% CI of tested IMD among the index cohorts, with and without MS.

<table>
<thead>
<tr>
<th>Immune-Mediated Diseases</th>
<th>Overall Unadjusted HR (95% CI)</th>
<th>Overall Adjusted for SEI HR (95% CI)</th>
<th>After diagnosis of MS, adjusted HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcerative Colitis</td>
<td>1.49 (1.22-1.82)</td>
<td>1.43 (1.11-1.83)</td>
<td>1.81 (1.38-2.39)</td>
</tr>
<tr>
<td>Crohn’s disease</td>
<td>1.45 (1.17-1.81)</td>
<td>1.45 (1.16-1.80)</td>
<td>1.70 (1.23-2.34)</td>
</tr>
<tr>
<td>Type 1 Diabetes</td>
<td>1.40 (1.31-1.50)</td>
<td>1.30 (1.22-1.40)</td>
<td>1.45 (1.34-1.57)</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>1.73 (1.42-2.10)</td>
<td>1.57 (1.29-1.91)</td>
<td>2.27 (1.81-2.84)</td>
</tr>
<tr>
<td>Temporal arteritis &amp; polymyalgia rheumatica</td>
<td>1.22 (0.97-1.54)</td>
<td>1.21 (0.96-1.53)</td>
<td>1.11 (0.85-1.44)</td>
</tr>
<tr>
<td>Eczema</td>
<td>0.97 (0.63-1.49)</td>
<td>0.99 (0.65-1.53)</td>
<td>1.25 (0.49-3.21)</td>
</tr>
<tr>
<td>Polyarteritis nodosa</td>
<td>4.13 (2.09-8.18)</td>
<td>3.86 (1.92-7.78)</td>
<td>6.18 (2.89-13.23)</td>
</tr>
<tr>
<td>Addison's disease</td>
<td>1.30 (0.72-2.33)</td>
<td>1.32 (0.73-2.39)</td>
<td>1.57 (0.81-3.06)</td>
</tr>
<tr>
<td>Pemphigoid</td>
<td>10.19 (6.38-6.28)</td>
<td>9.42 (5.84-15.20)</td>
<td>13.5 (7.92-23.16)</td>
</tr>
<tr>
<td>Rheumatoid Arthritis</td>
<td>0.85 (0.72-1.00)</td>
<td>0.77 (0.65-0.91)</td>
<td>0.89 (0.73-1.09)</td>
</tr>
</tbody>
</table>

The model was adjusted for age, sex, region of residence, and socioeconomic index. CI: confidence interval; HR= hazard ratio.
There was no notable temporal variation of the estimates before 1981 or subsequently. The analysis restricted to SMSreg did not change the estimations materially. There was no conspicuous variation in estimated risk for any of the diseases in parents or MS patients when the analysis was stratified by follow-up duration. Adjusting the models for age and SEI did not materially change the estimates.

11.3 Study III

The descriptive data about cohort with MS and cohort without MS is outlined in Table (3). Average follow-up time from the date of entry, for MS patients was approximately 8.3 years and for non-MS subjects 8.8 years.

<table>
<thead>
<tr>
<th></th>
<th>Without MS</th>
<th>With MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
<td>76,045</td>
<td>7,667</td>
</tr>
<tr>
<td>Female</td>
<td>53,592 (71%)</td>
<td>5,434 (71%)</td>
</tr>
<tr>
<td>Male</td>
<td>22,453 (29%)</td>
<td>2,233 (29%)</td>
</tr>
<tr>
<td>Mean age at entry</td>
<td>40.2</td>
<td>40.2</td>
</tr>
<tr>
<td>Mean age at CVD (SD)</td>
<td>54 (13.1)</td>
<td>57 (12.0)</td>
</tr>
</tbody>
</table>

The proportions with each MS course were RR-MS (42%), SP 26%, and PP 8%. A further 24% had no specified clinical course. MS patients without a known MS course were included in the overall risk estimations.

All cardiovascular diseases (I00-I99) regardless of MS course

A statistically significant increased risk for CVD-associated hospital admission was observed for MS compared with the non-MS cohort and the highest risk was for those aged 35 years or younger (RR=3.99, 95%CI 2.99-5.31). RR-MS, SP-MS and PP-MS were associated with raised CVD risks compared with those without MS (Fig. 6). The risk of CVD in RR-MS and SP-MS was statistically significant but for PP-MS, it was not statistically significant. After excluding the first year of follow-up, the association for RR-MS was no longer statistically significant, while SP-MS remained statistically significant with a lower magnitude estimate, with no notable change for PP-MS and PR-MS.

When MS courses were compared with each other (RR-MS as reference), the adjusted relative risks for overall cardiovascular disease (CVD) were (RR=1.40, 95%CI 0.97-2.05) and (RR=1.51, 95%CI 0.98-2.35) for SP-MS and PP-MS respectively.
Figure (6):
The figure depicts the risk of CVD by MS course, compared with the general population cohort.

**Hypertensive diseases (HT):**
Hypertensive diseases, including essential hypertension, were not associated with any MS course.

**Ischaemic heart disease (IHD):**
There was an overall non-statistically significant inverse association with IHD (RR=0.88, 95%CI 0.75-1.03). However, for some sub-analyses, differences reached significance as MS was associated with a decreased risk for angina pectoris (RR=0.72, 95%CI 0.58-0.88), most strikingly in SP-MS patients (RR=0.63, 95%CI 0.46-0.87), and an overall decreased risk of chronic ischemic heart disease (CIHD) (RR=0.73, 95%CI 0.59-0.91) was observed among males.

**Diseases of pulmonary circulation:**
MS was associated with significantly raised risks for pulmonary heart disease and diseases of pulmonary circulation and among MS courses significantly increased among patients with SP-MS. This observation was mainly explained by an increased risk of pulmonary embolism (RR=1.79, 95%CI 1.36-2.34), most prominent in SP-MS (RR=2.25, 95%CI 1.56-3.22) which also differed significantly from RR-MS (RR=1.39, 95%CI 0.71-2.71).
Other heart diseases:
This group of diagnoses includes atrial fibrillation, other dysrhythmia, diseases of the pericardium, endocardium cardiomyopathies and heart failure and has a significant inverse association with MS, most notably in SP-MS.
The risk estimation for specific CVD revealed: lowered risk of atrial fibrillation (RR=0.57, 95% CI 0.43-0.74), raised risks of acute infective endocarditis (RR=1.19, 95% CI 1.03-1.36) and lowered risk of heart failure (RR=0.82, 95% CI 0.50-1.41). The risks for atrial fibrillation were (RR=0.69, 95% CI 0.37-1.33) in RR-MS, (RR=0.46, 95% CI 0.30-0.69) in SP-MS and (RR=0.46, 95% CI 0.21-0.98) in PP-MS.
A significantly raised risk of infectious pericarditis was shown in RR-MS (RR=1.50, 95% CI 1.06-2.12) and in SP-MS it was (RR=1.18, 95% CI 0.98-1.43). Stratified analysis by sex showed that the risk was significant only in females with RR-MS.
The risks of heart failure in MS were insignificantly different from that among controls.

Cerebrovascular diseases:
MS was associated with statistically significant raised risks for cerebrovascular disease: (RR=1.32, 95% CI 1.14-1.55), most notably for RR-MS. In particular, there was an increased risk of cerebral infarction in MS compared with the general population and more pronounced in patients with RR-MS (RR=2.57, 95% CI 1.60-4.12) than those with SP-MS (RR=1.40, 95% CI 1.01-1.93).
However, when the first year of follow-up was excluded, the overall risk of cerebrovascular diseases (I60-69) and the increased risk of cerebral infarction (I63) in RR-MS and SP-MS were no longer statistically significant.

Vascular disease:
Diseases of arteries, arterioles and capillaries were not associated with any MS course, whereas diseases of veins, lymphatic vessels and lymph nodes showed a significantly increased overall risk in RR-MS, SP-MS and PP-MS.
There were overall raised risks of deep vein thrombosis (DVT) in extremities (I80), (RR=3.09, 95% CI 2.30-4.16) and haemorrhoids (RR=2.39, 95% CI 1.76-3.24). This increased risk of DVT was seen in RR-MS (RR=2.16, 95% CI 1.21-3.87), SP-MS (RR=3.41, 95% CI 2.45-4.75) and (RR=3.57, 95% CI 1.95-6.56). When MS patients were compared with each other, the risk of DVT in the extremities, revealed statistically significant raised risk in SP-MS and PP-MS, compared with RR-MS.

Other comparisons:
Where not mentioned above, stratification by sex or comparison between courses among MS patients did not reveal notable differences. Where not described above, all other CVD risks were largely unaltered when the first year of follow-up was excluded.
Using only the primary cause for hospital admission:
The analysis for risks of CVD as the primary cause for hospital admission produced a pattern of findings that was similar to the main analyses, in which both the primary and secondary causes for hospital admission were used, but the point estimates for overall (RR=1.24, 95%CI 1.04-1) and groups of CVD were of a somewhat lower magnitude (data not shown).

11.4 Study IV

All-cause mortality after a primary cancer diagnosis in individuals with and without multiple sclerosis

We estimated mortality among those with MS with and without cancer and similarly among those with and without cancer in the general population cohort.

A statistically significant raised mortality risk for cancer was observed in the MS cohort (RR=3.35, 95%CI 3.12-3.59), with a higher magnitude risk in general population cohort (RR=6.42, 95%CI 6.30-6.55). Interaction testing indicated significant effect modification for the association of cancer with all-cause mortality by presence of MS (P-value for interaction <0.001). The risk estimation for each organ or site of cancer indicated consistent associations.

Sensitivity analysis included division of the follow-up period by calendar year up to 1996 and from 1996, when immunomodulation therapies was recommended for IMT for MS in Sweden. The risk estimation for the periods before 1996 (number of events in subjects with multiple sclerosis 1,542) and from this time (number of events in subjects with multiple sclerosis 226) were broadly similar and statistically significant. The corresponding interaction terms were 0.50 (0.46-0.54), P <0.001) and (0.49 (0.46-0.53), P <0.001), respectively.
12 DISCUSSION

12.1 Study I

Overall, no association between acute appendicectomy and MS was determined. The results were equivocal due to a lack of statistical significance. There was a lower risk of MS associated with appendicectomy with a perforated appendix (the most reliable category to indicate acute appendicitis), raised risks for appendicitis with a non-perforated appendix and with a non-specific cause (least likely to be associated with acute appendicitis). This variation in association by underlying diagnosis may thus help to explain the contradictory findings reported by previous studies (148, 149).

An inverse association of MS with acute (perforated) appendicitis and the positive association with appendicectomy without appendicitis suggest that appendicitis could be a marker of immunological characteristics or exposures relevant to MS risk. The site of activation of peripheral blood mononuclear cells (PBMC) (186), including those causing inflammatory destruction of myelin, has been associated with different risk factors (187) and the appendix is one of the possible sites where PBMC may interact with antigen-presenting cells (188, 189). Acute appendicitis may potentially signal immunological characteristics relevant to MS risk, including those causing inflammatory destruction of myelin in the CNS (190, 191). It is of note that appendicectomy less likely to be due to acute appendicitis is associated with a raised MS risk, signalling other exposures or characteristics. However, the lack of statistical significance could also indicate that these are chance findings. These differences by appendicectomy category may indicate innate characteristics or exposures in earlier life relevant to MS risk.

12.2 Study II

Parents of PwMS did not show consistent evidence of an elevated risk for IMD, apart from MS. Despite sufficient statistical power, this study could not confirm the results of an earlier study that reported an increased risk of Crohn’s disease (CD) and Addison's disease (AD) among parents of individuals with MS (151). The associations in the previous study were based on relatively few events, so they may have been the result of chance findings among multiple tests.

Among the cohort of PwMS, there was a significantly raised risk for a number of other immune-mediated diseases. In contrast with their parents, MS patients themselves were found to have a significantly increased risk for several IMD, consistent with earlier studies (14, 151). The magnitude of associations between MS and other IMD, compared with before diagnosis of MS, was higher for those diagnosed after the MS diagnosis. In addition, an association between MS and PN was detected. As found by
an earlier study, an inverse association between MS and RA was observed (151). Higher magnitude risks were for ulcerative colitis, Crohn’s disease, psoriasis, polyarteritis nodosa and pemphigoid.

A relatively large number of genes, including many non-HLA genes, combine to increase MS risk, although individually most of these genes only increase the risk of MS modestly (75). The majority of these non-HLA genes involved in MS aetiology do not appear to be notably associated with other IMD and are unlikely to increase the risk of non-MS inflammatory diseases among parents. Alternatively, environmental exposures relevant to MS risk might also influence the risk of comorbidity. Another possibility is that symptomatic onset of the other diseases follows MS onset due to influences of MS disease activity. The significance of surveillance bias cannot be excluded, as those with a chronic disease like MS have more frequent contact with health services and are therefore likely to receive further diagnoses. Hemminki and colleagues examined MS risk among members of an index cohort and their first-degree relatives (14), where inclusion in the index cohort was defined by diagnosis of an immune-mediated disease other than MS. In contrast, we created an index cohort of patients with MS and examined the risk of other diseases. A study from Denmark found results similar to current findings among first-degree relatives (151). However, unlike this study, disease among relatives was ascertained by questioning the MS patients, which is a potential source of recall or reporting bias.

12.3 Study III

This study found an overall statistically significant increased risk for CVD diagnoses following an MS diagnosis. RR-MS was associated with higher magnitude risks, but the differences between courses were not statistically significant.

The risk of venous circulatory diseases in MS patients tended to be of the highest magnitude and the pattern of association by course was not identical to that for all CVD. The highest risks for CVD among MS patients were deep venous thrombosis (DVT), conditions highly associated with immobility and pulmonary embolisms (PE), a condition which often occurs as a consequence of DVT, when clots break off from the vein walls and travel through the heart to the pulmonary arteries (192). In this study, we could not assess possible associations with motor disability, although its influence on mobility might be less in RR-MS than other MS courses (37). An increased risk was also determined for haemorrhoids. This has also been associated with immobility, accompanied by constipation, which was reported earlier for the general population (193).

The risk of ischaemic stroke was significantly increased in MS and especially among patients with RR-MS, and in particular during the first year of follow-up. This is likely to be due, at least in part, to the identification of asymptomatic lesions during neuroimaging associated with MS diagnosis and management.
MS was associated with a decreased risk of angina pectoris, chronic ischaemic heart disease and atrial fibrillation, and some of these findings are consistent with previous reports (16, 194, 195). One speculative possibility is that immobility among MS patients renders them less prone to the physical strains required to precipitate angina pectoris. However, it is conceivable that neurological factors, such as dysregulation of the autonomic nervous system or even sensory disturbances could play a role (166), comparable to what has been reported for traumatic transection at the level of the cervicothoracic spinal cord (196). An alternative speculative explanation for the inverse association with AF is that decreased aerobic capacity due to immobility may be the opposite of the increased risk of atrial fibrillation evident in athletes (197).

12.4 Study IV

The overall result revealed a significantly lower magnitude raised risk of all-cause mortality in MS patients after diagnosis of cancer compared with people without MS. The results were consistent for cancer of the digestive organs, respiratory organs, breasts, female genitalia, male genitalia, blood and skin. This suggests that diagnostic neglect in MS patients does not account for the lower cancer incidence reported in MS.

Generally, the lifespan of patients with MS is shorter than that of the general population (173, 198). Patients with MS die of many causes, ranked as follows: multiple sclerosis, cardiovascular disease, infection, cancer, accidents and a 7.5 times higher risk of suicide (198-200). This finding in our study shows that average longevity in the population of patients with MS exposed to cancer is no worse than among the general population.

Interaction testing took into account the average mortality risk in those with and without MS and then any additional increase or decrease in mortality risk among those with cancer. This finding may be less biased than that reported by previous studies (201) since the relative all-cause mortality risk has some advantages over cause-specific mortality. All-cause mortality risk does not rely on the accuracy of the underlying causes of death assigned to the cancer patients (202).

There are several possible mechanisms to account for lower magnitude relative mortality risk following cancer diagnosis in MS and the previously reported reduced risk of cancer in MS is probably genuine (15). If immune cells such as monocytes react specifically against a malignant tumour, then this could represent an anti-cancer mechanism that operates more notably in MS (15).

Previous findings have shown an inverse association between cancer risk and MS (15, 176, 203, 204). It has been suggested that exposure to sun may account for reduced mortality after cancer diagnosis in MS (205), although this is not consistently reported (206). A lowered level of vitamin D has some commonality for both MS and cancer risk (207, 208). As MS is associated with low levels of vitamin D (209), and low levels
of this vitamin happen to correlate with higher cancer mortality rates (although only after cancer diagnosis) (210, 211), one would expect MS to be associated with a higher cancer mortality rate. However, this was not confirmed in this study.

Some reports implicate tobacco as a very well-known carcinogen factor associated with higher all-cause mortality risk in MS (212), but we observed a lower mortality after lung cancer diagnosis. This could again point to some specific immunological characteristic in MS, with an anti-malignant function (213, 214).

We speculate that the association with lower all-cause mortality risk in MS after cancer is consistent with earlier findings as MS patients on average have a lower body mass index (BMI) than the general population (215), and increased body mass index is a risk factor for some cancer types (216) with associated higher mortality (217). It could be suggested that lower body weight in MS may explain this risk reduction for all-cause mortality.

Previous investigations have reported a lower cancer risk for the alimentary tract in MS patients (218), and this risk is lowest in MS compared with other immune-mediated diseases (218). Consistent with this report, we demonstrated lower mortality risk for all types of digestive cancer. These two complementary findings could probably point to healthier dietary routines and less exposure to carcinogens in MS patients (219).

Another possibility is that, in contrast with diagnostic neglect, MS patients have more contact with health services and, on average, cancer is diagnosed at an earlier time, with benefits for prognosis. However, another report revealed larger tumour size in MS patients compared with the general population, thus contradicting the suggestion of earlier diagnosed tumours (176).

12.5 **Strengths**

The studies included in this thesis have a general population-based setting and include a large sample size of patients to improve precision of the estimates. The measures were recorded prospectively, reducing the influence of reporting bias and reverse causation. The data includes people with MS of all ages over an extended period of time. The data in the PR are estimated to include more than 99% of all discharge diagnoses since 1987 (15, 180). The PR is well established, with high data quality, and the diagnoses listed in the PR are reliable as the Swedish concept of MS is narrow and reflects diagnostic caution rather than over-inclusiveness (220). The overall quality for data from PR is that it is unselected. Another strength is access to detailed data on MS from the SMSreg, which has high validity and reasonable representativeness. There is high diagnostic accuracy for MS among patients included in the SMSreg, with confirmation through test results for CSF, OCB, as well as CNS-MRI findings. High diagnostic accuracy (over 90%) for MS among patients included in the SMSreg has been demonstrated in earlier reports (15, 221). To ensure that lower diagnostic accuracy did not influence the results in Studies I, II and IV, a sub-analysis used only diagnoses of MS identified through the MS Register. Another benefit of using the SMSreg to identify patients with MS is that those identified through the Patient Register may tend to have a greater disease burden (hospital admission for another disease may lead to the recording of MS in the register).
One of the strengths of Study IV is the use of the Cancer Registers, which provides national data that are considered to be of high quality, with the majority of diagnoses being histologically confirmed (183). The overall completeness of the cancer register in Sweden is high (182). Our study covered almost all cases of MS and cancer in Sweden (15).

Potential limitations
As is the nature of epidemiological observational studies, alternative explanations for the findings are always possible.

a. Selection Bias
Selection bias occurs primarily in the design stage since this type of bias arises from the procedures by which the study subjects are selected from the source population, or select themselves as being eligible for the study (222), potentially causing spurious associations. We controlled for this type of bias through the complete selection of patients with MS and a matched design to identify the general population cohort.

b. Information Bias
Information bias is the result of misclassification of study subjects, MS and non-MS, with respect to disease or exposure status. Thus the concept of information bias refers to those subjects actually included in the study, whereas selection bias refers to the selection of the study participants from the source cohort (223).

In Studies I, II and IV, because we were unable to divide MS into separate and meaningful distinct clinical subsets of MS (224), we may have missed an association with a subgroup.

As subjects with and without MS were at equal risk of exclusion because of the period of entry, and since all our analyses were internally stratified by risk-set through conditional modelling, the studies are unlikely to suffer from potential bias resulting from temporal or spatial differences in MS. The completeness of coverage for diagnoses in the PR varied over time, possibly influencing some associations, which is why stratification by period was performed.

In the current studies, it was not possible to examine the possible influence of immunomodulating treatments, such as interferon beta-1. The stratification by calendar year was used to control for this potential bias and the estimations remained in the same range. Other MS drugs such as Fingolimod have also been associated with CVD (225). However, the study period pre-dates the time of its introduction in Sweden.
It was not possible to examine influences of more recently introduced drug modifying therapy (DMT), as these data were not available from the registers for this study period. However, the risk estimation stratified when DMT was introduced in Sweden did not show any evidence of a major influence.

Despite our adjustment for potential confounding factors, other unmeasured characteristics, residual confounders, could still account for some of the results. Such confounding has been demonstrated for the association of multiple sclerosis risk (226) and decreasing BMI with all-cause mortality (217).

**b.1 Surveillance Bias**

MS is a chronic disease, usually resulting in more frequent contact with health services, and incidental diagnoses are more often recorded than among the general population, which can result in surveillance bias. This is a type of non-random misclassification bias, and might have occurred in Studies II-IV. As the majority of diagnoses for the outcome diseases were identified among hospital patients, any diagnoses made in primary care would not be included, thus reducing sensitivity. However, this was equal for both cohorts, possibly avoiding differential bias. Nevertheless, surveillance bias is a risk, where those with a chronic disease (MS) have more frequent contact with health services, with more opportunity for other diagnoses to be recorded.

In Studies II and IV, in order to reduce potential surveillance bias, an outcome was only included if it was recorded as the primary diagnosis, and thus likely to be the main reason for hospital admission. In Study III, where we included both primary and secondary diagnosis of CVD, a separate analysis was also performed with primary diagnosis of CVD alone. The estimation was comparable.

In Studies II-IV, this bias was also tackled by excluding events and follow-up (when diagnostic work-up for MS may result in other diagnoses being recorded) during the first year after MS diagnosis in the analysis.

In Study II, while not a concern among parents, surveillance bias is a concern among patients with MS. Not all parents could be identified, but as the exclusion criteria were identical for parents of those with and without MS, differential bias is unlikely.

Patients with MS were more likely to be diagnosed, correctly or incorrectly, with cerebral infarctions due to the investigations and brain imaging undertaken in a patient with recently diagnosed MS (227). This provides evidence of surveillance bias. This bias was tackled, as events in the first year after MS diagnosis were excluded and the excess risk was largely eliminated. An analysis of CVD outcomes was limited to the main discharge diagnosis, further reducing the risk of results entirely driven by surveillance bias.

**12.5.1 Confounding**

In general, confounding is when a confounding factor is associated with both the risk factor of interest and the outcome. This factor must be distributed unequally among the exposed and unexposed cohort (228). We dealt with the issue of confounding at the study design level.
and in the analysis phase. Several approaches have been used to control for confounding:

Matching— The subjects in all studies in this thesis were individually matched for equivalence. The matching criteria were based on characteristics at the time of the MS diagnosis. For each person with MS, individuals without MS who had the appropriate characteristics for matching were identified, and from them up to 12 subjects were selected at random.

Stratification— In Studies I-IV, the associations were measured within each well-defined and similar category (stratum), which was likely to be a potential confounding factor. For instance, if age was a confounder, the association was estimated separately.

Statistical modelling— statistical methods, such as regression (conditional logistic regression, Cox Hazard regression and Poisson regression) modelling, were used to control for confounding. These models were particularly useful when it was necessary to adjust simultaneously for several potential confounders in the model (229).

Choice of time-axis- The choice of model form should ideally be dictated by subject matter knowledge, biological plausibility, and the data (230). In Studies III-IV, the interest is in describing the increasing risk of outcomes with age in PwMS and that data on both variables have been gathered from a population-based cohort. Assumption of a linear relationship is proposed.

Adjustment: In Studies I-IV, there was no information on lifestyle and behaviour, such as smoking, which could have influenced the results. Smoking is positively associated both with acute appendicitis and with MS risk, as well as more rapid MS disease progression (231-235), CVD and all-cause mortality risk in MS (212). To tackle this issue, we adjusted for a measure of socioeconomic circumstances (associated with some behaviours). The limited influence of adjustment for socioeconomic index suggests behaviour with a socioeconomic gradient not due to MS is an unlikely confounding factor.

MS is heterogeneous in some disease characteristics, suggesting the possibility of heterogeneity in aetiology (236). As we were unable to divide MS into separate and meaningful distinct clinical subsets of MS (224), other than disease course, we may have missed an association with a specific subgroup in Studies I, II, and IV.
13 Conclusions

Study I
A non-statistically significant and therefore equivocal reduced risk of developing MS was observed in individuals who had prior appendicectomy due to perforated appendicitis, but not for those with other diagnoses underlying appendicectomy. These results may help to explain why earlier studies of appendicitis and MS risk have been inconsistent, as they did not examine the diagnosis underlying appendicectomy. The three underlying diagnoses showed different directions of risk associations, possibly signalling characteristics or exposures relevant to MS risk.

Study II
There may be an increased risk of a number of other immune-mediated diseases in patients with MS, but not in their parents. This suggests that the increased risk in those with MS is not due to conventional genetically inherited risk.

Study III
There appears to be a significantly increased risk of CVD in MS and the increased risk of venous thromboembolic disorders in progressive MS is of notably high magnitude. Immobility might be one of the characteristics that help to explain the findings for arterial and venous diseases. An increased frequency of ischaemic stroke in MS is most likely due to surveillance bias resulting from more frequent neuroimaging during the period when an MS diagnosis is first made. There is no increased risk for ischaemic heart disease in MS and atrial fibrillation is less common than among the general population.

Study IV
Lower all-cause mortality risk after cancer is confirmed for MS patients. The lower incidence of cancer diagnoses in MS is unlikely to be due to diagnostic neglect. Possible contributing factors worthy of further study are that MS patients have a hyper-vigilant immune system that renders them less vulnerable to cancer. However, other aspects of the disease, management or behaviour may explain the findings.
14 Future research

While the findings on appendicectomy are consistent with the assertions that early immune characteristics are important for determining MS risk, it seems unlikely that appendicitis will be a useful measure and other markers of risk are required. Non-Mendelian genetic and environmental risks for the range of other immunological abnormalities in MS deserve further investigation. The increased risk of CVD in MS is important and an understanding of the underlying mechanisms must be clarified. This may be in terms of specific MS characteristics, behaviour or treatment. This will be used to minimise the risk of CVD and possibly have an influence on treatment choices that may further influence CVD risk. The lower incidence of cancer and no increased risk of excess mortality following a cancer diagnosis is welcome news to the MS community. It may be that characterising immunological characteristics in MS may lead to improved cancer prevention therapies in the general population.
Syfte: Denna populationsbaserade avhandling beskriver några samexisterande sjukdomar hos patienter med multipel skleros (MS).

Metod: Uppsatsen använder register i Sverige för att undersöka samsjuklighet av MS, inklusive blindtarmsoperation, gemensamma genetiska faktorer, hjärt-kärl sjukdomar och dödlighet efter cancerdiagnosen hos MS-patienter. Unika svenska personnummer var tvärkopplade via olika register för att bygga upp datasets för olika sjukdomsuppgifter. De studieteknikerna som ingår i denna avhandling var, en nested fallkontroll studie och tre kohortstudier. I de två första arbeten undersökte vi hur samsjuklighet skulle kunna informera oss om etiologin av MS, medan vi i de återstående två arbeten undersökte samsjuklighet i förhållande till prognosen för patienter med MS.

Den första studien undersöker om patienter som haft akut blindtarmsinflammation har en sänkt risk att få MS. Den andra studien undersöker sambandet mellan MS och andra immunmedierade sjukdomar för att titta på eventuella genetiska riskfaktorer som kan vara förknippade med MS. I den tredje studien undersöker vi varför patienter med olika MS förlopp har en högre risk för hjärt-kärlsjukdom, medan vi i den sista studien undersöker om de har en lägre risk för dödlighet efter cancer.

Resultat:
Den första artikeln visade en lägre risk för MS- risken i gruppen med perforerad blindtarmsinflammation, men den var inte statistiskt signifikant. Studie två visade att patienter med MS bär egen högre risk för andra immunmedieradesjukdomar men deras föräldrar inte har någon ökad risk. Tredje studien visade att MS-patienter, oberoende av deras MS-förlopp, är mer benägna att drabbas av djup ventrombos än personer utan MS. I den sista studien, fann vi inte någon generell ökad risk för dödlighet för MS-patienter med cancer jämfört med den allmänna befolkningen med cancer, därav inga säkra tecken på negligerade cancer diagnoser hos MS patienter.

Slutsats: Sammantaget visar resultaten av denna avhandling att andra sjukdomstillstånd med eller efter MS genom miljö samt genetiska riskfaktorer har betydelse för MS-etiologi, men också bekräftar att MS i sig har konsekvenser för kardiovaskulära sjukdomar samt dödligheten hos patienter med diagnosen MS. Denna forskning visar att nya vägar behövs för att öka vår förståelse av MS.
16 ACKNOWLEDGEMENTS

This thesis is based on work carried out during the years 2007-2014 as a PhD project at the Department of Neurology, Karolinska University Hospital and at the Institute of Clinical Neuroscience, Section for Neurology, University of Karolinska (Karolinska Institutet) and the Clinical Epidemiology Unit in the Department of Medicine at Karolinska Institutet. I did some posters before I started research school proper at KI. Right from the start, I knew it would not be easy task to do research and work at the clinic at the same time and I could not have come this far without the support of help of other people.

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During my work with this thesis, I have also had the privilege of working with experts in different fields of scientific work. This work would not have been possible without their expertise and continued support. My mentor Dr. Rayomand Press introduced me to the existing field of neuroimmunology and its role in MS research and was involved in all aspects of the initial scientific planning and progress in my PhD programme. Dr. Press taught me how to interpret my initial findings. Professor Morten Andersen and Associated Professor Marjan Vaez introduced me to advanced STATA short-cuts for Study III and helped me to interpret the results in STATA. They also allowed me to use their research skills inclusive their own macros in STATA at Karolinska Institutet. Docent Magnus Andersson introduced me to the field of other co-morbidities of MS. Loretta Platts introduced me to better skills and techniques of writing and presentation for my half-time seminar.

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The days I discussed my finding and questions were many, and Dr. Hajeiebrahimi, Dr. Maghsoudlou, and Dr. Derogar at Z5, and colleagues at Department of Neurology never showed be hesitated to answer to my brainstorming, many thanks to them.

Also colleague who in my absent with good loyalty and sense of kindness took care of my patients. I do apology to all those of my patients who did probably suffered of not could meet me or talk to me when they needed me in the period when I was less available in my clinic.

Finally I am grateful to my family: to my mother and father for her simple continuous support and care for us; to my brother Dilan and his family, and step-father and step-mother Professors Herluf and Elisabeth Thieden for their care and support in my life; to my close family friends Inger Molich, Morgan Barbro Samuelsson, and Dr. Ege Schultz who never said no when we asked for their help; to Docent Mohsen Khademi who always gave a hand when I needed his support; to all people who had faith in me, believed in me and showed their friendship when I was having a very stressful time. Any person I do not mention here must rest assured that he/she is in my mind and I will return their kindness to thank them one day.

My wife Mitra has been a great inspiration and has always shown a deep interest in my research. She has read through many drafts for this thesis and this work would not have been possible without her patience, support and unconditional love. Mitra and my daughter Didé continuously remind me of what really matters in my life: patience and trust.
17 REFERENCES


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121  Smyk DS, Alexander AK, Walker M, Walker M. Acute disseminated encephalomyelitis progressing to multiple sclerosis: Are infectious triggers involved?


### Appendix 1: Disease groups and corresponding ICD codes

<table>
<thead>
<tr>
<th>Diseases</th>
<th>ICD-10</th>
<th>ICD-9</th>
<th>ICD-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheumatic heart diseases</td>
<td>I00-I09</td>
<td>390-398</td>
<td>390-398</td>
</tr>
<tr>
<td>Hypertensive diseases</td>
<td>I10-I15</td>
<td>401-405</td>
<td>400-404</td>
</tr>
<tr>
<td>Essential hypertension</td>
<td>I10</td>
<td>401</td>
<td>401</td>
</tr>
<tr>
<td>Ischaemic heart diseases</td>
<td>I20-I25</td>
<td>410-414</td>
<td>410-414</td>
</tr>
<tr>
<td>Angina Pectoris</td>
<td>I20</td>
<td>413</td>
<td>413</td>
</tr>
<tr>
<td>Acute myocardial infarction</td>
<td>I21, I22</td>
<td>410</td>
<td>410</td>
</tr>
<tr>
<td>Other acute ischaemic diseases</td>
<td>I24</td>
<td>411</td>
<td>411</td>
</tr>
<tr>
<td>Certain current complications following ST elevation (STEMI) and non-ST elevation (NSTEMI)</td>
<td>I23</td>
<td>429</td>
<td>422.97, 422.99, 4210, 4219</td>
</tr>
<tr>
<td>Chronic ischaemic heart disease</td>
<td>I25</td>
<td>414</td>
<td>412</td>
</tr>
<tr>
<td>Pulmonary heart disease and diseases of pulmonary circulation</td>
<td>I26-I28</td>
<td>415-417</td>
<td>426, 450</td>
</tr>
<tr>
<td>Other type of pulmonary heart disease and diseases of pulmonary circulation (pulmonary embolism excluded)</td>
<td>I27-I28</td>
<td>416-417</td>
<td>426</td>
</tr>
<tr>
<td>Pulmonary embolism</td>
<td>I26</td>
<td>415</td>
<td>450, 673</td>
</tr>
<tr>
<td>Other forms of heart disease</td>
<td>I30-I52</td>
<td>420-429</td>
<td>420-429 (exc. 426)</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>I480-I482, I489</td>
<td>427D</td>
<td>427.92-3</td>
</tr>
<tr>
<td>Heart failure</td>
<td>I50</td>
<td>428</td>
<td>427.00, 427.10, 428.99</td>
</tr>
<tr>
<td>Cerebrovascular diseases</td>
<td>I60-I69</td>
<td>430-438</td>
<td>430-438</td>
</tr>
<tr>
<td>Stroke</td>
<td>I60-I64</td>
<td>430-434</td>
<td>430-431</td>
</tr>
<tr>
<td>Ischemic Stroke</td>
<td>I63-I64</td>
<td>433-434</td>
<td>433-434</td>
</tr>
<tr>
<td>Cerebral infarction</td>
<td>I63</td>
<td>433</td>
<td>433</td>
</tr>
<tr>
<td>Other type of cerebrovascular diseases (stroke excluded)</td>
<td>I65-I69</td>
<td>433-438</td>
<td>432-438</td>
</tr>
<tr>
<td>Diseases of arteries, arterioles and capillaries</td>
<td>I70-I79</td>
<td>440-448</td>
<td>440-448</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>ICD-10-AM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------------------------------------------------------</td>
<td>-----------</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Other disorders of the circulatory system, inclusive lymph circulation</td>
<td>I80-I99</td>
<td>451-459</td>
<td>451-459</td>
</tr>
<tr>
<td>Diseases of veins, lymphatic vessels and lymph nodes, not elsewhere classified</td>
<td>I80-I89</td>
<td>451-458</td>
<td>451-458</td>
</tr>
<tr>
<td>Deep Vein Thrombosis (all extremity)</td>
<td>I80</td>
<td>451</td>
<td>451</td>
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<tr>
<td>Haemorrhoids</td>
<td>I84</td>
<td>455</td>
<td>455</td>
</tr>
<tr>
<td>Multiple Sclerosis</td>
<td>G35</td>
<td>34.0</td>
<td>34.0</td>
</tr>
</tbody>
</table>

Where specific CVD diagnoses generated fewer than 50 events, the results for such rare outcomes are not presented. The following diagnoses generated fewer than 50 events and the results are not presented: rheumatic heart disease, post thrombotic syndrome, sexual organ varicose veins, oesophageal varicose veins, varicose veins of lower extremities, portal vein thrombosis, truncal vein thrombosis and other less common diagnoses.
Appendix 2: The following ICD-codes have been used to identify cancer diagnosis used in the analysis.

<table>
<thead>
<tr>
<th>Type of cancer</th>
<th>ICD-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digestive cancer</td>
<td>(150–159)</td>
</tr>
<tr>
<td>Respiratory cancer</td>
<td>(160–164)</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>(170)</td>
</tr>
<tr>
<td>Female genital cancer</td>
<td>(17, 172, 175)</td>
</tr>
<tr>
<td>Male genital cancer</td>
<td>(177, 178)</td>
</tr>
<tr>
<td>Kidney cancer</td>
<td>(180)</td>
</tr>
<tr>
<td>Urinary organ cancer ex. kidney</td>
<td>(181)</td>
</tr>
<tr>
<td>Endocrine cancer</td>
<td>(194, 195)</td>
</tr>
<tr>
<td>Bone &amp; Connective tissue cancer</td>
<td>(196, 197)</td>
</tr>
<tr>
<td>Blood cancer</td>
<td>(200–202, 203, 205)</td>
</tr>
<tr>
<td>Skin cancer</td>
<td>(190, 191)</td>
</tr>
<tr>
<td>Eye, Nose, middle ear cancer</td>
<td>(160, 192)</td>
</tr>
</tbody>
</table>