Epidemiological Studies of Multiple Sclerosis in Sweden with focus on the County of Värmland

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ABSTRACT

The aim of this thesis was to study the frequency of MS in the suggested high-risk area of Värmland county by investigating the current prevalence and analyzing the development over time; secondly to investigate some aetiological factors – the pattern of industry, both in relation to MS in Värmland and in the whole country, and also to examine the possible relation between the distribution of MS and ALS, as it has previously shown a positive correlation in two studies; finally to analyze the women-to-men ratio of MS in Sweden because of international reports of an increasing ratio.

Clinical data was collected from hospitals and other health care units in the county of Värmland, to calculate the prevalence ratio. The prevalence was 170.07 per 100,000 population on the prevalence day, 31 December 2002. The incidence rates analysed during ten years was 6.46 per 100,000 person-years (1991-1995) and 6.39 (1996-2000).

The ecological study used data from the beginning of the 20th century on industries in Värmland and in all Sweden, which were correlated with the two MS prevalence studies (1925-1934 and 2002), and the mortality study on the time period 1952-1990. There was a statistical significant association between large sawmills and the prevalence 1925-1934 (p = 0.022). For all Sweden, wood-pulp factories and paper-mills correlated significantly with MS mortality 1952-1990 (p = <0.05).

Collected data from Causes of death Register and from the Total Population Register were used when analysing mortality from ALS and MS. The previously shown correlation between ALS and MS mortality distribution in the Swedish counties was not confirmed in this study. However, the mean MS mortality rate was still highest in the county in Värmland. The mean MS mortality rates for whole Sweden was increased from 1.65 per 100,000 person-years (1952-1992) to 2.04 (1990-2010). For analysing sex ratio in MS, data from the Swedish Multiple Sclerosis Register and data from Total Population Register of the Swedish Statistics Office were used. These data was analysed by birth day cohort and by year of onset. The sex ratios in Sweden showed a stable women-to-men ratio.

These investigations give indication that Värmland is a high-risk region of multiple sclerosis, and particularly the municipality of Säffle.

We conclude that Värmland is a suitable area for continued epidemiological studies with both an environment and genetic focus.
## ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ALS</td>
<td>Amyotrophic lateral sclerosis</td>
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<tr>
<td>CDMS</td>
<td>Clinically definite multiple sclerosis</td>
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<td>CPMS</td>
<td>Clinically probable multiple sclerosis</td>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
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<td>CIS</td>
<td>Clinically isolated syndrome</td>
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<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<td>EDSS</td>
<td>Expanded Disability Status Scale</td>
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<td>HLA</td>
<td>Human leukocyte antigens</td>
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<td>ICD</td>
<td>International Classification of Diagnosis</td>
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<td>LSDMS</td>
<td>Laboratory-supported definite multiple sclerosis</td>
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<td>LSPMS</td>
<td>Laboratory-supported probable multiple sclerosis</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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<td>OCB</td>
<td>Oligoclonal bands</td>
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<tr>
<td>PCP</td>
<td>Pentachlorophenol</td>
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<td>SALAR</td>
<td>Swedish Association of Local Authorities and Regions</td>
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<td>SMSreg</td>
<td>The National Swedish MS Register</td>
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<td>SIR</td>
<td>Standardized incidence ratio</td>
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INTRODUCTION

The disease of multiple sclerosis (MS) was first described in clinical terms in the middle of the 19th century. It was studied and described by Jean-Martin Charcot in the last decades of that century. Thereafter further reports of the disease were published from other countries. At this time there was no consensus as to the term given to this disease. First in the 1950s was there consistency in the nomenclature of multiple sclerosis (Compston 2007 p3).

Within MS epidemiology, important research has been carried out by many contributors. John F Kurtzke is one of them and he has worked hard since the 1950s, collecting and analysing epidemiological information regarding multiple sclerosis. He has developed the methodology for describing/presenting the occurrence, distribution and disability of multiple sclerosis (Compston 2007 p57). The Kurtzke Expanded Disability Status Scale (EDSS) has, in the following years, been widely used (Kurtzke 1983).

MS epidemiology in Sweden has been developed by several investigators, first the epidemiological studies of MS by Sällström (1942), and later by Kurtzke (1967, 1968, 1974) with his interest in the "Fennoscandian focus". Later Broman established the Gothenburg cohort (Broman et al. 1981), which was succeeded by Andersen and collaborators Svenningsson, Runmarker, Lycke, Ahlgren and others. Other studies focusing on the distribution of MS in Sweden have been carried out by Landtblom (2002, 2005), who also studied the presumed high risk county of Värmland (Landtblom and Callander, 2006) with the aim of finding possible risk factors and/or causes of MS. There has also been an important contribution from the north of Sweden in terms of studies from Umeå University by Forsgren, Svenningsson, Salzer, Sundström and co-workers.

This thesis includes further studies of MS in Värmland and throughout Sweden, with the purpose of analysing the current and the previous distribution, to determine the sex ratio, and to investigate some potential etiological factors.
The science of epidemiology

The overall aim of epidemiological studies is to increase knowledge needed to determine the cause of the disease, to find risk factors involved, and thus prevent disease and illness. Studies in epidemiology are also important for the provision of services and allocation of resources.

There are many definitions of epidemiology. A widely cited definition by MacMahon and Pugh (1970) is “The study of the distribution and determinants of disease frequency in human population”. Another definition describes epidemiology as the study of the occurrence of illness (Cole 1979). Greenland and Rothman defined epidemiology as “the study of the distribution of health-related states and events in population” (Rothman 2008 p32). Their definition gives the possibility of capturing physiologic states and psychological measures such as blood pressure and depression score. The epidemiological studies always focus on the distribution of all these measures in populations.

It is important to use sound epidemiological principles and precise definition of cases is a prerequisite. Epidemiologic principles and methods encompass three closely interrelated components: distribution, determinants and frequency (Hennekens 1987 p3).

Distribution of disease considers questions such as who is getting the disease within a population and also where and when the disease is occurring. Knowledge of distribution is essential to describe patterns of disease and formulate hypotheses concerning possible causal or preventive factors. Disease frequency involves quantification of occurrence of disease. The determinant of the disease derives from the knowledge of frequency and distribution of the disease, e.g. in order to test an epidemiological hypothesis (Hennekens 1987 p54).

Common concepts in epidemiology when measuring the frequency of a disease are the number of prevalent cases, incident cases and deceased individuals with a certain disease (numerators) amongst a population at risk (denominator) (Hennekens 1987 p58, Kurtzke 2005).

Prevalence quantifies the proportion of individuals (numerator) in a population (denominator) who have the disease at a specific point in time. Prevalence ratio is often called prevalence rate, although by strict definition it is a proportion, not a rate.
The point prevalence refers to the number of people affected within the community at one point in time, per unit of population (Hennekens 1987 p57, Kurtzke 2005). Prevalence proportion is the proportion of a population that has the disease at a given instant (Rothman 2008 p33).

Incidence quantifies the number of new cases of disease that develop in a population of individuals at a specified time interval. The mortality rate is the number of deaths from a disease within a unit of time per population (Kurtzke 2005). Incidence or mortality rates are often expressed as cases per 100,000 person-years (Rothman 2008 p35).

The time factor is of vital importance for results of the different studies of prevalence, incidence and mortality.

For measure of disease frequency, precise definition of the denominator is important for both accuracy and clarity (Hennekens 1987 p56). When interpreting maps of disease distribution, it is necessary to be aware of variations in the population structure. There can be an uneven distribution of disease between sexes and at different ages, and between one population and another there can also be a variation in age and sex structure. This variation requires standardization for age and sex, which can be done by direct or indirect standardization (Barker 1998 p52).

In epidemiological studies, it is sometimes possible and necessary to firstly conduct ecological studies, where the unit of analysis is a population rather than an individual. In ecological studies there is no information available about the individual members of the population. Individual-level data is usually unavailable and impractical to gather. Environmental data is an example of exposures that are measured at the level of groups. This data can be compared with disease distributions in each exposed group. To quantify disease occurrence in groups, data of prevalence, incidence or mortality rates are commonly used. Ecological studies can be useful for detecting associations of exposure distributions with disease occurrence. If a strong association is observed, the results of ecological studies can provide the first signs of a causal association, and indicate further investigation using a much more sophisticated case-control, or cohort design (Rothman 2008 p99).
Geographical distribution of MS

Descriptive epidemiological studies have shown a large world-wide variation in the distribution of MS rates. Reviews of the distribution of MS were published by Kurtzke (1975, 2000) and Pugliatti et al. (2006). In Kurtzke’s review (1975) he classified prevalence as indicating high (>30 per 100,000 population), medium (5-30 per 100,000) and low risk areas of MS (<5 per 100,000). High prevalence of MS was found in the northern parts of Europe and North America, medium prevalence was found in southern USA and southern Europe, and low prevalence was found in Asia and South America (Kurtzke 1975). Later Kurtzke updated the prevalence of MS in Europe and the Mediterranean basin (2005), where he modified epidemiological data on MS by Firnhaber and Lauer (1994). They showed that most of the countries in Europe, at that time, belonged to an area of high MS frequency.

In the study by Pugliatti et al. (2006) whose aim was to describe the current burden of MS in Europe, previous information on MS epidemiology in Europe was updated. Among other things, they calculated the total prevalence rate (when age-specific prevalence rates were given) for the countries in Europe and standardized them to the European population 1966, together with age-specific prevalence rates. Their results of standardized prevalence are shown in Figure 1. Prevalence rates, shown for all the Scandinavian countries, indicated a high prevalence throughout, as shown earlier by Kurtzke (1968, 2000).

Figure 1 Prevalence ratios of multiple sclerosis in Europe, adjusted for the European population (in brackets crude ratios). Reprinted from Pugliatti et al. 2006. Published with permission from Copyright Clearance Center.
The latitudinal effect on the MS distribution has been reported in several studies (Kurtzke et al. 1979, Taylor et al. 2010, Pugliatti et al. 2002, Ahlgren et al. 2011). However, some hesitation about the latitude effect has been reported. A large meta-analytical study of the association between MS prevalence and latitude was recently published by Koch-Henriksen and Sörensen (2010). Results from that analysis confirmed a latitude effect on MS in Australia and New Zealand, but in Europe and North America the latitudinal effect seemed, at best, to be modest. Thus, this issue is presently under discussion.

In Sweden, a nation-wide period prevalence study of MS (1925-1934) by Sällström (1942) showed a prevalence of 21.2 per 100,000 population. He collected data from MS patients from 87 different hospitals all over Sweden over a ten year period. In 1988 the prevalence in Gothenburg city, on the Swedish west coast, was 96 per 100,000 population. Incidence rate, in the same area was 4.2 per 100,000 person-years (1950 to 1964) and 2.0 per 100,000 (1974 to 1988) (Svenningsson et al. 1990). The county of Västerbotten, in northern Sweden, showed a rather high MS frequency, indicating a “cluster” in the north. The prevalence was 154 per 100,000 population (1997) and the incidence rate was 5.2 per 100,000 person-years during the period 1987-1997 (Sundström et al. 2003). Recently, a nation-wide prevalence study was published which presented the MS prevalence in Sweden of 188.9 per 100,000 population (Ahlgren et al. 2011).

The county of Värmland in Sweden has been pointed out as a high risk area for MS in the following studies: the period prevalence study by Sällström, 1925 - 1934 (1942); the Fennoscandian focus in Scandinavia by Kurtzke (1968, 1974); the mortality investigation 1952 - 1992 by Landtblom et al. (2002); the study of a cluster of MS in Lysvik, a parish in the northern Värmland, by Callander and Landtblom (2004). However, the most recent study of MS prevalence by Ahlgren et al. (2011) does not fully support this notion.
Aetiology

The aetiology of MS is not definitely known, but it is generally believed that both genetic and environmental factors contribute to the disease susceptibility. Both environmental and genetic factors can be both harmful (noxious) or protective.

Environmental risk factors

Different environmental factors, of both infectious and non infectious origin, have been proposed as risk factors for MS.

The role of infectious agents in MS has been studied by for example Kurtzke (1995) from the “epidemic” in the Faroe Islands, and he suggests a viral cause of MS. The Norwegian studies by Riise (1992) using a space-time-cluster methodology also give support to an infectious origin. Several studies have confirmed that Epstein-Barr virus is a risk factor for MS (Haar et al. 1997, Ascherio 2007). Individuals who have never been infected by the Epstein-Barr virus have a reduced risk of contracting MS, and those infected as young adults have a greater risk than those who had it at a younger age (Ascherio 2007).

Decreased sunlight exposure has now been linked with a higher risk of MS (Marrie 2004) and decreased vitamin D intake and production has been the mechanism used to explain the higher risk among those less exposed to sunlight (Marrie 2004, Ascherio 2007, 2010). Cigarette smoking has also been shown to be a risk factor for developing MS (Riise 2003, Ascherio 2007).

The risk of MS in relation to industry has also been reported, e.g. to the textile industry (Stocks 1971), to the paper industry (Lauer 1998, Böhme 2005) and the wood industry (Darmstaeder 2008, Lauer 2010). Elements of diet have also been proposed as risk factors (Lauer 2010, 2011).

Exposure to organic solvents has been evaluated (Hawkes 1989, Landtblom 1993, 1996, 1997, 2006) but no clear conclusions have been reached (Marrie 2004). Vaccinations have previously been considered as potential causal factors for MS but present investigations show no association between MS and vaccines (Marrie 2004, Ahlgren 2009).

Results from migrant studies support the role of an environmental factor by showing that the individual risk of MS may be altered by changing residence before adolescence, leading to adoption of the incidence of the new area (Kurtzke 1995, Dean and Kurtzke 1971). Immigrants from Iran to Gothenburg, Sweden showed a significantly high MS rate, higher than the country of origin and also higher than
the general population of Gothenburg (Ahlgren et al. 2010). Immigrants with MS onset after immigration to Canada were examined (Orton et al. 2010) showing that the women-to-men ratio gradually and progressively increased in a rather similar way to the Canadian-born MS patients. The women-to-men ratio and also its rate of change were variable by the immigrants’ region of origin.

Genetic risk factors
The genetic effect on MS risk has been discussed for a long time (Oksenberg 2010). The risk of getting MS is higher in relatives of a person with the disease than in general population, particularly for siblings, parents, and children (Dyment et al. 2004, Compston 2002). There is robust knowledge that the human leukocyte antigen (HLA) is involved in the genetics of MS (Jersild 1973). Differences in the HLA system moderate the probability of getting MS (Compston 2007 p57). A study by Chao et al. (2010) has shown that patients with the MS-associated allele HLA-DRB1*15 had a higher women-to-men ratio versus those lacking it. Poser (1994) has presented a theory on the Vikings as a genetic pool for dissemination of MS throughout the world. In MS there is, according to new findings, also an overrepresentation of the genes for the alpha chains of interleukin receptors IL-2 and IL-7 (Olsson and Hillert 2008). Other studies with primarily genetic focus regard the risk in relatives of a MS patient to develop pathological signs, the so called MS trait (Haghighi et al. 2000, Poser 2004). A large population-based study by Hemminki et al. (2009) investigated the familial risk of MS versus other autoimmune disease, amyotrophic lateral sclerosis (ALS) and asthma. The standardized incidence ratio (SIR) was 5.94 when a parent was diagnosed with MS and an almost doubled risk of getting MS was found when a parent was diagnosed with ALS.

The sex ratio
The sex ratio is also interesting from an aetiological point of view. Female sex is a powerful risk factor for MS and studies show an odds ratio of about 5. There is a distinct female preponderance of autoimmune diseases in general during reproductive ages. Sex hormones and/or sex chromosomes may be responsible for this enhanced susceptibility. In general, women have more robust immune response than men (Voskuhl 2011). The risk by female sex in MS may be the effect of both environmental (internal or external) and genetic factors, or combinations. The women-to-men ratio of multiple sclerosis in Europe shows a variation from 1.1 to 3.4 (Pugliatti 2006). Several studies in the latest decade have shown an increased women-to-men ratio in
MS (Hirst et al. 2009, Koch-Henriksen 1999, Bentzen et al. 2009). This is due to an increased incidence in women rather than a decline in men. Interestingly, authors in Canada have presented results that showed a significant increase in women-to-men ratio (Orton et al. 2006). However, from Alberta, Canada (Warren et al. 2008) where the incidence of MS is very high, no changes of women-to-men ratio from 1990 to 2004 were seen. Results from a follow-up of the incidence in MS in Hordaland, Norway (Grytten et al. 2006) can be interpreted as showing an increase of the women-to-men ratio from 1.27 (1953-1957) to 2.4 (1978-1982), which thereafter decreased to 1.22 (1998-2002). The sex ratio from different prevalence studies in Sweden has shown the following women-to-men ratios: 1.9 in the county of Västerbotten (1997); 2.3 in the county of Värmland (2002); and 2.4 for the whole Swedish nation (2008) (Sundström et al. 2003, Boström et al. 2009, Ahlgren et al. 2011).

Gene-environment interaction
Furthermore, an attractive hypothesis is that the causes of MS certainly interact, in the form of gene-environment interaction, for example regarding smoking and HLA type (Hedström et al. 2010), but it may also be in the form of gene-gene interaction or interaction between several environmental factors.

Epidemiological trends and questions
For some time several investigators have found indications of an increasing frequency of MS. It is however difficult to determine if this is a genuine increase or due to improvement in diagnostic methods, MRI above all. A change in sex ratio is also debated during the last years. Such a change may be the effect of environmental factors, see above, but one cannot exclude that also diagnostic improvement can influence sex ratio. Finally, immunomodulating drugs are expected to change the natural course of MS, because of their properties to decrease bouts and affect inflammatory and other mechanisms in MS. A Swedish study has shown that disability due to secondary progression is decreased in treated patients (Tedeholm et al. 2010).
MS Registers

Denmark
In 1949 a Danish nationwide prevalence survey was conducted. This was the start of The Danish Multiple Sclerosis Registry, which was formally established in 1956. Since then, the register has continually collected data from new and old cases of MS or suspected MS. The long-term nationwide Danish register has proved to be a valuable instrument for monitoring incidence and prevalence, analysing survival, performing genetic analysis, providing unselected patient samples for clinical analyses, performing case-control studies and prospective studies and estimating the need for treatment and care (Koch-Henriksen et al. 2001, Bentzen et al. 2010).

Norway
The National Multiple Sclerosis Registry of Norway was established in 1998. Through a national collaboration, the register aims for inclusion of a biobank unit for collection of cerebrospinal fluid and serum, DNA, and tissue samples (Myhr et al. 2006).

Sweden
The National Swedish MS Register, SMSreg started in 1996 and later developed into a web-based (www.msreg.net) version in 2001 covering the geographical area of the whole of Sweden. The intention of the SMSreg is to support patient-related work as a Register of Good Health Practice (Kvalitetsregister), but it also allows local units to manage quality control and improvement.

The aim of the SMSreg is:
• to contribute towards high-quality, equitably distributed MS care in Sweden
• to assure that prevailing treatment indications for MS are followed
• to assess the long-term effects of modern drugs that modify the progression of MS
• to produce and broadcast new knowledge of MS by research and information

All counties in Sweden are involved and there are in total about 50 regional departments/units in the registry. In total there is information from 13,000 patients with MS which is more than 70 percent of the estimated Swedish MS patient population. Important information is demographic variables, history of the disease (e.g. age of onset, onset symptoms, type of progression, heredity), diagnostic investigations (e.g. magnetic resonance imaging (MRI) and spinal fluid testing), immune modulating treatment, Expanded Disability Status Scale (EDSS) and other rating scales for functional level, quality of life, fatigue, and cognition.
Healthcare professionals report data directly during the MS patient’s visit and continuously update the information in the internet-based register. Participating caregivers can directly find statistical information about their own patients and the entire country. There are also possibilities to search for data on the patients at the practise or county level. Reports of national data are annually presented.

The SMSreg is included in the National Healthcare Quality Registries in Sweden, Swedish Association of Local Authorities and Regions (SALAR) (www.skl.se). These National Quality Registries contain individual-based data on symptoms or diagnoses, treatment interventions and outcomes. Data is useful for multiple purposes in the development of a health care of good quality and security, for example to evaluate a disease-modifying drug, natalizumab, the use of which is registered continuously in accordance with a special, expanded protocol to assess risks and benefits.

**Diagnostic criteria**

The diagnostic criteria for MS have been changed over time. Before 1983 the diagnosis of MS was made using the criteria of Schumacher (Schumacher et al. 1965). In 1983 the Schumacher criteria were updated by the Poser criteria and these included paraclinical data such as magnetic resonance imaging (MRI), and cerebrospinal fluid analysis (CSF) examination (Poser et al. 1983). An International Panel on the Diagnosis of MS presented new diagnostic criteria for MS in 2001, known as “McDonald Criteria”. These criteria have been extensively assessed and used (McDonald et al. 2001). Later, in 2005 these criteria were revised (Polman et al. 2005). The latest revision was made in 2010 by Polman et al. (2011). Common to all these diagnostic criteria are that they need to demonstrate dissemination of lesions in both space and time and to exclude alternative diagnosis. Revised diagnostic criteria allow earlier diagnosis in some patients.

The clinical entity of clinically isolated syndrome (CIS), was introduced in 2001 (McDonald et al. 2001).

In paper IV on sex ratio in MS, cases from the SMSreg have been collected, and both the Poser and the McDonald criteria are used in the register. There is a continuous upgrading towards use of McDonald criteria.
### The Poser criteria

In paper I the diagnostic criteria of Poser were employed (Poser et al. 1983).

**A. Clinically definite MS (CDMS)**
1. Two attacks and clinical evidence of two separate lesions.
2. Two attacks, clinical evidence of one lesion and paraclinical evidence of another, separate lesion.

**B. Laboratory-supported definite MS (LSDMS)**
1. Two attacks, either clinical or paraclinical evidence of one lesion, and CSF oligoclonal bands (OCB)/intrathecal IgG synthesis (IgG).
2. One attack, clinical evidence of two separate lesions, and CSF=OCB/IgG.
3. One attack, clinical evidence of one lesion and paraclinical evidence of another, separate lesion; and cerebrospinal fluid (CSF) OCB/IgG.

**C. Clinically probable MS (CPMS)**
1. Two attacks and clinical evidence of one lesion.
2. One attack and clinical evidence of two separate lesions.
3. One attack, clinical evidence of one lesion and paraclinical evidence of another, separate lesion.

**D. Laboratory-supported probable MS (LSPMS)**
1. Two attacks and CSF OCB/IgG

### The McDonald criteria

The McDonald criteria consider the radiological lesions as having a high predictive value in the course of MS. The diagnoses are: MS, Possible MS, Primary progressive MS.

**Relapsing remitting MS**
At least one episode with neurological symptoms interpreted as MS-relapses. The following investigation includes MRI of the brain, CSF analysis. Four situations can be identified:

**A.** The history contains two relapses; the clinical examination reveals two lesions. Criteria for MS diagnosis are fulfilled even if MRI and CSF examinations have not been performed. If these examinations have been performed and are normal, the diagnosis should be questioned. In order to secure a diagnosis of MS, proof is needed for dissemination in space and time: at least two lesions appearing with 30 days interval. The lesions are demonstrated through clinical symptoms (relapses) and clinical examination, sometimes in combination with MRI according to table 1 and 2.

**B.** The history contains two relapses, the clinical examination reveals one lesion: Dissemination in space must be demonstrated in order to fulfill the criteria for MS diagnosis. Multifocality can be shown in three ways:
   a. a new lesion in another region can be demonstrated at a later clinical investigation
   b. the MR image shows lesions that fulfill the MR criteria in table 1
   c. the MR image shows at least two lesions that fit well with MS and CSF analysis is “positive”, i.e. shows oligoclonal bands and/or a high IgG index.

**C.** The history contains one relapse, the clinical examination indicates two lesions: Dissemination in time must be demonstrated:
   a. dissemination in time with MR (table 2) or
   b. a new relapse compatible with a new lesion
Table 1. MR criteria on dissemination in space in MS
Dissemination in space is based on MR criteria. Three of the following should be fulfilled:
1. A gadolinium enhancing (Gd+) lesion or nine T2 hyperintense lesions if no Gd+ lesions exist. Also spinal lesions count.
2. At least one infratentorial or spinal lesion
3. At least one juxtacortical lesion (“u-fibers”)
4. At least three periventricular lesions

D. The history contains one relapse, the clinical examination shows only one lesion: dissemination in time and space must be demonstrated which can be done according to B (b or c) and C, see above. According to these criteria, a positive CSF finding subsequently gets an important diagnostic impact for those patients where multifocality is shown on MR, but where the spread of the disease on MR is sparse and cannot give diagnostic accuracy on its own. In practice all patients with a neuroinflammatory bout and positive CSF findings should be looked upon as possible MS patients and be followed up as such.

Table 2. Evidence of dissemination in time using MR
1. A Gd+ lesion demonstrated at an examination performed at least three months after the onset of a relapse in a localisation that does not correspond to the clinical symptoms
2. A new T2 lesion on a new MR examination. The MR examination used as a reference must be performed at least 30 days after the relapse onset.

Primary progressive MS
For diagnosis is required:
Disease progression during >1 year (retrospective or prospective), and two of the following:
   a. Positive MRI of the brain (>9 T2 lesions or >4 T2 lesions and positive VEP)
   b. Positive spinal MR (>2 focal lesions)
   c. Positive CSF examination, i.e. oligoclonal IgG bands and/or elevated IgG index.

Source: Translated Swedish text in Metodboken, Svenska MS-sällskapet, 2012: http://www.mssallskapet.se/
This text was based upon Recommended diagnostic criteria for multiple sclerosis: Guidelines from the International panel on the diagnosis of multiple sclerosis (McDonald et al. 2001)
AIMS

The aim of this thesis was (1) to study the frequency of MS in the suggested high-risk area of Värmland county by investigating the current prevalence and analyzing the development over time; (2) to investigate some aetiological factors – the pattern of industry, both in relation to MS in Värmland and in the whole country; (3) to examine the possible relation between the distribution of MS and amyotrophic lateral sclerosis (ALS), as it has previously shown a positive correlation in two studies; and (4) to analyze the women-to-men ratio of MS in Sweden because of international reports of an increasing ratio.

The specific aim of each paper was:

• to determine the current prevalence of MS in the county of Värmland;

• to study the pattern of industry in relation to MS, firstly in the high-risk county of Värmland, and secondly in the whole of Sweden;

• to investigate if an association between ALS and MS, which has been described earlier, could be demonstrated at a later period using national mortality statistics;

• to analyse the sex ratio in MS birth cohorts in the Swedish population by (a) year of birth and (b) year of onset;
METHODS AND MATERIALS

To determine the prevalence ratio in Värmland (paper I), medical files were checked for International Classification of Diagnosis (ICD) codes corresponding to the diagnosis of MS from all hospitals, large health care centres and a private neurologist practice in the county of Värmland. These files were then collected and scrutinized by two of the authors, who are specialists in neurology (AML, MC). The diagnostic criteria of Poser (1983) with the categories definite, probable and possible MS were used. The study was based on the onset of disease which was defined as the year of initial symptoms. All patients had symptoms of the disease prior to the prevalence day, 31 December 2002 and were living in the county of Värmland on that day, and fulfilled the diagnostic criteria of Poser for CDMS and probable MS (1983). The data was collected between November 1998 and August 2005.

On the prevalence day 31st December 2002 (paper I), the population consisted of 273,419 inhabitants with a women-to-men ratio of 137,728 / 135,691, i.e. 50.4% / 49.6%. During the year 2000, approximately 7,000 people immigrated to the study area, and approximately the same number emigrated. Immigration and emigration, according to Statistics Sweden, have been in this range for some years. The county of Värmland has a high proportion of inhabitants who are professionally active in industry, forest-related industry being the major one. However, there has been a decrease in industry-related professions from 35% in the 1970s to around 20% in the year 2005. The county of Värmland is located in western Sweden on the 60° N latitude. It borders to Norway and has an area of 22,000 km². The county is divided into 16 municipalities.

Three population-based investigations on MS frequency in Värmland (Sällström 1942, Landtblom et al. 2002, Boström et al. 2009) were used to make an ecological study (paper II) on the correlation of MS with the density of specific industries, as an indicator of environmental pollution. The MS period prevalence 1925-1934 (Sällström 1942), the MS point prevalence in 2002 (Boström et al. 2009), and the annual age- and gender-adjusted MS mortality 1952-1992 (Landtblom et al. 2002) were correlated with industries in 14 different sectors in 1913 (Guinchard 1914). These were shown in the form of maps in the original data source. Industry in the 1950s was derived from an extended encyclopaedia of Sweden (Carlquist 1959-61), and classified in the same dichotomous way. Northern latitude and percentage of the population engaged in forestry-related industries, by population 1960, was derived from an extended monograph of forest industries in Värmland (Edberg and Arnell 1973).
Selected industries were also tested in all Sweden. The industries were taken from the works of Guinchard (1914) and Sømme (1961), where all factories were presented as dots. For the evaluation of all Sweden, these dots were divided by county population in 1913 (Statistiska Årsbok 1915) and 1950 (Statistical abstract 1970), respectively.

The study of the potential association between MS and ALS was based on mortality statistics of both diseases for all counties in Sweden during the period 1990 to 2010 (paper III). Data from Causes of Death Register was collected from the National Board of Health and Welfare and data from the Total Population Register was collected from the Swedish National Statistics office (Statistics Sweden) (www.socialstyrelsen.se, www.scb.se). During the study period both the Ninth Revision and the Tenth Revision of the International Classification of Diseases were used. For the death certificates, both underlying and contributing cause-of-death were considered in the analysis. The mortality rates of MS and ALS in 21 counties were adjusted for age and sex, using the direct standardization method (Hennekens 1987) with the population of Sweden 1998 as the reference population (www.scb.se).

Three papers concerned the whole nation Sweden (paper II, III, IV); Sweden is geographically located between latitudes 69°03’ N (north) and 54°37’ N (south) and longitude 10°33’ E (west) and 24°50’ E (east). The centre is in 61°50’ N - 17°42’ E. The total area is 449,965 km² and the southern part of the country has a long coastline of 3,218 km. In the north-east, the country is adjacent to Finland and in the north-west to Norway (www.sverigeatlas.se). The country is now divided into 21 counties. The average annual population varied during different study-time periods from 6,912,724 in the years 1946-1950 to 8,976,946 in the years 2001-2005 (paper IV). In the study of MS and ALS mortality (paper III), the average annual population during the 21 years of study (1990-2010) was 8,943,330.

In the study of sex ratio of MS (paper IV), determined by year of birth and by year of onset, data from the Swedish MS Register (SMSreg) (www.msreg.net) and from the Total Population Register of the Swedish National Statistics Office (Statistics Sweden) (www.scb.se) was used. Possible MS and the clinical entity of clinically isolated syndrome (CIS) were not included. Sex ratio by year of birth included all persons who were born in Sweden during the period from 1921 to 1989. Information on the number of persons with MS by year of birth, sex and whether they were born in Sweden was taken from SMSreg. The study period was divided into 11 five-year sub-periods, 1931-1935 to 1981-1985. Prevalence proportions were calculated as the cases of MS, who contracted the disease, from each year-of-birth cohort, (numerator)
divided by members of the birth cohort (denominator) for each five-year intervals for both sexes. Then sex ratios were calculated.

All persons with a diagnosis of MS who were registered in the SMSreg, both people born in Sweden and immigrants, were included in the study of Sex ratio by year of onset. Individuals with MS were extracted and analyzed by sex and by year of onset. The population number for each year 1941 to 2005 was collected from the Total Population Register (Statistics Sweden, www.scb.se). The study-time periods were divided into 12 five-year periods, from 1946-1950 to 2001-2005. For each five-year period, the age- and sex-adjusted incidence rates were calculated. Thereafter the sex ratios were determined covering onset periods from 1946 to 2005.

**Diagnostic criteria**

In the prevalence study (paper I) the diagnostic criteria of Poser were applied (1983). Although new diagnostic criteria were introduced during the study period, the above criteria were retained. The clinical entity of CIS, introduced in 2001 (McDonald et al. 2001), had been used for some time by some physicians in Värmland at the time of the prevalence investigation, but was not included in the present study. The data from SMSreg which was used to analyse sex ratio (paper IV) comprised cases of MS diagnosed by Poser and/or McDonald criteria (but before 2010 revision).

**Statistics**

For statistical evaluations, SPSS 13.0 was used in paper I, III, IV and the software Statistica for Windows™ (StatSoft 1994) was applied in the ecological study, paper II. The Poisson distribution was used for calculation of 95% confidence intervals in paper I and IV. Age- and sex-adjusted prevalence ratios, mortality rates and incidence rates were calculated using the direct standardisation method in paper I, III and IV. To test whether or not the difference between prevalence in the municipalities of Värmland 2002 (paper I) and differences in prevalence between 1933 and 2002 were statistically significant, the chi-square test (X²) was performed.
For industrial variables (paper II), the non-parametric Kruskall-Wallis test was applied, and the rank correlation coefficient according to Spearman was used for the wood-related industrial pattern in Sweden in 1913 and in 1950s, and the forestry data and northern latitude in Värmland (paper II). Considering the low number of geographical units (paper II) (n = 11, or n = 16, respectively) and the still early phase of hypothesis generation in MS, a p-value of ≤ 0.10 was considered significant, and of 0.10 < p ≤ 0.15 indicating a trend towards an association in Värmland. For the correlation between the mean mortality of ALS and MS (paper III) and to test if the sex ratio in MS (paper IV) had significantly changed, the non-parametric Spearman’s rho was used.
RESULTS

County of Värmland

Prevalence and incidence, paper I

At the time of the prevalence day, there were 465 patients who fulfilled the criteria for MS diagnosis (Poser 1983), lived in Värmland county, were alive and had onset of the disease prior the prevalence day. Patients with possible MS were not included. On the prevalence day, the population was 273 419 which gave the prevalence of 170.07 per 100,000 inhabitants (95% CI 154.5-185.5), women 235.97 per 100,000 inhabitants (95% CI 210.3-261.5) and men 103.17 (95% CI 86.1-120.2). After sex- and age-adjustment to the national population in 2002 (www.ssd.scb.se), the prevalence in Värmland was 168.3 per 100,000 (women 234.6, and men 101.4). The mean age was 50.49 years, for women 50.27 and men 51.00, respectively.

Distribution of MS prevalence in the municipalities in the county of Värmland showed a variation of 112.7 to 277.1 per 100,000 population, but the variation was not statistically significant.

In 1933 Sällström described the distribution of MS in Värmland in four cities and in seven rural districts (härad). These rural and judicial districts no longer exist, but their boundaries, 1933 can still be found in the archives. To illustrate the occurrence of MS in the county, Sällström’s collected data from judicial districts (domsaga) was presented as percentages of the total prevalence ratio in the county. The variation in the prevalence of MS between judicial districts in 1933 was not statistically significant. When the high prevalence ratio in 2002 was extrapolated back to 1933, keeping the proportion of cases by their geographical distribution, a highly significant difference from homogeneity was obtained. Since the current distribution in 2002 is not statistically significantly different from homogeneity, this gives evidence that - at the county level - in Värmland, there has been diffusion or spread of disease so that all parts are more equal.

The incidence rates were calculated for the period 1991-2000 in two 5-year periods. The average annual population during the incidence study periods was 284,721 in 1991-1995 (143,371 women and 141,350 men) and 278,448 in 1996-2000 (140,404 women and 138,044 men). In all, 181 patients with MS had onset of disease within this period, 66 women and 26 men during 1991-1995 and 62 women and 27 men during 1996-2000. In 1991-1995 the average annual incidence rate was 6.46 per 100,000 person-years (95% CI 5.14-7.78), women 9.21 (95% CI 6.99-11.43) and
men 3.68 (95% CI 2.26-5.09). In 1996-2000 the average annual incidence rate was 6.39 (95% CI 5.06-7.72), women 8.83 (95% CI 6.99-11.43) and men 3.91 per 100,000 person-years (95% CI 2.26-5.09). The mean age of onset was 34.9 years (men 36.2 and women 34.3).

Mortality, paper III

The study of ALS/MS generated basic epidemiological data that was used to study mortality in Sweden. The sex- and age-adjusted mean mortality rates for MS in the county of Värmland through the period of 1990 to 2010 was 2.76 per 100,000 person-years (95% CI 2.28-3.22), women 3.18 (95% CI 2.54-3.81) and men 1.97 (95% CI 1.46-2.48). The crude death rates in the municipalities of the county are presented in Table 1. The death rates varied from 1.92 (Årjäng) to 4.48 per 100,000 person-years (Torsby), but this variation was not significant. This mortality data shown by municipalities was not included in Paper III, only here.

Table 1. Mean mortality rates of multiple sclerosis per 100,000 person-years by municipalities in the county of Värmland, during time period 1990-2010. (O = Observed, E = Expected).

| Municipality | Number of deaths | Population | Rate | P | O-E/E | X^2 | p
|--------------|-----------------|------------|------|---|-------|-----|---
| Torsby       | 13              | 13 817     | 4.48 | 1.445 | 1.724 |   |   |
| Grums        | 9               | 9 719      | 4.41 | 1.413 | 1.086 |   |   |
| Säffle       | 15              | 16 755     | 4.26 | 1.365 | 1.472 |   |   |
| Hagfors      | 12              | 14 390     | 4.00 | 1.282 | 0.745 |   |   |
| Filipstad    | 9               | 11 992     | 3.75 | 1.164 | 0.165 |   |   |
| Kjelltorp    | 9               | 11 992     | 3.75 | 1.164 | 0.165 |   |   |
| Kristinehamn | 18              | 24 749     | 3.46 | 1.109 | 0.198 |   |   |
| Forshaga     | 8               | 11 710     | 3.25 | 1.042 | 0.013 |   |   |
| Arvika       | 18              | 26 491     | 3.24 | 1.038 | 0.131 |   |   |
| Esa          | 6               | 8 891      | 3.21 | 1.029 | 0.006 |   |   |
| Hammarö      | 9               | 14 256     | 3.01 | 0.965 | 0.012 |   |   |
| Storfors     | 3               | 4 807      | 2.97 | 0.952 | 0.007 |   |   |
| Sunne        | 8               | 13 661     | 2.79 | 0.894 | 0.101 |   |   |
| Karlstad     | 39              | 80 655     | 2.30 | 0.737 | 3.625 |   |   |
| Munkfors     | 2               | 4 272      | 2.23 | 0.715 | 0.229 |   |   |
| Årjäng       | 4               | 9 697      | 1.92 | 0.615 | 0.949 |   |   |
| County of Värmland | 192 | 192 | 277 807 | 3.12 | 1.000 | 10.655 |   |   |

X^2=10.655; p=0.05 at X^2=24.096
Ecological study of industry in Värmland 1913 and 1950s, paper II

The three MS rates available for Värmland in the past 90 years, i.e. the MS mortality 1952 - 1992, the MS period prevalence 1925 - 1934 and the MS prevalence 2002, were uncorrelated with each other, when Yates’ correction was made.

The MS period prevalence 1925 - 1934 was associated with “large sawmills” and a possible correlation of that factor was shown for the MS prevalence 2002. The MS period prevalence 1925 - 1934 and the MS mortality 1952 -1992 showed a possible association with “wood-pulp factories”.

When the number of wood - pulp factories was related to population 1913, there was a significant association ($r_S = 0.6636; p = 0.0260$).

The association between industry in the 1950s, and the MS point prevalence 2002 and the MS mortality 1952 - 1992 were also evaluated. Here, “textile factories” were positively correlated, and “wood-pulp factories” and “food industry” showed a tendency towards association with the MS point-prevalence 2002. “Northern latitude” was correlated with MS mortality 1952-1992, and the combinatorial term “sawn timber 1967 - 1971 and/or cellulose factories 1965, both by population 1960 showed a trend towards a correlation with the MS mortality 1952 - 1992.

ALS/MS in Värmland, paper III.

The sex- and age-adjusted mean mortality rate for ALS in Värmland during 1990-2010 was 2.28 per 100,000 person-years (95% CI 1.96-2.59), i.e. 76% of the total mean mortality rate for the whole nation (2.98). The mortality rate in MS was 2.76 per 100,000 person-years (95% CI 2.28-3.22), i.e.135.2% of the total rate. Mortality rates in both ALS and MS decreased – from 125.5% to 76% of the total rate in ALS, and from 171.5% to 135.2 % in MS.
Sex ratio, paper 1

The frequency of MS in the prevalence study 2002 in Värmland, paper I, was 2.3 times higher amongst women than men. The age-specific prevalence and women-to-men ratio are shown in Figure 2. The highest women-to-men ratio was in age-group 25-34 and 65-74 years.

The women-to-men ratio of the mean incidence rates during 1991 to 2000 in Värmland was 2.38.

The data collected for the prevalence study in 2002, paper I, was also used to analyse sex ratio by year of birth during the period from 1931 to 1975. No clear trend was found for women-to-men ratio, (Spearman’s rho = 0.383, p = 0.308, n = 9). Also sex ratio by patient’s year of onset, Figure 3 was calculated and this did not show any trend either (Spearman’s rho = 0.000, p = 1.000, n= 8). This data is not presented in paper I, only here.
**Sweden**

MS distribution in the country of Sweden.

Mortality rates, **paper III**

The study of MS/ALS generated a large amount of epidemiological data: The age- and sex-adjusted national mean mortality rate for MS in Sweden during 1990 to 2010 was 2.04 per 100,000 person-years (CI 95% 1.95 – 2.12), in women 2.43 (CI 95% 2.31-2.56) and in men 1.64 (CI 95% 1.54 – 1.74). Mortality rates for both sexes and sex ratios for each calendar year are shown in Figure 4.

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*Figure 3. Incidence rate of multiple sclerosis per 100,000 person-years and women-to-men ratio in the county of Värmland analysed by year of onset*
The mean mortality rates in the Swedish counties (Figure 5) showed a significant variation, ($\chi^2 = 119.46$, d.f. = 20, $p < 0.001$). The MS mortalities 1952-1992 (Landtblom et al. 2002) and 1990-2010 were significantly correlating (Spearman’s rho = 0.593; $p = 0.005$; $n = 21$). The mean age at death was 66.0 years (women 66.5, men 65.2).

Figure 4. Mean mortality rates of multiple sclerosis in both sexes and women-to-men ratios in Sweden from 1990 to 2010.

Figure 5. Mean mortality rates of multiple sclerosis in the counties in Sweden during 1990-2010.
In paper III the MS mortality rates were analyzed to see if there was an association with ALS mortality rates. There was no correlation between these mortality rates in the 21 Swedish counties during the period of 1990 to 2010 (Spearman’s rho = -0.052; p = 0.822; n = 21).

Ecological study of select industries in Sweden 1913 and 1950s, paper II

Wood-pulp and paper-mills industries showed an association with the MS mortality 1952-1992. From the 1950s, only MS mortality 1952-1992 was available for comparison. Pulpmills, paper and cardboard industries and sawmills, all by population 1950, were unrelated to the MS mortality. When subcategories by calendar period were formed, however, the paper and cardboard industry of 1951 showed a borderline correlation with the MS mortality 1952-1962 and 1963-1972 (rs = 0.3486 and 0.3951, respectively; p=0.0968 and 0.0561, respectively). The wood-pulp, papermills, large sawmill industries, mechanical works and selected miscellaneous industries in Sweden in 1912 were all unrelated to the MS period prevalence 1925-1934.

ALS/MS, paper III

The MS mortality rates were analyzed to see if there was an association with ALS mortality rates. There was no correlation between these mortality rates in the 21 Swedish counties during the period of 1990 to 2010 (Spearman’s rho = - 0.052; p = 0.822; n=21). Detailed results regarding MS, see above.

The sex- and age-adjusted national mean mortality for ALS was 2.98 per 100,000 person-years (95% CI 2.87-2.08) in women 2.72 (95% CI 2.58-2.86), and in men 3.23 (95% CI 3.07-3.40). The mean age at death in ALS was 70.4 years (women 71.9 and men 69.2). The mortality rates of ALS in Västerbotten and Norrbotten were 3.48 per 100,000 person-years (116.7% of the total nation rate), and 3.49 (117.1% of total) respectively. Both these counties have low MS mortality rates, Västerbotten 1.86 per 100,000 person-years (91.1% of total national mean), and Norrbotten 2.00 (98% of total).

Sex ratio, paper IV

Analyzed by year of birth

Extracted from the SMSreg, there were 8,834 patients (6,271 women and 2,563 men) with MS diagnosis born in Sweden during the period 1931 to 1985. Patients born before the year of 1931 and after 1985 were excluded because of their low number.
The number of women and men who were born between 1931 and 1985 and the number of individuals with MS, were divided into five-year periods. The average annual prevalence proportions of MS by year of birth were increased from the period of 1931-1935 up to 1956-1960 in women, and in men up to 1951-1955. Thereafter the prevalence proportion was decreasing in the both sexes. The variation of the women-to-men ratios were marginally changed throughout the period with a mean value of 2.62 (CI 95% 2.48 – 2.74) and there was no clear trend noted for the women-to-men ratio by year of birth (Spearman’s rho = 0.345, p = 0.298, n = 11).

**Analyzed by year of onset**

The number of MS patients, analyzed by year of onset from SMSreg, was 9,098 patients (6,452 women and 2,646 men) during the study period from 1946 until 2005. The average annual population and the total number of cases of MS for were divided into five-year periods, before incidence rate by year of onset was calculated.

The average annual sex- and age-adjusted incidence rates of MS in five-year periods and subdivided by gender and by period of onset, have continually increased from 1946-1950 until 2001-2005, both for women and men. In women, the incidence rate has increased from 0.14 to 6.16 per 100,000 person-years and for men from 0.06 to 2.57. The mean incidence rate during the whole period was 1.75 per 100,000 person-years (95 % CI = 1.00-2.51), in women 2.48 (95% CI = 1.12-3.85) and in men 1.03 (95% CI = 0.44-1.61).

The mean women-to-men ratio for the whole period, 1946 to 2005 was 2.57 (95% CI 2.24-2.91). This ratio by year of onset was highest during the period from 1956 to 1970. Later, it was rather stable. There was no significant change of the women-to-men ratio during this time (Spearman’s rho = -0.007, p = 0.983, n = 12).
DISCUSSION

On the prevalence and mortality of MS in Värmland as a suggested high risk region

The results of the studies of prevalence ratio, incidence rate in paper I and mortality rate of MS in Värmland, paper III, support the previous notion that Värmland is a high-risk region for MS in Sweden (Kurtzke 1974, Landtblom et al. 2002, 2005). The MS prevalence ratio in Värmland county was 170.07 per 100,000 inhabitants. Comparisons show that the prevalence ratio in Gothenburg, southern Sweden, was lower, 96 per 100,000 population (Svenningsson et al. 1990). However, this data is approximately 20 years old. Västerbotten county also has a high prevalence, 154 per 100,000 population in 1997 (Sundström et al. 2003). This region has previously been pointed out by Kurtzke (1974) as another high-risk region for MS. Other published prevalence figures from the Nordic countries reveal high prevalences (Norway, 150.8 (Grytten et al. 2006), Finland 93-188 (Sumelahti et al. 2001), and Denmark from 56.2 to 173.3 per 100,000 population (1950 to 2005) (Bentzen et al. 2010). Still, Värmland clearly keeps its position at the upper level. A recently published study of Sweden, by Ahlgren et al. (2011) showed an unexpectedly high nationwide prevalence of 188.9 per 100,000 population, higher than the Värmland prevalence in paper I but also performed some years later. One municipality in Värmland, Säffle, showed a very high occurrence in this study, as well as in several of our studies, see Table 2.

The incidence rates, 6.46 and 6.39 per 100,000 person-years in Värmland county in 1991-95 and 1996-2000, respectively, are relatively high and nearly the same as in Västerbotten, Sweden 1987-1997: 6.7. These are higher compared with rates reported from Norway 1993-1997: 6.0 per 100,000 person-years (Grytten et al. 2006). Other areas in Europe (Pugliatti et al. 2006) with high (crude) incidence rates were: eastern Norway 8.7; Seinäjoki in Finland 11.6; south-eastern Scotland 9.3; and northern Sardinia, Italy with 6.8 per 100,000 person-years. This data still supports Värmland as a rather high incidence area.

Värmland county, paper III, has a high MS mean mortality rate, 2.76 per 100,000 person-years, during the period 1990-2010, which was highest amongst the Swedish countries.
The mortality rates in Värmland were also analyzed by municipality, data which was not presented in the papers but is shown below. The crude mortality rates are shown in Table 2, where also the previously published mortality rates from 1952-1992 (Landtblom et al. 2002) are presented, as well as the local prevalence 2002. Observe that prevalence is a term that actually should not be used in too small populations. The municipalities that had high rates in two of three studies were Sunne, Torsby and Hagfors. The municipality of Säffle had a high rate in all three studies, Table 2, which also was demonstrated by Ahlgren et al. 2011.

The variation of MS mortality rates between the municipalities observed during 1990-2010 was not statistically significant. Further, the MS mortality in the municipalities of Värmland 1990-2010 and 1952-1992 were not significantly correlated (Spearman’s rho 0.453, p=0.078, n = 16).

Table 2. Average mortality rates in multiple sclerosis per 100,000 person-years from 1952-1992 (data of Landtblom et al. 2002) and 1990-2010; prevalence ratios in the municipalities of Värmland, 2002. (The four highest rates/first in each investigation are marked in bold).

<table>
<thead>
<tr>
<th>Municipality</th>
<th>Average death rates per 100,000 person-years, 1952-1992</th>
<th>Prevalence ratios per 100,000 population, 2002</th>
<th>Average death rates per 100,000 person-years, 1990-2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>Årjäng</td>
<td>0.9</td>
<td>277.1</td>
<td>1.92</td>
</tr>
<tr>
<td>Säffle</td>
<td>4.1</td>
<td>245.6</td>
<td>4.26</td>
</tr>
<tr>
<td>Sunne</td>
<td>5.2</td>
<td>213.7</td>
<td>2.79</td>
</tr>
<tr>
<td>Arvika</td>
<td>3.3</td>
<td>182.8</td>
<td>3.24</td>
</tr>
<tr>
<td>Karlstad</td>
<td>2.2</td>
<td>181.6</td>
<td>2.30</td>
</tr>
<tr>
<td>Munkfors</td>
<td>2.6</td>
<td>171.3</td>
<td>2.23</td>
</tr>
<tr>
<td>Kil</td>
<td>3.1</td>
<td>199.8</td>
<td>3.57</td>
</tr>
<tr>
<td>Hagfors</td>
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<td>4.00</td>
</tr>
<tr>
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<td>152.1</td>
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</tr>
<tr>
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<td>3.21</td>
</tr>
<tr>
<td>Torsby</td>
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</tr>
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<td>Forshaga</td>
<td>1.8</td>
<td>130.7</td>
<td>3.25</td>
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<td>130.3</td>
<td>2.97</td>
</tr>
<tr>
<td>Hammarö</td>
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<td>119.4</td>
<td>3.01</td>
</tr>
<tr>
<td>Kristinehamn</td>
<td>1.9</td>
<td>112.7</td>
<td>3.46</td>
</tr>
<tr>
<td>County of Värmland</td>
<td>2.8</td>
<td>170.1</td>
<td>3.12</td>
</tr>
</tbody>
</table>

The county of Värmland had the highest MS mortality rates in both investigated periods: 1990 - 2010, (2.76) and in 1952 – 1992 (2.83) (Landtblom et al. 2005). This can be explained by the fact that there has been a high frequency of MS in Värmland for a long time (Sällström 1942, Kurtzke 1967). One can also not exclude increasing incidence as a cause of such an increase of mortality. A third factor that may be involved is the increasing survival time in later periods. The increase in mean age for MS may support such a cause.
National distribution of MS

The mean mortality rate in all Sweden was 2.04 per 100,000 person-years in the present study (paper III) and this was significantly higher than in the earlier investigation, 1.65 per 100,000 person-years (Landtblom et al. 2002) (chi² = 4.36; d.f. = 1; p = 0.037). Both studies of mortality rates in the counties (1952-1992 and 1990-2010), (mean mortality rates are shown in Figure 6) showed a significant variation between the counties. Further, when this data was compared, there was a significant correlation between the rates for each county (Spearman’s rho = 0.542; p = 0.011, n=24), indicating that the variation was stable over time. In a previous investigation from our group the concept of diffusion of MS in a slowly spreading pattern was discussed (Landtblom et al 2005) and the present findings do not contradict such a possibility.

Mortality statistics is, however, a crude instrument, and several factors could have influenced the results. Although death rates are an indirect and crude measurement of disease frequency several studies demonstrate that they have a rather high diagnostic precision (Johansson. 2009, Midgard et al. 1996). Both underlying and contributing cause-of-death were used in the mortality studies 1952-1992 (Landtblom et al. 2002) and in 1990-2010, which has been reported as important (Malmgren et al. 1983, Goldacre et al.2003).

The adjusted prevalence rates in Europe, presented in a review article by Pugliatti et al. (2006) showed a great variation of prevalence ratios. This review of prevalence ratios in Europe shows that the prevalence ratios differ not only between countries, but that there is also a great variation within countries, e.g. in three different locations in Norway with 74, 121 and 165 per 100,000 population. Similar variations have been shown in Italy with 55, 61, 65 and 140 per 100,000 population. Also for incidence rates, there has been reported a great variation as mentioned before (Pugliatti et al. 2006).

Differences in incidence rates and prevalence ratios from various studies, should however be interpreted with caution. The studies represent different geographical areas, points of time and period and the collection of data in different sources, – e.g. from hospitals or from registers. Changes in diagnostic criteria and new developments in examination and treatment throughout time might also have influenced estimated rates and make comparisons difficult. Despite this, we propose that the studies show that Värmland has a continuously high occurrence of MS, demonstrated in earlier studies by Sällström (1942) by Kurtzke (1968, 1975) and by Landtblom (2002) as well as in the present
Figure 6. Mean mortality rates of multiple sclerosis per 100,000 person-years, in counties of Sweden, in two investigated time periods: 1952-1992 (data of Landtblom et al. 2002) and 1990-2010.
studies of prevalence 2002 and mortality 1990 – 2010, Table 2. Particularly the area of the Säffle municipality has shown a high mortality rate during both study periods for mortality 1952 – 1992 and 1990 – 2010 and also a high prevalence in 2002.

**On the aetiological studies**

Industry in Värmland, **paper II**

MS prevalence and mortality in Värmland were associated with sawmills and wood-pulp factories. Throughout Sweden wood-pulp factories were correlated with MS mortality 1952-1992. The data confirms a relation of MS frequency to wood processing, or wood-related industries that has been shown in other studies (Lauer 2010, Darmstaedter 2008, Howe et al. 2007).

The importance of the study of environmental factors must be seen in the light of present focus on gene-environment interaction in MS (Landtblom et al. 2005). An example of studies that have shown such an interaction concerned HLA and smoking in MS (Hedström et al. 2010). Another study showed a negative interaction between smoking and EBV in MS (Riise et al. 2011). Furthermore, a gene-environment interaction has been discussed in connection to the MS cluster in Lysvik, a high risk area in Värmland county, which is studied by our group (Callander and Landtblom 2004, Imrell 2009 pp19-20) The preponderance of Finnish immigrants in the MS patients suggest a genetic component in this cluster (Geidraitis et al. 2003, Imrell 2009). Interestingly, in this area drinking water revealed high levels of pentachlorophenol (PCP) at some occasions (Lysviks Water Administration, 2005) and as possible source, a former sawmill was identified. Amphiphatic phenols e.g. nitrophenols can lead to immunological alterations such as autoimmune reactions (McConnachie and Zahalky 1991, Vojdani et al. 1992).

**ALS/MS, paper III**

Our study on mortality distribution of MS and ALS did not show any significant correlation, like in the previous study (Landblom et al 2002). Värmland, however, demonstrated high mortality in both time periods, but ALS, which had a high mortality in the early period had dropped. For the whole country, it was interesting to see that MS mortality still showed a significant variation between municipalities, but stable. ALS mortality pattern also showed a high variation; the distribution, however, had changed. The evidence for a relation MS/ALS could not be supported in this study.
Diffusion, paper 1
The prevalence study was compared with previous results (Kurtzke 1967), and the possibility of a slow spreading pattern of MS, a diffusion was demonstrated (Landtblom et al. 2005). This might be compatible with an infectious aetiological factor, and it has been suggested that virus is the best fitting agent to explain the risk variation over time.

Sex ratio, paper IV
An increase in the female/male ratio has been observed in Canada (Orton et al. 2006), in Denmark (Bentzen et al. 2009, Koch-Henriksen 1999) and in Japan (Osoegawa et al. 2009). A recently published review (Sellner et al. 2011) of possible aetiologies for the gender disparity in MS, suggested that decreased sun exposure may be a critical factor. Sunlight influences the level of vitamin D, which may have an effect on the immune system. Later childbirth as a factor for increased MS in women, has been discussed, but this was not supported by findings in a recently published study in Canada (Ramagopalan et al. 2010). A Swedish study revealed that the use of combined oral contraceptives and childbirth before onset of MS seemed to delay the onset of disease (Holmqvist et al. 2010). In our study (paper 4) the Swedish sex ratio showed no increased women-to-men ratio either when analyzed by birthday or by year of onset. One study in Australia (Simpson et al. 2011) has presented a similar result with a stable ratio during the time period 1951-2009.

Age specific incidence rates in the Swedish population, analyzed by year of onset, increased in all age groups and in both sexes during the period from 1951 to 2005 (Figure 7), with the highest increase in the age-groups 20-29 years and 30-39 years. The women-to-men ratios in these age-groups were rather stable (Table 3).
Figure 7. Age-specific incidence rate of multiple sclerosis per 100,000 person-years, in both sexes, by ten year calendar periods, from 1941 to 2005, in Sweden.


<table>
<thead>
<tr>
<th>Year</th>
<th>20-29 years</th>
<th>Sex ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1951-1960</td>
<td>3.03</td>
<td>3.84</td>
</tr>
<tr>
<td>1961-1970</td>
<td>3.44</td>
<td>4.02</td>
</tr>
<tr>
<td>1971-1980</td>
<td>2.28</td>
<td>2.13</td>
</tr>
<tr>
<td>1981-1990</td>
<td>2.56</td>
<td>3.27</td>
</tr>
<tr>
<td>1991-2000</td>
<td>2.71</td>
<td>2.66</td>
</tr>
<tr>
<td>2001-2005</td>
<td>2.62</td>
<td>2.50</td>
</tr>
</tbody>
</table>
CONCLUSION

The county of Värmland represents a high risk area for MS in Sweden and contains municipalities with very high frequency of the disease, as shown by the investigations of prevalence, incidence and mortality rates. The prevalence in 2002 was 170.07 per 100 000 population. The development of frequency in the municipalities fits with a slowly progressing pattern, diffusion. The development of MS mortality in the counties of Sweden may also fit into such a pattern.

The national MS mortality data reveals a statistically significant increase of mortality and the mean age at death has increased. This might be partly related to an increased survival time in the Swedish population, but likely also reflect a general increase in the incidence of the disease.

The sex ratio in Sweden taken from material in the National Swedish MS Register and the Swedish National Statistics Office did not show any significant change, as has been reported from some other countries, with an increased women-to-men ratio. A change of the sex ratio in MS can be related to environmental factors, internal or external. The stable ratio in Sweden, does not point to any specific risk factor.

The results from the ecological study included in this thesis might indicate a possible association with wood-related industry in Värmland and in the whole country. Previous studies from our group have pointed to possible genetical risk factors in Värmland and we hypothesize that there may exist gene-environment interactions.

Exposure to pentachlorophenol has previously been associated with the MS cluster of Lysvik in Värmland.

The study of MS/ALS ratio was performed in order to follow-up a positive correlation in our previous epidemiological studies. This correlation had disappeared in the present study and both diseases, interestingly, demonstrate a more homogenous pattern in the country.

This thesis is an example of epidemiological studies representing a tool for aetiologic investigations. Värmland county is suitable for further MS studies, investigating both genetic and environmental factors with a particular focus on gene-environment interaction.
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