Multiple Sclerosis in Västerbotten County, Northern Sweden

AKADEMISK AVHANDLING
som med vederbörligt tillstånd av Rektor vid Umeå Universitet
för avläggande av medicine doktorsexamen
offentligen försvaras i sal B, 9 tr, tandläkarhögskolan
lördagen den 7 juni 2003, kl 13.00

av

Peter Sundström

Fakultetsopponent:
Professor Oluf Andersen
Institutionen för klinisk neurovetenskap
Göteborgs Universitet

Institutionen för Farmakologi och Klinisk Neurovetenskap
Umeå Universitet
Umeå 2003
ABSTRACT
Multiple Sclerosis in Västerbotten County, Northern Sweden
Peter Sundström, Department of Pharmacology and Clinical Neuroscience, Neurology
University of Umeå, Sweden

One out of several distinguishing features of multiple sclerosis (MS) is the epidemiological variation of geographic distribution. Population-based studies on the prevalence and incidence of MS in Sweden have previously been performed only in Göteborg. Another feature of MS is the clinical variation between individuals. To a large extent data on the clinical characteristics of MS come from studies on cases frequenting MS clinics and therefore, may be biased. Also rare are population-based studies of the consequences of MS-related incapacity on socio-economic factors. As for MS aetiology, both environment and genes are involved. Human herpesviruses are often the main suspected environmental aetiological agents.

Our aim was to estimate the prevalence of MS in Västerbotten County for 1 January 1990, the incidence during a 10-year period 1988-97, and the prevalence 31 December 1997; and also to present detailed clinical data including onset symptoms and the disability distribution for the latter two MS populations. Furthermore, we wanted to estimate the prevalence of sick leave, professional assistance, and housing; and also, to study the risk factors for sick leave. In order to investigate the association between MS and human herpesviruses, samples were identified in two regional population-based serum bank registers. This linkage identified samples collected from before MS-onset in 73 MS cases and after MS onset in 161 cases.

The prevalence and incidence populations were identified through multiple sources. Diagnostic ascertainment, the reliability of clinical data, and additional information were assured from a questionnaire with follow-up interview and neurological examination.

The onset adjusted crude prevalence of MS was 125/100,000 (95% CI: 112-140) in January 1990, and 154/100,000 (95% CI: 139-170) in December 1997. The increase was mainly attributable to a higher incidence than mortality. The crude incidence rate 1988-97 was 5.2/100,000 (95% CI: 4.4-6.2). The disability distribution in the 1997 prevalence population in Västerbotten was compared to the disability distribution in a Canadian MS population, which has been used for publications on the natural history of MS. One difference from the Canadian studies appears to be the better recognition of cases with more benign disease. Nevertheless almost half of prevalent MS cases aged 18-64 years were fully sick-listed, and one-fourth of all prevalent cases received professional assistance. High disability level was the strongest predictor for sick leave. All MS cases showed signs of past Epstein-Barr virus (EBV) infection. High activity to EBV (EBNA-1 but not VCA) and human herpesvirus 6 (HHV-6) significantly (borderline significance for HHV-6) increased the risk to develop MS.

These estimates show that Västerbotten County is a high risk area for MS. Both incidence and prevalence were significantly higher when compared to estimates from Göteborg. The comparison with the Canadian MS population shows that MS might be a slightly more benign disease than previously recognized. Still, the consequences of MS regarding socio-economic aspects are considerable. We suggest that EBV is a prerequisite for the development of MS. Individuals that will develop MS exhibit an altered immune response against the EBV virus characterised by high activities to EBNA-1 in the absence of high VCA activities, this being most pronounced in the five-year period preceding MS onset. A pathogenetic role is suggested for EBV and remains possible also for HHV-6.

Key words: Multiple sclerosis, epidemiology, prevalence, incidence, onset symptoms, disability, sick leave, professional assistance, Epstein-Barr virus, and HHV-6.
Multiple Sclerosis in Västerbotten County, Northern Sweden

Peter Sundström

Umeå 2003
"Know then thyself, presume not God to scan,
The proper study of mankind is Man.
Placed on this isthmus of a middle state,
A being darkly wise and rudely great:
With too much knowledge for the Sceptic side,
With too much weakness for the Stoic's pride,
He hangs between, in doubt to act or rest;
In doubt to deem himself a God or Beast;
In doubt his mind or body to prefer;
Born but to die, and reas'ning but to err;
Alike in ignorance, his reason such,
Whether he thinks too little or too much;
Chaos of thought and passion, all confused;
Still by himself abused or disabused;
Created half to rise, and half to fall:
Great lord of all things, yet a prey to all;
Sole judge of truth, in endless error hurl'd;
The glory, jest, and riddle of the world!

Go, wondrous creature! mount where Science guides;
Go measure earth, weigh air, and state the tides... "

From "Essay on Man", Epistle II (Of the Nature and State of Man, With Respect to Himself as an Individual) following Argument I: "The business of Man ... to study himself. His middle nature; his powers and frailties." by Alexander Pope (1688-1744).
Abstract

One out of several distinguishing features of multiple sclerosis (MS) is the epidemiological variation of geographic distribution. Population-based studies on the prevalence and incidence of MS in Sweden have previously been performed only in Göteborg. Another feature of MS is the clinical variation between individuals. To a large extent data on the clinical characteristics of MS come from studies on cases frequenting MS clinics and therefore, may be biased. Also rare are population-based studies of the consequences of MS-related incapacity on socio-economic factors. As for MS aetiology, both environment and genes are involved. Human herpesviruses are often the main suspected environmental aetiological agents.

Our aim was to estimate the prevalence of MS in Västerbotten County for 1 January 1990, the incidence during a 10-year period 1988-97, and the prevalence 31 December 1997; and also to present detailed clinical data including onset symptoms and the disability distribution for the latter two MS populations. Furthermore, we wanted to estimate the prevalence of sick leave, professional assistance, and housing; and also, to study the risk factors for sick leave. In order to investigate the association between MS and human herpesviruses, samples were identified in two regional population-based serumbank registers. This linkage identified samples collected from before MS-onset in 73 MS cases and after MS onset in 161 cases.

The prevalence and incidence populations were identified through multiple sources. Diagnostic ascertainment, the reliability of clinical data, and additional information were assured from a questionnaire with follow-up interview and neurological examination.

The onset adjusted crude prevalence of MS was 125/100,000 (95% CI: 112-140) in January 1990, and 154/100,000 (95% CI: 139-170) in December 1997. The increase was mainly attributable to a higher incidence than mortality. The crude incidence rate 1988-97 was 5.2/100,000 (95% CI: 4.4-6.2). The disability distribution in the 1997 prevalence population in Västerbotten was compared to the disability distribution in a Canadian MS population, which has been used for publications on the natural history of MS. One difference from the Canadian studies appears to be the better recognition of cases with more benign disease. Nevertheless almost half of prevalent MS cases aged 18-64 years were fully sick-listed, and one-fourth of all prevalent cases received professional assistance. High disability level was the strongest predictor for sick leave. All MS cases showed signs of past Epstein-Barr virus (EBV) infection. High activity to EBV (EBNA-1 but not VCA) and human herpesvirus 6 (HHV-6) significantly (borderline significance for HHV-6) increased the risk to develop MS.

These estimates show that Västerbotten County is a high risk area for MS. Both incidence and prevalence were significantly higher when compared to estimates from Göteborg. The comparison with the Canadian MS population shows that MS might be a slightly more benign disease than previously recognized. Still, the consequences of MS regarding socio-economic aspects are considerable. We suggest that EBV is a prerequisite for the development of MS. Individuals that will develop MS exhibit an altered immune response against the EBV virus characterised by high activities to EBNA-1 in the absence of high VCA activities, this being most pronounced in the five-year period preceding MS onset. A pathogenetic role is suggested for EBV and remains possible also for HHV-6.
Original papers


IV Sundström P, Nyström L, Svenningsson A, Forsgren L. Sick leave and professional assistance for multiple sclerosis individuals in Västerbotten County, northern Sweden. Submitted


The papers were reproduced with permission from the respective publisher.
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADEM</td>
<td>Acute disseminated encephalomyelitis</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CIS</td>
<td>Clinically isolated syndrome</td>
</tr>
<tr>
<td>CMV</td>
<td>Cytomegalvirus</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DSS</td>
<td>Disability Status Scale</td>
</tr>
<tr>
<td>EBNA</td>
<td>EBV-determined nuclear antigen</td>
</tr>
<tr>
<td>EBV</td>
<td>Epstein-Barr virus</td>
</tr>
<tr>
<td>EDSS</td>
<td>Expanded disability status scale</td>
</tr>
<tr>
<td>ESS</td>
<td>Environmental Status Scale</td>
</tr>
<tr>
<td>GP</td>
<td>General practitioner</td>
</tr>
<tr>
<td>HHV-6</td>
<td>Human herpes virus 6</td>
</tr>
<tr>
<td>HLA</td>
<td>Human leukocyte antigens</td>
</tr>
<tr>
<td>HSV</td>
<td>Herpes simplex virus</td>
</tr>
<tr>
<td>ICD</td>
<td>International classification of diseases</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>IM</td>
<td>Infectious mononucleosis</td>
</tr>
<tr>
<td>MBP</td>
<td>Myelin basic protein</td>
</tr>
<tr>
<td>MONICA</td>
<td>Monitoring Trends and Determinants in Cardiovascular Disease</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>MS</td>
<td>Multiple sclerosis</td>
</tr>
<tr>
<td>NHR</td>
<td>Swedish Association of Neurologically Disabled</td>
</tr>
<tr>
<td>NMO</td>
<td>Neuromyelitis optica</td>
</tr>
<tr>
<td>NSHDS</td>
<td>Northern Sweden Health and Disease Study</td>
</tr>
<tr>
<td>NSMC</td>
<td>Northern Sweden Maternity Cohort</td>
</tr>
<tr>
<td>OAPR</td>
<td>Onset adjusted prevalence</td>
</tr>
<tr>
<td>OCB</td>
<td>Oligoclonal bands</td>
</tr>
<tr>
<td>ON</td>
<td>Optic neuritis</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>PPMS</td>
<td>Primary-progressive MS</td>
</tr>
<tr>
<td>PRMS</td>
<td>Progressive-relapsing MS</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>RRMS</td>
<td>Relapsing-remitting MS</td>
</tr>
<tr>
<td>RR/SPMS</td>
<td>MS with relapsing-remitting onset</td>
</tr>
<tr>
<td>SPMS</td>
<td>Secondary-progressive MS</td>
</tr>
<tr>
<td>SSPE</td>
<td>Subacute sclerosing panencephalitis</td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
</tr>
<tr>
<td>VCA</td>
<td>EBV viral capsid antigen</td>
</tr>
<tr>
<td>VIP</td>
<td>Västerbotten Intervention Program</td>
</tr>
<tr>
<td>VZV</td>
<td>Varicella-zoster virus</td>
</tr>
</tbody>
</table>
Introduction

Multiple sclerosis – general background

Multiple sclerosis (MS) is a chronic human disease characterized by recurrent inflammations in the central nervous system (CNS). The term "multiple sclerosis" refers to the histopathological finding of multiple lesions containing scar tissue, sclerosis, being the result of previous inflammations at these localizations. In 1866, the French counterpart of the term multiple sclerosis – “sclerose en plaque disseminé” - was coined by Vulpian, being one member of the circle surrounding Charcot at the hospital la Salpêtrière in Paris. The first printed clinical descriptions of the disease came more than fifty years earlier, in the beginning of the 19th century. MS must be regarded an important disease in the western world society. Although too rare to be referred to as a national disease (sv. folksjukdom), MS has been recognized by the World Health Organisation as a “public health problem of considerable significance for individuals and society alike” (http://www.who.int). MS is second only to trauma as cause of neurological disability arising in young and middle-aged adults in the Western world. Both environmental and genetic factors are required for the acquisition of MS. MS is considered to be an autoimmune disease, and as for most autoimmune diseases females are more commonly affected, about twice as often as males. The socio-economic costs for MS in Sweden were estimated to 5,000 million Swedish Crowns in 1998. These costs were dominated by the costs of personal assistants, drugs, and loss of production. Studies show that MS has a major impact on the quality of life. From 1995, MS-specific medication has been available in Sweden. However, the effect is modest, and several trials searching for more effective medical treatments are ongoing.

Clinical characteristics

One distinctive feature of MS is the marked variability between individuals. This is reflected by observations ranging from the finding of asymptomatic MS lesions at autopsy in the elderly to the recognition of “malignant MS” where exceptional cases may die within months after the disease onset. In addition, different subtypes can be distinguished from a qualitative point of view. This categorisation of clinical course usually follows the results of a survey among neurologists. The aim was to standardize the terminology used to describe the clinical course of MS and resulted in the proposal of four clinical subtypes.

- Relapsing-remitting
- Primary-progressive
- Secondary-progressive
- Progressive-relapsing

Figure 1. The clinical subtypes in MS.
To understand these subtypes based upon the time aspect of MS-symptoms, one has to distinguish the two entities *attacks* and *progression*.

An **MS-attack** (or if not the first attack: *relapse*) denotes symptoms that come and go in a smooth fashion, which, if present, is almost unique for MS. Typically, the onset of the attack is not sudden, nor is the end of the symptoms abrupt. The attack symptoms may or may not disappear completely. A new inflammatory lesion is always responsible for the MS-attack, whereas the opposite is not true in general.

![Figure 2. The MS inflammatory lesion and its relation to the MS attack.](image)

Most new inflammatory lesions are asymptomatic. A ratio of $\sim 10:1$ for asymptomatic vs. symptomatic lesions was suggested early in the magnetic resonance imaging (MRI) era.$^9$ The MS-attack must not be confused with the *pseudo-attack*. The latter is not caused by a new CNS-lesion but by a transiently decreased function of nerve tracts previously injured from MS-lesions (conduction blockage).$^1$ These symptoms will cease when the cause of the pseudo-attack is removed. Typically, the pseudo-attack occurs during an infection with raised body temperature. In the MS-attack, a *new* MS-lesion is responsible for the symptoms, which are therefore likely to also be *new*. The MS-attack symptoms correspond to the anatomical localisation of the new lesion. Of course it is possible that two and even more lesions may occur simultaneously and produce new multifocal symptoms. The pseudo-attack on the other hand produces symptoms from old lesions. Therefore the pseudo-attack is characterised by the worsening of old symptoms.

We now consider the average MS patient early on in the disease course. Every new MS inflammation is not likely to produce focal neurological symptoms, but it is experienced as an attack when it does occur (relapsing-remitting MS, RRMS). This process goes on for several years and will for the great majority of cases reach a threshold where the remaining functional loss from earlier inflammations can no longer be compensated for, and it will be revealed and experienced as a continuous worsening of neurological function. The patient has then entered the secondary-progressive disease course (SPMS).

**Progression** in MS is characterised by continuous worsening of neurological symptoms. In the majority of MS individuals, progression is due to SPMS. About 10-15% have primary progressive MS (PPMS), and by definition do not experience attacks. Even fewer have progression from onset with superimposed relapses (progressive-relapsing MS, PRMS).
evaluation of disease progression when SPMS is suspected may be difficult both for neurologists and patients. To detect progression not attributable to attacks puts demands on the history given from the patient and the terminology used in the patient-physician communication. One patient may be prone to seek events responsible for his or her deterioration and may refer to these occasions as “attacks”. A state of continuous progression may be difficult to accept, or, if a cognitive dysfunction is present, difficult to detect. Another MS-individual may be depressed and may report a tainted picture of her recent neurological history, with emphasis on those symptoms experienced to be worsening. As the neurological examination may contain semi-objective items, where the cooperation of the patient is vital, the history may falsely be “supported” by findings in the neurological examination. Thus, the neurologist may underestimate the presence of progression in the first example and overestimate the presence of progression in the second example.

The MS-lesion may be located at any anatomical site within the CNS, but some locations are more common. CNS areas containing more myelin, especially the white matter, are more prone to inflammation. However, the myelin content in a specific CNS area is not entirely what decides the risk to be the subject of an MS-inflammation. Factors responsible for where MS-inflammations will occur are not completely known, but the predisposing sites are reflected in the diagnostic MRI criteria below. From a clinical point of view, one may therefore speak about neurological symptoms as being more or less typical for MS. This distinction is based upon clinical experience and is mirrored by the typical or atypical localization of the corresponding MS lesions. Examples of typical symptoms are optic neuritis, partial myelitis, and some brainstem/cerebellar symptoms. Examples of atypical symptoms for MS are cortical symptoms (for example apraxia, aphasia, hemianopsia), extrapyramidal symptoms, and radicular symptoms.

The consequences of MS

The consequences of MS may be estimated in several ways. Examples of different measures are: quality of life, disability level, sick leave prevalence, assistance usage, and the prevalence of changed residence. A Swedish socio-economic study showed a significant association between increased disability and higher society costs. About one-third of the total costs of MS in Sweden in 1998 were owing to sickness absence and early retirement. About one-fifth of the costs were due to personal assistance care. MS disability is most commonly evaluated by using the Expanded Disability Status Scale (EDSS). EDSS emphasizes ambulation as an endpoint while upper extremities or cognitive functions have less influence. Publications on the EDSS distribution are available from several MS populations. Data on disability distribution in a prevalence population are difficult to interpret when data on the disease duration is missing. If a low disability level is reported in an MS prevalence population with short disease duration, the suspicion arises that ascertainment of old cases has not been complete. In Olmsted County, Minnesota, a median EDSS of 3.5 (preserved full ambulation) was found in a cross-sectional MS survey where median disease duration was 15.4 years. Longitudinal data from Göteborg have shown that every second case reaches EDSS 6 (needs one crutch to walk 100 meters) by 15 years disease duration. Population-based data for sick leave and the usage of professional assistance are rare. In a Norwegian study, 62% of MS cases had not received disability pension after 10
years disease duration.\textsuperscript{16} In a prevalence study in N. Ireland, 18\% had changed residence due to MS, 5\% were institutionalized, and 35\% required assistance for at least 1 h/day.\textsuperscript{17}

Epidemiology

Epidemiology refers to the branch of science that estimates the occurrence of diseases in populations.\textsuperscript{18} The epidemiologist identifies a number of afflicted cases (numerator) which are divided by the number of individuals in the population (denominator, population at risk). Two ways to describe disease occurrence are prevalence and incidence. The incidence estimates the number of cases that get a disease during a time period in a population. The prevalence measures the number of individuals who have a disease at given point in time in a population. The prevalence (cross-sectional) study will provide low numbers when a lethal disease or a disease with short duration is studied. Data from epidemiological studies may be used for aetiological research, for example.

Out of many distinguishing features of the MS disease, the most striking observation of all, according to many, is the variation of geographic distribution.\textsuperscript{19} Already commented upon as a clinical impression by Charcot, these variations are not restricted to differences between, but also differences within many countries.\textsuperscript{20} The United States (USA) and the United Kingdom (UK) are countries for which a north-to-south gradient (=a higher occurrence in the northern than in the southern part of the countries) has been observed, whereas the opposite gradient has been observed for Australia. The proposed explanations have been genetic factors for both the USA and UK\textsuperscript{21,22}, and environmental factors for Australia.\textsuperscript{23} Anticipating the hypotheses on the MS aetiology given below, a common notion is that both environmental and genetic factors determine geographical variations.\textsuperscript{24} Methodological differences have also been suggested as an explanation of the observed differences in the results from epidemiological studies.\textsuperscript{25} Earlier performed epidemiological research has shown MS to be almost non-existing among the black race in Africa, but more than one per thousand individuals in the western world have the disease. This notion has been adjusted somewhat since the report of some certain MS cases in black Africans, but still the view holds that being a black African infers a very high resistance to MS.\textsuperscript{20} Moreover, two large door-to-door surveys carried out in Africa did not identify any MS cases.\textsuperscript{26,27} Black individuals native in the USA have MS about half as often as white individuals, which may at least in part be attributed to the admixture of white genes.\textsuperscript{28-30} Limburg studied mortality statistics in the USA and Europe and concluded in 1950 that the colder the climate, the higher was the MS rate.\textsuperscript{19} Kurtzke later discerned three categories of areas regarding their risk for MS: low risk, medium risk, and high risk areas.\textsuperscript{31} However, the identification of ethnic groups with low incidence of multiple sclerosis within areas with high risk for MS argues against factors associated with latitude.\textsuperscript{32}

Thirty-five years ago Kurtzke pointed out some areas in Scandinavia and Finland as the “Fennoscandian focus”. In Sweden, Västerbotten and especially Umeå was identified as the “northern Swedish focus”.\textsuperscript{33}
Already in 1922 Davenport associated high MS rates in the USA with Scandinavian ancestry. The highest MS rates were found in Michigan and Minnesota; “One thinks of the big Swedes that live in these parts of the country”. Even the Vikings have been accused for the genetic dissemination of the disease.

Variations in MS prevalence and incidence are not only restricted to space, but also temporal variations have been described. Kurtzke’s observations on the Faroe islands, where the term “epidemic” has been used, are perhaps the most famous example of temporal variation. Temporal variations have been reported also from Scandinavia, with either decreasing or increasing trends. The Mayo Clinic has surveyed Olmsted County and Rochester, Minnesota since 1905. The latest report in 1990 indicates an increase in MS incidence, but the authors are not convinced that the increase is biologically true. Methodological issues such as new techniques facilitating the making of an MS diagnosis may be responsible.

These data do favour population studies as the proper MS research level. However, one argument against that view may be that although an abundance of epidemiological MS studies have been performed, the key questions on MS aetiology are still unanswered. At the time of writing, a web search using Pub Med on “epidemiology and multiple sclerosis” generates
almost 2000 hits. In 1994, the number of surveys on MS epidemiology carried out since 1950 was more than 300. Still, the same author requested further “carefully planned” MS epidemiological studies to create a multipoint prevalence map. The quote given in the beginning of this thesis: “The proper study of mankind is Man” harmonizes with this request. In this sentence, “mankind” has in previous quotations made in scientific lectures been substituted for “human disease”. This seemingly tautological sentence may certainly be interpreted as such. Surely the time and effort invested in experimental animal models have produced valuable knowledge. However, it may seem plausible that key questions in the understanding MS might be difficult to answer from animal models created on the basis of the available knowledge on MS. Ten years ago there were 7000 papers on the animal model of MS, Experimental Allergic Encephalomyelitis, which was created in 1935.

What is then known from Sweden? Sällström’s dissertation in 1942 with the subtitle “zur geographischen Pathologie der multiplen Sklerose” was an analysis of patients with MS frequenting Swedish hospitals 1925-34; 3641 medical records on 2100 patients. All these MS patients received questionnaires (“Diese Korrespondenz war sehr zeitraubend”). The responder rate was 66%. Sällström identified 1365 cases that were regarded as certain MS cases. Umeå and Skellefteå were recognised as a “frequenzstarkes Gebiet”. One of the conclusions was that there were variations in the geographical distribution in Sweden that(46,628),(966,734)

Population-based epidemiological studies had before the present study only been performed in Göteborg, southwestern Sweden. The Göteborg region has a long tradition of MS-epidemiology going back to the identification of the 1950-64 incidence cohort, which is followed-up repeatedly.

Aetiology

Still today in the very beginning of the 21st century, the major part of the MS aetiology remains to be revealed. Few, if any, MS-researchers believe that the cause of MS is purely genetic or purely environmental. The most commonly encountered opinion is that MS is a polygenetic disorder, that is: several genes are required for MS acquisition. An MS-susceptible genotype, which may show inter-MS-individual variation, may be a necessary but not sufficient condition for the development of MS. In addition environmental factors are needed, the most suspected agents being common viruses. Most researchers also believe that MS should be categorized among the autoimmune disorders.

MS is referred to as a demyelinating inflammatory CNS disorder. It seems that the autoimmune attack targets components of the myelin sheath, which surrounds the CNS axons. CNS is capable of remyelination. Therefore in part, the pathophysiology of MS may be explained by an insufficient ability to remyelinate relative to the demyelinating mechanisms. The inflammatory attacks lead not only to the loss of myelin, but also the axon may be damaged, leading to irreversible damage. Axonal damage can be demonstrated early in the clinical course. Other mechanisms than inflammatory, for example degenerative, have been postulated. There are also findings supporting the view of heterogenic mechanisms in
different MS individuals. However, the latter results, to a great extent, come from autopsies and biopsies made on patients with more malign course and may not be entirely representative of MS.

Environmental factors

Several environmental factors have been studied in the search for exogenous causes of MS. A majority of the determinants identified have not been confirmed. The grand old man in MS epidemiology, Kurtzke, still advocates his view of an infectious agent being responsible for MS, an idea substantiated in his famous observation of the Faroe Islands “MS-epidemic” which is referred to in the MS chapter in most neurological text books. Briefly, there were no MS cases observed on these islands before the 2nd world war. Coinciding with British troops occupying the Faroe Islands in 1940-45, the first MS cases appeared. In the analysis of subsequent MS cases, temporal clusters were identified. The existence of a Faroe Islands epidemic have since been criticized from different points of view.

The hypothesis of an environmental component in MS aetiology is supported by migration studies which have shown that the migrant adopts the risk of the new area if the immigrant is young. The notion that MS, from a biological point of view, “starts” many years earlier than the appearance of symptoms originates from these studies, suggesting that the age when MS is acquired is below 16 years. These studies have been criticized from a methodological point of view, mostly because those who choose to emigrate may not be representative.

Studies on monozygotic twins - which share the same genes - also support the hypothesis of an environmental component. The concordance rate, that is the risk of MS when your twin sibling has MS, is only about 35%.

Viruses are the environmental agents where the strongest suspicion of an association with MS has been proposed. Evidence supports the view that the agents associated with MS are common and act on a population level. Viruses have been shown to trigger MS-attacks. The relationship has been studied between several viruses and MS. Many previous studies on the association between viruses and MS may be criticized for poor matching of referents. Furthermore, with two exceptions, all studies are retrospective, that means performed on serum samples collected after the onset of MS, which makes the interpretation difficult.

According to many, some of the viruses belonging to the human herpes virus family are the most suspected (Table 1).

Epstein-Barr virus (EBV) was discovered in 1964 when analysing sera from patients with Burkitt Lymphoma. The discovery involved a technician who developed infectious mononucleosis (IM) while using her own serum as negative control. EBV is one of the eight DNA viruses constituting the family of human herpesviruses. EBV is often regarded as the virus having the strongest association with MS. The association with MS was proposed more than 20 years ago from serological studies and from similarities between MS and IM epidemiology. EBV virus is tropic for B-cells. The virus persists normally throughout life as a latent infection in some of these B-cells making them immortal. EBV also resides in the oropharyngeal and genital areas.
Table 1 The human herpes virus family.

<table>
<thead>
<tr>
<th>Subfamily</th>
<th>Virus</th>
<th>Latent in cell type</th>
<th>Examples of diseases associated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha herpes virinae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSV-1</td>
<td>Dorsal root ganglia</td>
<td></td>
<td>Gingivostomatitis, herpes labialis, encephalitis</td>
</tr>
<tr>
<td>HSV-2</td>
<td>Dorsal root ganglia</td>
<td></td>
<td>Genital herpes</td>
</tr>
<tr>
<td>VZV</td>
<td>Dorsal root ganglia</td>
<td></td>
<td>Varicella (chicken pox), herpes zoster (shingles)</td>
</tr>
<tr>
<td>Beta herpes virinae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMV</td>
<td>Monocytoid cells</td>
<td></td>
<td>Mononucleosis</td>
</tr>
<tr>
<td>HHV-6</td>
<td>T lymphocytes</td>
<td></td>
<td>Exanthema subitum (roseola)</td>
</tr>
<tr>
<td>HHV-7</td>
<td>T lymphocytes</td>
<td></td>
<td>Exanthema subitum (roseola)</td>
</tr>
<tr>
<td>Gamma herpes virinae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EBV</td>
<td>B lymphocytes</td>
<td></td>
<td>Infectious mononucleosis, certain lymphomas, nasopharynx cancer</td>
</tr>
<tr>
<td>HHV-8</td>
<td>T lymphocytes (?)</td>
<td></td>
<td>AIDS related Kaposi’s sarcoma</td>
</tr>
</tbody>
</table>

Unlike in the developing countries, approximately half of the population in developed countries have their primary EBV infection after childhood (Figure 4). This difference between countries may explain the findings in MS-migration studies. Several case-referent studies have shown an association with MS. EBV seropositivity has been reported consistently in close to 100% of MS cases, compared to about 90% for referents. Some of these studies also compared virus activities (titres) and found higher activities in MS cases than in referents. Reactivation of EBV has been associated with MS disease activity. A single EBV subtype has been associated with a Danish small MS cluster, and a clustering in time and space at 13-20 years of age (suggestive of EBV as infectious agent) has been demonstrated in a Norwegian study. Epidemiological and serological observations have prompted the experimental immunological search for a link between EBV and MS studies. This research has yielded interesting results and further support for a role for EBV in MS pathogenesis.

In the last ten years the cause of roseola (exanthema subitum), Human herpes virus 6 (HHV-6), has received much attention and is presently often regarded as an equally strong candidate as EBV in MS pathogenesis. HHV-6 was discovered in 1988 and consists of two subtypes, HHV-6A and HHV-6B. Whereas HHV-6A has not been shown to cause disease in humans, HHV-6B causes the usually benign infection occurring in the very first few years of childhood. HHV-6B is a recognised cause of febrile seizures, and it has been implicated as a cause of meningoencephalitis. HHV-6 replicates preferably in CD4-positive T-cells, but it may be found in several tissue types, including the brain. Although an association with the geographical distribution of MS is not seen for HHV-6 (the seroprevalence is near 100% worldwide), there are several studies using different methods that suggest a relationship with MS. Twenty-eight studies performed up to year 2000 have been critically evaluated, and in summary they were regarded to provide “at least some evidence” for an association between HHV-6 and MS. As is the case for EBV, there is experimental support for a potential immunological role for HHV-6 in MS pathogenesis.
The support for an association between *Herpes simplex virus* (HSV) and MS is weaker than for EBV and HHV-6. HSV consists of two closely related viruses, HSV-1 and HSV-2. Although not strictly connected to the specific subtype, HSV-1 causes gingivostomatitis, most frequently in children aged < 5 years, and HSV-2 causes genital herpes.\(^7\) The most common presentation of HSV-1 reactivation is herpes simplex labialis. HSV is neurotropic and establishes latency in dorsal nerve root ganglia after ascending there through sensory nerves. HSV is a frequent cause of encephalitis and may cause myelitis.\(^8\) A possible association between HSV infection and MS has been suggested from the ability of HSV to induce demyelination.\(^8\) HSV-1 has also been isolated during the first attack of MS.\(^8\) However, herpetic eruptions were significantly rarer in MS patients compared to referents in a prospective study.\(^5\)

Primary infection of *Varicella-zoster virus* (VZV) causes chickenpox, mostly in young children, whereas reactivation produces shingles.\(^7\) VZV is neurotropic and has been identified as the main viral agent associated with CNS complications such as myelitis.\(^8, 8\) A significant correlation between MS rates and VZV incidence has been found in the USA.\(^8\) One paper reviewed 40 studies of the association between MS and VZV. The authors concluded that there was insufficient evidence to support an important aetiological role for VZV.\(^8\)

No association has been shown for the remainder of viruses in the human herpesvirus family.\(^8\) The herpesvirus trail has led to recent clinical trials on MS with antiviral agents.\(^8, 8\) Although both these studies were negative regarding the primary outcome measures, subgroup analyses showed possible treatment effects.
The measles virus, *Morbilli*, is a paramyxovirus. Measles is unlike the herpesviruses RNA-encoded and does not induce latency. Since the 1970s, Swedish children have been vaccinated for measles. However, vaccination does not seem to protect against MS. Measles is able to induce subacute sclerosing panencephalitis (SSPE), a rare but well-known complication with inflammatory demyelination in the CNS. SSPE arises a few or several years after measles infection. Two other types of autoimmune encephalitis occurring within months after infection also exist. In the early 1960s, MS individuals were shown to have higher levels of intrathecal antibodies against measles. An association between risk for MS and late measles infection has been proposed, but a recent study failed to show any significant association.

Two other environmental factors will be mentioned. One recent study in females found an association between smoking habits and risk for MS. The risk increase was significant for smokers with a cumulative exposure of 10 pack-years or more, and it was highest among those with ≥ 25 pack-years: odds ratio (OR) = 1.7 (95% confidence interval (CI): 1.2-2.4) compared to never-smokers. Mercury, however, has been a subject of controversial opinions regarding several chronic (neurological) diseases including MS. This has also resulted in alternative MS treatments. Today there are two case-referent studies, the largest encompassing 132 MS cases and 423 referents, and both failed to show any significant association between MS and mercury (the number of dental amalgam fillings).

**Genetic factors**

Evidence for a genetic basis for MS comes from several types of studies, each producing convincing arguments. The results of studies support the involvement of more than one gene, and there may be an overlap in genetic susceptibility factors in an MS population. A positive family history of MS is encountered more often than would be suspected from the prevalence in the population. In these studies, 15-20% of MS cases had one or more relatives with MS. Moreover, there is a convincing association between the percentage of genes shared and the risk increase for the relatives. The recurrence risk is therefore the highest for monozygotic twins, and it is lower the more distant is the relation with the affected relative. A possible objection to the second argument would be that the environment also is shared to a greater extent when more genes are shared. This has been studied in dizygotic twins, who share the same percentage of genes, as do siblings (first-degree relatives). Although the extent to which environment is shared is greater for dizygotic twins, the recurrence rate is the same as for siblings (first-degree relatives). In a Canadian study this issue was also studied on half-siblings, who often do not share the same environment. The study showed that halving the number of genes shared reduced the recurrence risk for MS to an extent consistent with oligo/polygenic inheritance. There was no difference in recurrence rates between maternal and paternal half-siblings or between half-siblings who were raised together or apart, thus speaking against maternal effects such as perinatal factors or breast feeding, and (Canadian) environmental factors, respectively. Further support for a genetic cause for the increased recurrence risk for relatives to MS cases comes from a study on adopted relatives, which have no increased risk for MS. The recurrence rate has also been established for conjugal MS (both parents have MS).

However, results of the search for genes contributing to MS aetiology have been disappointing since the demonstration of an association between the HLA class II allele DR 2 and MS thirty years ago. This endeavour continues and linkage analysis in multiplex
families has identified several genomic regions (6p, 17q, 19q, and 1p) where genes influencing susceptibility to MS are likely to be located.\textsuperscript{101} Not only susceptibility genes are searched for. Also, genetic research aiming to identify the disease modifying genes is ongoing.\textsuperscript{102} One may argue that the latter search does not have to differ in a qualitative way from the search for susceptibility genes.

Pathogenesis

The pathological hallmark of MS is the demyelinated plaque. These lesions characterized by loss of myelin and the relative sparing of axons are scattered throughout the CNS, localised preferably in those parts with higher myelin content, the white matter. The MS plaque is believed to be the result of an autoimmune inflammatory attack directed towards antigens in myelin proteins.\textsuperscript{45} The series of events participating in the production of MS inflammations is likely to include:\textsuperscript{3,103}

- Activation of CD4 positive myelin autoreactive T-cells outside the CNS, possibly by molecular mimicry,\textsuperscript{70}
- Activated T-cells cross the blood-brain barrier (venules),\textsuperscript{104,105}
- T-cells will re-encounter the myelin antigen inside the CNS via antigen-presenting cells,\textsuperscript{3}
- The inflammation escalates through the production of pro-inflammatory cytokines,\textsuperscript{3} and
- New antigenic myelin structures are exposed (uncertain relevance).\textsuperscript{106}

The inflammation will result in myelin damage, subsequent remyelination, axonal loss, and scar tissue replacement. However, inflammation may dissolve completely, leaving no trace.\textsuperscript{107} Axonal damage has been shown to occur early in MS, and its relation to inflammations visualized on MRI is not quite understood.\textsuperscript{46,108} It is still unclear if the above mechanisms may sufficiently explain tissue destruction in MS or if a degenerative component exists, at least for a subgroup of patients.\textsuperscript{109} It has been suggested that inflammations may not contribute to clinical progression. This hypothesis would harmonize with clinical data, where relapses were shown not to significantly influence the evolution of irreversible disability.\textsuperscript{110} The MS lesion has been shown to differ in appearance between individuals but not within the same individual.\textsuperscript{47} There was also a hint of an association between the type of appearance and clinical subtype, supporting the view that MS is not a single entity.

Several elements involved in the different steps in mechanisms leading to tissue destruction have been proposed to have central roles in MS pathogenesis.\textsuperscript{111,112} Further studies will show whether they do have key roles, or just constitute parts of an immunological or physiological response following an earlier causal event.

Diagnosis

The making of an MS diagnosis in an individual is generally straightforward for the experienced neurologist, but it can be tricky. Several alternative diseases may have to be considered. MS is a clinical diagnosis, which may be supported by cerebrospinal fluid (CSF) and MRI results. Different diagnostic criteria have been used over time, but the common denominator has been to show “dissemination in time and space”, which may be exemplified
by the following case. Last summer, a patient experienced double vision for three weeks. An ophtalmologist made an examination and concluded that the cause was a lesion in the brainstem, probably of inflammatory origin because the symptoms eventually disappeared and had no abrupt onset. After this episode, the patient was doing well until last week. Since then she has had tingling in her right leg up to the right arcus, and for two days she also has had numbness in her left foot together with slight balance problems. If the latter symptom can also be documented by neurological examination and if there is no reason to suspect any other cause of her symptoms, the patient fulfills criteria for MS because “dissemination in time and space” has been proven.

Different criteria have allowed for the results of clinical, paraclinical (MRI), and CSF examinations to be included in the diagnostic procedure. The latest contribution, the McDonald criteria, came two years ago. Unlike earlier criteria, these criteria satisfactorily encompass the clinical subtype PPMS. Furthermore, the results of MRI are emphasized, and no distinction is made between different levels of certainty for the MS-diagnosis. MRI criteria for dissemination in time and space are included. These originate from studies on individuals having experienced a single attack, which could be the onset of MS. These MRI studies were carried out to optimize MRI criteria with respect to their ability to predict the clinical conversion to MS. With these criteria a proportion of the responsibility in making an MS diagnosis is moved from the neurologist to the neuroradiologist. This is certainly true if the diagnostic procedure takes place after only one attack, in which case the criteria allows for the neuroradiologist to decide if there is dissemination in time and space. The neuroradiologist must, for example, take care not to assign any diagnostic value to lesions attributable to age or ischemic disease. The ability for these criteria to identify other diagnoses mimicking MS is not known.

The Poser criteria published in 1983 barely mentioned MRI, which was a new technique at that time. The results of CSF, clinical examination and paraclinical tests were incorporated into the diagnostic procedure. The Poser criteria allowed dissemination in time and space to be documented by clinical or paraclinical findings, which may have been asymptomatic. The paraclinical tests referred to in the Poser criteria were, above all, the evoked (sensory-, motor-, visual-, and brainstem auditory-) potentials, which have largely been abandoned with the introduction of MRI.
Aims


- To describe clinical characteristics in a prevalence and incidence MS population (Paper III).

- To estimate the prevalence of sick leave, professional assistance, and housing in an MS prevalence population, and to identify risk factors for sick leave (Paper IV).

- To estimate the impact on the development of MS from the viral activity to Epstein-Barr virus, human herpes virus 6, herpes simplex virus, varicella-zoster virus, and measles virus, in prospective and non-prospective serum samples (Paper V).
Materials and methods

Prevalent cases 1990-01-01 n=313

Incident cases 1988-87 n=133

Prevalent cases 1997-12-31 n=399

Working age and known vocational status n=307

Professional assistance and housings data known for n=375

Serum donators n=187

Serum donators not from epidemiological survey n=47

Serum donators total number n=234

Figure 5. Populations studied in paper I-V.

Background

Population-based studies on the prevalence and incidence of MS in Sweden were before the present studies performed only in Göteborg, southwestern Sweden. A prevailing impression among neurologists at the Department of Neurology at the University Hospital in Umeå was that the MS occurrence in this part of Sweden was high. It would therefore be of interest to perform an MS survey in the area. In addition, interferon beta treatment was approved for RRMS in 1995 in Sweden and we also considered participating in interferon beta trials evaluating the effect on SPMS. Therefore, an investigation of the number of MS patients became all the more important. The process of identifying MS cases started in 1995. January 1, 1990 was chosen as prevalence day because some years usually pass between onset and diagnosis. The initial plan was to make a database search using the diagnostic (International Classification of Diseases, ICD) codes corresponding to "Multiple Sclerosis" and "Demyelinating diseases in the CNS" for patients having visited the neurological department for the last ten-year period. It soon became evident that epidemiological data of interest such
as time for and type of onset, clinical course, and the present disability level were lacking or important details were missing. Most important, the MS-diagnosis was, in several cases, not completely ascertained, and for many patients a question mark following the MS diagnosis had never been deleted. We also suspected that the considered identification process was not sufficient.

It was therefore necessary to extend our search to other sources and to collect more information through sending out a questionnaire to all patients with MS or where MS was suspected. In the covering letter we asked for the individual’s participation in a follow-up interview and neurological examination. The purpose was to confirm or reject a diagnosis of MS and to complete missing clinical data together with epidemiological data of interest. The study design was finalized in 1997 and included the decision to perform an incidence study for the five-year period from 1988 to 1992. In the end of the process of performing that study, it became evident that the number of MS-individuals in that incidence population was too small for an incidence study. In early 2001 it was therefore decided to extend the study period through 1997. We also realized that the number of individuals with MS at the end of that incidence period would be greater than the corresponding number January 1, 1990, and therefore we decided to estimate the prevalence December 31, 1997, as well.

The study area

The Västerbotten County is located in northern Sweden at 64 - 65°N latitude. The mean daily temperature is -6 to -10°C in January and +15°C in July. The mean annual rainfall is 500 mm. The total population was 250 134 on the first prevalence day January 1, 1990, and 259 163 on the second prevalence day December 31, 1997. The three health care districts each have one hospital. These hospitals are located in the only three urban areas of Umeå, Skellefteå, and Lycksele, with populations of 90,004, 77,720, and 14,186 respectively on the prevalence day 1990, together accounting for 72% of the total population of Västerbotten.

The department of neurology started in 1960. The neurological rehabilitation unit Björkgården, located in Sävar 15 km north of Umeå, was started in 1980 and serves the northern Swedish health care region. The University hospital in Umeå has the only neurological department and neurologists in the study area. In the past, only two neurologists have been working outside the department. One neurologist worked at the internal medicine department in Skellefteå for some years during the 1990s. The other neurologist, who worked privately in Umeå 1985-89, has informed us that he referred every case with a suspected MS to the department of neurology. MRI has been available at the radiology department in Umeå from 1986 (0.02 Tesla, upgraded to 0.04 Tesla in 1988, exchanged in 1991 [0.5 Tesla], and complemented with a second 1.5 Tesla imager in 1998). Since 1993 one MRI imager is also available in Skellefteå (0.5 Tesla).

The sources

ICD codes: The most important source was the inpatient database. The ICD codes corresponding to the following diagnoses were used: MS, demyelinating disorders in CNS, optic neuritis, spastic paraplegia, ataxia, myelopathy, spinocerebellar disease, and myelitis. The search was done for the following clinics at the three hospitals in Västerbotten County: neurology, neurosurgery, neurorehabilitation, internal medicine, ophthalmology, paediatrics,
and geriatrics. The rehabilitation unit, Björkgården, was included in this search. Data on outpatients for the clinics at Umeå University hospital (except for the department of ophthalmology) were available from 1997, and from 1984 for the neurological department.

**CSF electrophoresis analyses:** All analyses with immunopathy suggestive of MS were recorded. This was in accordance with the Poser criteria defined as the presence of oligoclonal (> 2) bands found exclusively in CSF or increased production of IgG (IgG-index > 0.7). It was not feasible to check the results from CSF analyses performed earlier than 1988, as these were sent to different laboratories in Sweden. We also recorded the results for CSF protein, CSF cell count, and the albumin quotient. Any abnormal values were noted and this was kept in mind for the concluding diagnostic decision. One secondary product of the CSF electrophoresis analyses inventory was that we found that not only the past but also the present local method (silver staining) used for the detection of immunopathy had too low sensitivity. This was obvious from a clinical point of view, where too many with apparent MS had no intrathecal production of oligoclonal bands (OCB). This notion was strengthened by the fact that several of the OCB-negative analyses had an elevated IgG-index. Usually OCB are detected more often than an elevated IgG-index, and the reverse finding is very rare. For the 1990 prevalence study, we re-evaluated CSF samples from 14 cases where CSF analysis had been OCB-negative. Nine out of these were found OCB-positive at the Huddinge University hospital where isoelectric focusing followed by immunofixation is used.

**General practitioners and district head nurses:** In April 1998, we contacted all 186 general practitioners (GPs) working at the 37 health centres in the county by letter. We asked for information on patients with MS or inflammatory disorder of the CNS for the past 10-year period. Non-responders received a reminder five months later. Sixteen municipally employed head nurses responsible for 169 nursing homes, sheltered homes, and residential homes were contacted with the same request as for the GPs.

**Hereditary cases and “additional cases”**: From 1997 and onwards the author identified these cases in clinical work or from the study interviews.

**The Swedish cause of death registry:** All cases that died in Västerbotten County since 1990 with MS or demyelinating disorder in the CNS as the underlying or contributory cause of death were selected.

It was possible to collect information enabling a decision about whether there was reason to suspect a diagnosis of MS for every individual identified by the above sources.

Data from MRI records could not be used as a source for case identification as our neuroradiologists, by tradition, use an unspecific code for documentation of MRI interpretations when MS lesions are encountered. The disability and wheelchair services did not have any user register. There was no classification available on the results from evoked potential investigations made at the Department of Neurophysiology, Umeå University Hospital. We assumed that it would not be purposeful to go through all the neurophysiological investigation results. The autopsy register from the pathology department contained a few MS cases, but no cases were found exclusively from this source. Lists of members of the Swedish
Association of Neurologically Disabled (NHR) were of no use because the neurological diagnosis, if any, of the respective member was not known.

Patients with an MS-diagnosis or with medical records indicating possible symptoms of MS were contacted if there was a possibility, from the available data, that the patient would qualify in either of the epidemiological studies from a geographical point of view. In order not to make any unnecessary contact with individuals, we used lists available from the Oncology Department for the Västerbotten County total population to identify those not qualifying geographically. The geographical localization of identified individuals was decided upon from the place in which the individual was registered. We chose not to contact those having medical records indicating only one symptomatic lesion. Our judgement was that a large proportion of these might not be aware of the risk that their previously experienced symptoms could be caused by a chronic disease. For the same reason, we chose not to contact individuals who had only been in contact with an ophthalmologist for optic neuritis, even if dissemination in time and space could be suspected from medical records. At the prospect of the 1990 prevalence study, when the purpose was also to estimate the incidence for 1988-92, we did not contact individuals where the onset of symptoms according to medical records was in 1995 or later. Individuals with later onset were contacted in the year 2001.

The follow-up

A covering letter explained the purpose of the study. We chose not to use the word “multiple sclerosis” in the covering letter because we suspected that some patients had not been informed about the diagnosis. Neither did we want to worry individuals were the diagnosis was uncertain. Instead we used the more neutral term “inflammatory neurological disorders”. In the covering letter, individuals were asked to fill in the questionnaire and were informed that we would phone them in a few days to ask for their participation in a follow-up interview with an neurological examination, and if they were willing to donate blood for future research. In the three-paged questionnaire we requested information on the following questions/items.

- Your name.
- Your identification number.
- Your address and telephone number.
- The date on which you fill in this questionnaire.
- For which neurological disease have you been informed that you have (or have had)?
- When did you receive your diagnosis?
- Where were you residing when you received your diagnosis?
- When did you, for the first time, have symptoms from your disease (if possible year and month)?
- Do you have any relatives with similar symptoms? Please specify name and relationship.
- Do you have any other relatives with neurological diseases? Please specify name, relationship, and diagnosis.
- Does your spouse have any neurological disease, and if so, what?
- Do you have any relatives with porphyria?
- Do you have any other disease? If so, please specify. Use the backside of the page if necessary.
- Are you a twin?
• Are your parents related?
• Is any close relative of yours Sami (mother, father, grandmother, grandfather)?
• Where is your place of birth?
• Please specify at which locations you have been resident (for more than 6 months) since you were born until now. Specify where and between which years. Use the backside of the page if necessary.
• Where you resident in Västerbotten County 1 January 1990?
• Are you or have you been a smoker?
• Do you use or have you used smokeless tobacco?
• Please specify which professions you have had in your life. Specify the main tasks and between which years. Use the backside of the page if necessary.

We started making follow-up interviews and examinations in September 1997. For the estimate of the incidence 1988-92 and the prevalence on January 1, 1990, this work ended January 1999. As the survey was extended for reasons mentioned above, the majority of incident MS-individuals 1993-97 and those being prevalent December 31,1997 (but not being prevalent January 1, 1990) were contacted in 2001. The last follow-up interview and examination was made in December 2001. The paraclinical support for MS diagnosis was far better in cases belonging to the incidence population as compared to the 1990 prevalence population. Therefore we did not urge for an individual follow-up examination on a routine basis.

If there were any unclear points from the questionnaire, these were discussed at the interview. If data from the questionnaire or interview were in conflict with data from medical records, clinical judgement decided which data were used. In addition to the clinical data received from the questionnaires, we also discussed or asked about the following items (when applicable) at the interview:

• The time and type of first and second attack (the second attack had to demonstrate dissemination in space),
• The duration of the first attack,
• The grade of remission of first attack (total or partial),
• The absence or presence of progression,
• The absence or presence of fatigue, and
• The absence or presence of temperature sensitivity.

At the interview we also supplemented data from the questionnaire on:

• To what extent the patient at present was (partially) sick listed or received (temporary) disability pension,
• In patients who received full (temporary) disability pension, the onset of persistent full sick leave was notated,
• Education level (years),
• To what extent the patient received any professional assistance, and
• Type of housing.

We noted if the individual had other diseases the presence of which made an MS diagnose questionable (for example systemic diseases, malignancy, infectious diseases, or
thromboembolic disease), or if symptoms emerged that were possibly attributable to a diagnosis other than MS. We also asked questions about which physician the patient turned to if neurological problems were encountered, if and when the patient had received rehabilitation at Björkgården, and recorded the last time the patient had been to the Neurology Department in Umeå.

The neurological examination included an assessment of the degree of disability as measured with EDSS and its seven Functional Systems. For the prevalence 1990 study, the follow-up examinations (n=235) were performed at an extra hospital/health centre visit (75%), at a regular visit to the hospital (14%), or at a home visit (13%). The presence or absence of the following MS-atypical signs were recorded:

- Absence of objective findings in the neurological examination?
- Presence of down beat nystagmus or Cranial nerve III paresis?
- Signs of polyneuropathy, fasciculations or atrophy?
- Early psycho-organic affection? Aphasia?
- Skin symptoms? Photosensitivity?
- Absence of eye findings?
- Radicular pain? Sensory level?
- Localized disease?
- Extrapyramidal symptoms?
- Systemic disease symptoms? Elevated sedimentation rate?

Case definition

For the prevalence studies, we adopted the concept “onset adjusted prevalence”, which means that an individual was included if he or she:

- had experienced an onset symptom before the prevalence day,
- was resident in the study area at the prevalence day, and
- fulfilled MS diagnostic criteria at the time of data collection

The same principle was used for the incidence study: the onset symptom had to occur during the incidence period, the individual had to be resident in the study area at that time, and fulfil diagnostic criteria at the time of data collection. Resident was defined as being registered in Västerbotten County. University students were to a large extent excluded by the use of this definition.

Alternatively, one might only include individuals who have received an MS diagnosis according to medical records or fulfil diagnostic criteria before the prevalence day. One may argue that both these alternatives estimate neurological services and are therefore less relevant.

If the preceding concept is used, the onset of MS has to be defined. There have been several publications on the spectrum of symptoms encountered in MS. This is the case also for onset symptoms. However, there has been little debate about which symptoms that may be regarded as onset symptoms in the epidemiological MS study. The following situation illustrates the problem (Figure 6).
How the onset of MS is defined is often not commented upon at all in MS epidemiological studies. This is true despite the fact that the determination of MS onset is crucial for all the study results. This problem arises owing to the variability of MS symptoms, both qualitatively (more or less typical for MS) and quantitatively (more or less subtle). A frequent situation is that the patient, after having received a diagnosis of MS, may report earlier MS-symptoms than previously reported. Since MS may produce most symptoms, some guidelines are needed for which symptoms that may be attributed to MS.

Medical records were carefully studied in order to reveal any eventual signs for explanations other than MS for the patient’s neurological symptoms. Results possibly indicating or corresponding to differential diagnoses were recorded, for example, sedimentation rate, blood cell count, pulmonary X-ray, Anti-nuclear antibodies (ANA), Extractable nuclear antibodies (ENA), cardiolipin antibodies, serology for Borrelia, vitamin B12, Human T-cell lymphotrophic virus type I (HTLV-I), and Human immunodeficiency virus (HIV). For the 1990 prevalence study, 25 individuals were excluded after interview and
examination. Furthermore, 16 MRI examinations, 13 lumbar punctures, and additional blood sample analyses from 43 individuals were performed to clarify diagnoses and exclude differential diagnoses.

An important point is that individuals without MS diagnosis had to have medical records indicating possible symptoms of MS, that is, more than one symptom was required for being contacted. While this sentence is given in the first paper, some confusion may arise from the subsequent sentence in the paper; “those having medical records indicating only one lesion were not contacted”. This sentence should be interpreted in the context of the preceding sentence in the paper, that is, “those having medical records indicating only one symptomatic lesion were not contacted”.

Thus we did not contact the category that has been referred to as “clinically isolated syndrome (CIS)”. This term was actualized after the two interferon beta trials where treatment was initiated after one attack only. The risk for this category to develop MS is high (probably >50%), and the prediction may be further specified individually by MRI and with additive information from CSF examination. As a result of the criteria for contacting individuals, even those without MS diagnosis but with MRI documented dissemination in space and positive CSF examinations were not contacted if only one attack had occurred. This was only true for less than a handful of individuals, as many in this category actually had received an MS diagnosis based upon a not too infrequent misinterpretation of the diagnostic criteria. The size of this “possible MS” population was not estimated. The reason was that we considered it unethical to contact these individuals because a significant proportion of these may not be aware of the risk for MS. We also thought it would be too arbitrary to estimate the frequency of possible MS from information in medical records only.

The virus study

The design of the study on virus activities was suggested close to 40 years ago by Kurland in a discussion reproduced in print. For this purpose the MS database used to identify cases for the MS epidemiological survey was linked with the databases of: (i) the Northern Sweden Health and Disease Study (NSHDS) Cohort (Medical Biobank of Umeå University) containing three sub-cohorts: The Västerbotten Intervention Program (VIP) cohort, the MONICA cohort, and the Mammography Screening cohort, and (ii) the Northern Sweden Maternity Cohort (NSMC). This enabled us to identify serum samples collected before MS onset (prospective samples) and after MS onset (retrospective samples). For 234 MS cases were serum samples from cases and matching referents available (82 from VIP, 5 from MONICA, 23 from the Mammography Screening cohort, and 124 from NSMC).

We analysed serological IgG activities to five viruses with a suspected MS association: Epstein-Barr virus (EBV), human herpes virus 6 (HHV-6), herpes simplex virus (HSV), varicella-zoster virus, and measles virus. Blood samples from MS cases and matched referents (3:1) were derived from the two cohorts. EBV was analysed for EBNA-1 (EBV-determined nuclear antigen 1), and VCA (viral capsid antigen). The HSV response was analyzed with a group-reacting antigen to HSV-1 and HSV-2. The method used to estimate HHV-6 response measured activity to HHV-6B.
Results

The prevalence of MS 1990

We identified 313 individuals fulfilling the criteria used for case definition, giving a crude onset adjusted prevalence of 125/100,000 (95% CI: 112-140) of MS in Västerbotten County 1 January 1990. The prevalence was higher than previously reported from other major Scandinavian or Finnish areas. After adjustment for age, sex, and as far as possible for methodology, the prevalence was significantly higher than the 1988 prevalence in Göteborg (117/100,000 vs. 96/100,000, p=0.011). Göteborg remains the only other Swedish area where population-based studies of MS epidemiology have been performed.

The incidence of MS 1988-97 and the prevalence 1997

The same method for case finding and the same case definition was applied in estimating the incidence of MS in Västerbotten County during a 10-year period 1988-97, and the prevalence 31 December 1997. We identified 133 cases with onset of MS during the 10-year period, giving a crude incidence rate of 5.2 (95% CI: 4.4-6.2). The crude onset adjusted prevalence based on 399 identified MS cases was 154/100,000 (95% CI: 139-170) 31 December 1997. A comparison with epidemiological MS studies performed in Scandinavia and Finland for the last 25 years was performed. The Västerbotten age-, sex-, and methodology-adjusted incidence rate 1988-97 was significantly higher than both the Göteborg crude incidence rate 1950-64 (4.2/100,000), and 1974-88 (2.6/100,000) (p=0.017, and p<0.001, respectively). The incidence rate was comparable with other recent Fennoscandian incidence rates.

Clinical characteristics of MS

Clinical data derived from the 10-year incidence cohort and the 1997 prevalence population are described. The significance of how onset is determined is elucidated by the significant difference found between median age at “possible onset” and age at “defined onset” (27 vs. 29 years for prevalent RR/SPMS cases; p = 0.010). The clinical spectrum of the first attack (defined onset) is presented in detail. The median duration between first and second manifestation of RR/SPMS was 2 years (range 0-9 years) for the 1988-97 incidence cohort (median year of follow-up 1998). We found a 40% increased risk of having the first and second attack in the same anatomical region (p = 0.004 for prevalent RR/SPMS cases). Half of initially RRMS cases in the prevalence population had entered SPMS at follow-up. Median duration from the onset attack to SPMS was 10 years (range 0-36). There was no significant difference between the median age at onset of SPMS (42 years, range 14-74) and PPMS prevalent cases (39 years, range 15-68). Gender and age at onset were associated with anatomical localization of the onset attack. Optic nerve onset was an uncommon presentation in males with high age at onset, and spinal cord onset was more frequent in younger females. The disability distribution for both populations is presented; median EDSS in the prevalence population was 3.5.
The consequences of MS

Almost half (45%) of MS cases aged 18-64 years in the 1997 prevalence population were fully sick listed, and only 35% were not sick listed at all. Every fourth individual in the total prevalence population received professional assistance, and 9% were living in care homes or special apartments for the disabled. Multiple logistic regression analysis identified EDSS as the strongest predictor of sick leave. Fatigue is a risk factor for sick leave mainly because of a higher prevalence among the partially sick listed. The time from symptom onset to full sick leave leading to temporary or permanent disability pension was significantly shorter for cases with progressive onset, higher age at onset, and in males. The risk of full sick leave due to MS was six times higher than in the general population.

The impact of viruses on the development of MS

All studied MS cases showed signs of past EBV, HHV-6, and measles infection. High activity to EBNA-1 and HHV-6 significantly increased the risk for MS (OR=4.5; 95% CI: 1.9-11, and OR=2.3; 95% CI: 1.0-5.1 respectively) vs. low activity in prospectively collected samples. A discrepancy between activities to EBNA-1 and VCA was found in cases. This was striking in samples collected less than five years before relapsing-remitting MS onset, where high activity to EBNA-1 increased (OR=11; 95% CI: 1.5-75), and high VCA activity decreased (OR=0.16; 95% CI: 0.032-0.86) the risk for MS. There was no support for major causal roles for HSV, VZV, or measles.
Discussion

Case identification

The number of sources used was higher than in most epidemiological MS studies. We chose not to include the diagnostic code corresponding to “paraesthesias” in the diagnostic code search of the in- and outpatient hospital registry. This symptom diagnosis is frequently used and the patients are often not investigated as thoroughly as are the patients receiving the diagnostic codes being used in this study. We assumed that the information in many medical records would not allow the exclusion of MS as a possible explanation. However, we checked the ICD code corresponding to paraesthesias for one year (1988) to elucidate this issue. We found only one case with possible MS, which was not identified by any other source. Furthermore, of the cases identified by sources other than ICD codes, only two had received a “paraesthesia” diagnosis. This favours the view that the exclusion of this ICD code did not considerably underestimate the number of MS cases.

A diagnosis of MS in cases originally identified from the source “hereditary cases” could later be substantiated in all but one case. Because about one out of five cases had relatives with MS, this may support the view that the number of MS cases was not underestimated.

When the follow-up was extended through 2001, another 16 cases were identified. Only five of these would have qualified for the 1990 prevalence population follow-up based on data in medical records. However, these records were from before the start of the search period.

Case ascertainment

Table 2 shows to what extent the 313 MS cases in the 1990 prevalence population had MS according to their medical records, that is according to the front page ICD code. Ten out of 14 cases with “other diagnosis” had symptom-describing diagnoses. In the remaining four cases, the medical record diagnoses specified concurrent diseases. The table also shows the diagnoses reported by cases at the follow-up interview. The follow-up procedure performed on the 1990 prevalence population interfered with both diagnoses in medical records and patient’s knowledge about their diagnosis. Therefore, the results for these two parameters are not commented for the incidence population or 1997 prevalence population, as many of these were a part of the 1990 prevalence population.

Table 2. Diagnoses in medical records and from patient reports at follow-up interviews.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Medical records</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>221</td>
<td>70.6</td>
</tr>
<tr>
<td>Suspected MS</td>
<td>28</td>
<td>8.9</td>
</tr>
<tr>
<td>(Suspected) Demyelinating CNS disease</td>
<td>24</td>
<td>7.7</td>
</tr>
<tr>
<td>Monofocal disease (e.g. myelitis)</td>
<td>21</td>
<td>6.7</td>
</tr>
<tr>
<td>Other diagnosis</td>
<td>14</td>
<td>4.5</td>
</tr>
<tr>
<td>No medical record/information</td>
<td>5</td>
<td>1.6</td>
</tr>
<tr>
<td>Follow-up interview not performed</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>313</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 2 illustrates the need for the follow-up procedure. The available paraclinical diagnostic support was scarce. MRI supported the MS diagnosis in half (n=155) of the cases, and was not performed/available in 45.4% (n=142) of cases. The result of CSF examinations supported MS diagnosis in 68.1% (n=213) of cases. Some of these were performed with old techniques such as Mastix (n=42). For both the negative MRI and negative CSF examinations, these results could to a large extent be attributed to sub-optimal methods. CSF electrophoresis analysis with isoelectric focusing was negative for 2.6% (n=8) of the cases.

Do these data suggest that the 1990 prevalence of MS was over-estimated? Our interpretation is that these data just depict the starting-point given in the “Material and methods”. To date, we only suspect one case to be incorrectly included (true for all the three populations). This case was judged to fulfil the Poser criteria for clinically probable MS (one attack, clinical evidence of two lesions, “CPMS C2”) and had been included first after some consideration. No paraclinical investigations had been made previously at inclusion, but subsequent MRI and CSF were both normal. This category must be regarded one of the Poser criteria categories supplying the weakest support for MS, and it does not satisfy the new diagnostic MS criteria either.

Another argument speaking against any overestimation of the number of MS cases comes from unpublished data on the prevalence of the HLA class II allele DRB1*1501, which is similar to the prevalence in a previously studied Swedish MS-population identified through the two largest neurological clinics in Stockholm.125

What is the true prevalence of MS in Västerbotten? The prevalence population 1990 with follow-up through 1997 was estimated to 313 MS cases. When the follow-up was extended through 2001, another 16 cases were identified, and one case was excluded. Without having scrutinized the sources again, the author was in December 2002 aware of another 3 cases that belonged to the 1990 prevalence population (Figure 7). This expected phenomenon is related with the well-known fact that repeated surveys yield rising prevalence, until the point in time when the prevalence population becomes saturated.25

Figure 7. The number of MS cases in Västerbotten county 1990 as estimated with follow-up through 1997, 2001, and 2002.
Statistically significant differences in MS occurrence do not necessarily reflect significant differences in the biologic sense. Arguments that Västerbotten County is a higher risk area for MS than Göteborg are as follows:

- The adjusted prevalence was significantly higher in Västerbotten County 1990, as compared with Göteborg 1988.
- Repeated surveys yield higher estimates (due to longer follow-up), thus underestimation is probably a greater problem in Västerbotten as compared to Göteborg, where a successively updated MS-register was initiated already in 1950.
- The adjusted incidence rate in Västerbotten 1988-97 was twice as high as compared with Göteborg 1974-88 and also higher than the incidence in Göteborg 1950-64, both differences being statistically significant.
- In Kurtzke’s presentation of the distribution of MS in Sweden based on Sällströms data from 1925-34, the prevalence in Västerbotten county was 145%, and Göteborg-Bohus county 88% of the national mean.
- In Landtblom’s investigation of the MS distribution in Sweden based upon mortality statistics 1952-92, Västerbotten county had an average annual MS morbidity expressed as the proportion of the national mean of 1.20, while the county of Göteborg had 1.05.

However, the implicit conclusion may be questioned. Firstly, the studies presented in this thesis were carried out in a more Assyrian fashion, adopting Kurtzke’s terminology. That is, the number of sources was greater in the present study, as compared to Göteborg. Secondly, any differences found from estimates of MS occurrence based on hospital registers, disability registers, or mortality registers, should be interpreted with greatest caution owing to the insensitive methodology. Landtblom’s study also contained data on disability pensions because of MS 1971-94, which in fact showed higher rates in Göteborg compared to Västerbotten.

Many more factors than these affect the outcome of epidemiological MS studies. In contrast to the Göteborg area, there has been no tradition of MS research in the Västerbotten area. One might suspect that ongoing MS research at a department may increase the awareness among neurologists and also neuroradiologists for when to suspect MS. The existence of private neurologists may also affect estimates, if all their cases are not identified. Adjacent neurology departments outside the study area may also affect estimates if these departments are not included in the search. Whereas the Department of Neurology at Sahlgrens Hospital in Göteborg was the only neurological service during the identification of the 1950-64 cohort, several clinics served outpatients when the 1974-88 cohort was identified. This may have affected case retrieval, and may have contributed to the lower incidence 1974-88, as compared to 1950-64, in Göteborg.

For some differential diagnoses and MS-variants, the lack of universal criteria, or the interpretation of these, pose a problem in the comparison of epidemiological MS studies. Acute disseminated encephalomyelitis (ADEM) is one example. Some authors claim that these patients may have new symptoms disseminated in time and space, “multiphasic DEM”, which will be almost impossible to differentiate from MS. In the present study, one case, where both ADEM and the onset of MS were discussed in medical records after the first
attack, was found to have neurological signs from new areas at follow-up and was included. The distinction between Devic’s syndrome (neuromyelitis optica [NMO]) and MS with predominating optico-spinal symptoms is another example. One patient in the present material with exclusively optico-spinal symptoms was included since both CSF immunopathy and a relapsing-remitting disease course were present. However, that case also fulfils the recently proposed diagnostic criteria for NMO. The inclination to diagnose systemic diseases with predominant or exclusively neurological symptoms on the basis of the presence of autoantibodies and some atypical clinical features differs between neurologists, and this may account also for some of the observed differences between MS epidemiological studies.

The interpretation of MS diagnostic criteria may not be uniform. In the present study all PPMS cases were included in the clinically definite MS group in the Poser criteria. One of the reasons for the revised diagnostic criteria for MS was the lack of a scheme for PPMS classification. The inclusion of PPMS in the clinically definite group may be justified from the long disease duration (shortest disease duration nine and four years in the 1990 and 1997 prevalence populations, respectively) at data collection, which in every case had allowed for the appearance of symptoms supporting dissemination in time and space.

Is it important whether a difference in MS occurrence within Sweden exists or not? Is the presumption of an equal distribution of MS in Sweden sounder than the opposite presumption? Should reports on smaller Swedish areas having a very high MS prevalence be explained by random fluctuations, or biological differences not being relevant for the total population? These questions relate to the question about whether or not all these epidemiological MS studies add important knowledge. It also relates to the need for uniform criteria enabling the comparison of studies. The creation of a global multipoint prevalence map using standardized methodology could provide for each area the prevalence and incidence of MS, the prevalence of suspected genes and estimates of suspected environmental factors, for both cases and referents, and would indeed increase our understanding of MS.

Clinical characteristics of MS

We describe the clinical data in an MS population consisting of not only the MS cases seen by the neurology department in the last 10-15 years, but also of cases identified from other sources. The first USA population-based MS survey threw light on this issue by showing a twice as high prevalence as previously reported, and a more optimistic prognosis than previously estimated from hospital-based series.

For about one-third of the cases, a possible onset symptom preceded the defined onset symptom. The significant difference between median age at possible (27 years) and definite onset (29 years) in RR/SPMS shows the importance of standardized onset criteria when comparing MS epidemiological studies. The original list of onset symptoms used serves this purpose. However, “transverse myelitis” was changed to “myelitis”. Transverse myelitis seldom represents the onset of MS, whereas partial myelitis does.

Onset symptoms in MS have been reviewed in several studies. This presentation of onset symptoms (Table 3 in paper III) is restricted to RR/SPMS cases and follows the suggested definition of onset symptoms. Sensory symptoms were more common than motor symptoms, and this has been the case in studies performed in later years. The adopted list also allows for categorization based on the probable anatomical localization of the lesion.
responsible for the onset symptom in a majority of cases. The onset symptom “gait ataxia” was attributable to spinal dysfunction in the present cases, but may, of course, otherwise be attributed to cerebellar involvement.

An anatomical association between first and second attack was found. This finding may be related to clinically recognised subtypes of MS such as cerebral, spinal, or cerebellar MS. Support for the existence of subtypes of this kind also come from autopsy studies. Genetic factors have been shown to affect clinical expression in MS, and pathogenic mechanisms involving intraaxonal spread of a noxious agent or antigen have been proposed.

Age and sex seem to affect the onset of MS with regard to anatomical region. The ratios in the incidence population were similar to the ratios in the prevalence population (Table 3). In a study on optic neuritis (ON), the female to male ratio was 4.1:1. This ratio was 4.5:1 in cases with onset ≥40 years, and 3.6:1 in cases with onset <40 years.

Table 3. The female to male ratio (F/M) by age at onset and first attack anatomical region in 106 incident RR/SPMS cases.

<table>
<thead>
<tr>
<th>Age at onset</th>
<th>Optic nerve</th>
<th>Brainstem/cerebellar</th>
<th>Spinal cord</th>
<th>Cerebral/unclear</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex ratio</td>
<td>n</td>
<td>Sex ratio</td>
<td>n</td>
<td>Sex ratio</td>
</tr>
<tr>
<td>0-28</td>
<td>2.0</td>
<td>1.3</td>
<td>3.0</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>4F/2M</td>
<td>4F/3M</td>
<td>9F/3M</td>
<td>8F/2M</td>
</tr>
<tr>
<td>29+</td>
<td>6.0</td>
<td>2.6</td>
<td>1.2</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>6F/1M</td>
<td>13F/5M</td>
<td>12F/10M</td>
<td>16F/8M</td>
</tr>
</tbody>
</table>

Recent presentations of the distribution of clinical subtypes usually follow the proposed classification criteria. The study reporting the highest prevalence (37%) of PPMS does not report the sources used for case identification. The prevalence of PPMS was high in one hospital-based case series (26%). One population-based incidence study used central hospital registers (ICD 9: 340-341) for case identification and showed slightly lower prevalence of PPMS (22%), whereas even lower prevalence was seen when several sources were used for case identification (14%). The prevalence of PPMS was 14% in a prevalence population in Olmsted County, Minnesota, in 1991. In two prevalence studies where multiple sources were used for case identification, the prevalence of PPMS was 18% and 22%, respectively.

In the discussion in the third paper we try, without the use of statistic methods, to elucidate the difference regarding the disability distribution between the Västerbotten and London, Ontario populations and give some observations related to this difference. We propose that the differences observed might be owing to selection bias that may have occurred in the London, Ontario MS population because it consists of cases referred to a tertiary referral centre specializing in MS.

The knowledge on clinical characteristics and natural history of MS is to a large extent derived from studies on cases frequenting MS clinics. The most elaborate and cited study of the natural history of MS constitutes a follow-up of cases from 1972-84 at the MS clinic at the University Hospital in London, Middlesex County, Ontario, Canada. To this date, this material has been used in a series of eight papers on the natural history of MS. Keeping the pronounced inter-individual variations of MS in mind, it is reasonable not to assume that the individuals who visit or have visited a neurological department on a regular basis are
completely representative of the total MS population. The prognosis in the London Ontario total population has been compared to the prognosis in the Göteborg 1950-64 incidence cohort. While both materials show that 50% of patients reach DSS 6 by 15 years of disease duration one should keep in mind that the London population used in the comparison is not an incidence cohort.

Referral bias may thus affect the generalizability of the results. One study showed that patients referred to a university based MS referral center were significantly younger and had significantly more impairment for their age, as compared to the non-referred patients. We compared EDSS estimated in median year 1998 for cases with “MS” according to medical records (n=221) to EDSS for cases without a clear MS diagnosis in medical records (n=82) in the present 1990 prevalence population. Median EDSS was significantly higher in the first group compared to the latter (6.5 vs. 3.5, p<0.001, Mann-Whitney test).

As this methodological difference between the London, Ontario and the Västerbotten study, does exist, one does not have to assume another difference, such as a more benign MS phenotype in Västerbotten. “Plurality should not be assumed without necessity” (Occams Razor).

In contrast to the differences found in the comparison to the London, Ontario population is the similarities found between the present population, and the prevalence population from Olmsted County, Minnesota (Table 4).

Table 4. Comparison of clinical data from two prevalence populations.

<table>
<thead>
<tr>
<th></th>
<th>Västerbotten County</th>
<th>Olmsted County</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence</td>
<td>154/100,000</td>
<td>168/100,000</td>
</tr>
<tr>
<td>Median age at onset (range)*</td>
<td>30 (7-68)</td>
<td>28 (14-66)</td>
</tr>
<tr>
<td>Median number of years from onset (range)</td>
<td>15 (0-62)</td>
<td>15 (0.7-63)</td>
</tr>
<tr>
<td>Percentage with RR/SPMS</td>
<td>84%</td>
<td>86%</td>
</tr>
<tr>
<td>Median EDSS (range)</td>
<td>3.5 (0-9.5)</td>
<td>3.5 (1.0-9.5)</td>
</tr>
</tbody>
</table>

* The median age of onset in the Västerbotten population was 28 years if possible onset was applied

The consequences of MS

The choice of MS population (the case retrieval procedure) affects not only clinical data, but also data on the consequences of MS. Estimates on the prevalence of sick leave are therefore varying. In one hospital-based case series, 40% had a job last month, of which 40% was full-time. Twenty-five percent of MS individuals in a prevalence population in N. Ireland were working essentially full-time. One study reported the functional status of all 162 definite MS individuals belonging to the prevalence population 1991 in Olmsted County, Minnesota. A self-reporting interview using the Environmental Status Scale (ESS) showed that 53% of cases worked full-time (ESS 0-1). One review on this subject provided unemployment rates from seven US studies ranging between 74-81% among persons with MS. In the present study 35% of MS cases aged 18-64 years were not sick listed at all, and 23% out of all MS cases were working full time.
Studies on risk factors for sick leave in an MS prevalence population may identify risk factors associated to better outcome and longer survival. Moreover may identified risk factors be non-specific for MS. Nevertheless do data from the prevalence population picture the impact of a disease at given point in time, which may be a useful supplement to data from longitudinal studies. Employer attitudes have been identified as one factor associated with employment in MS.\textsuperscript{154}

One study used data from a national US MS survey to identify factors that might influence an MS individual's employment status.\textsuperscript{155} The investigators identified female sex as a risk factor for unemployment. Another group analysed factors associated with time to unemployment, and identified male sex as a risk factor for shorter time to unemployment (however not significant in the multivariate analysis).\textsuperscript{156} The results of previous studies and the present study support the view that the study design and choice of case definition will affect these estimates. Male sex is associated with worse prognosis in MS, and is therefore more likely to be a risk factor when time to unemployment is used, while cross-sectional studies are more likely to identify female sex as a risk factor for present unemployment, by reasons discussed above.\textsuperscript{157}

The present study found a six-fold increase of the risk for full sick leave among MS cases in comparison to the general population. The corresponding risk increase from MS would certainly be higher if the life-time risk for full sick leave had been studied. The prevalence of sick leave in Västerbotten county is also among the very highest in Sweden (www.rfv.se).

Population-based data on the consequences of MS for the usage of community services and housing are rare. The Minimal Record of Disability for MS includes the ESS, and has been used to estimate these issues in two prevalence populations. The prevalence study from N. Ireland reported that 35\% of MS individuals required assistance for at least 1 h/day (ESS≥2; major help, relatives involved according to the original definition), 18\% of individuals had changed residence due to MS, and 5\% were institutionalized.\textsuperscript{17, 152} In Olmsted County, Minnesota, a self-reporting interview over the MS individuals belonging to the prevalence population showed that 38\% required more than minor personal assistance (ESS ≥2), and 8.0\% were institutionalized.\textsuperscript{14} The prevalence of ESS≥3 regarding personal assistance (the need of outside help according to the original definition) in the two populations was 24\% and 30\%, respectively. These estimates are similar to the 25\% of MS cases receiving professional assistance in the present study. Yet while 9\% of MS individuals were living in care homes or special apartments for the disabled, only 2\% resided permanently in nursing homes. The Swedish practice of allowing for extensive professional assistance, without changing residence, affected these estimates. For example, 2\% of the MS cases had professional personal assistance more than 70 hours per week while residing in their own homes.

The impact of viruses on the development of MS

The work from the preceding studies gave access to a well-characterized MS population, which could be subgrouped based on several clinical epidemiological factors. This population as well as collected blood samples may be used for further research projects. One example hereof is the virus study, made in collaboration with the Medical Biobank, Umeå University, and the NSHDS and NSMC cohorts.
As serological studies investigate the immune response, all conclusions rely on the premise that the results relate to the particular infectious antigen. Thus, an association with a particular microorganism cannot be proven by serological results only. The interpretation of serological analyses do become complicated when ubiquitous pathogens are studied. The ideal prospective case-referent MS study on the viruses studied here would require samples from a younger population with a higher prevalence of seronegative individuals. This would also be more appropriate in the case of MS, for which many years of subclinical disease is believed to precede clinical onset.

It may be appropriate to recapitulate Koch’s Postulates, which read as follows:

1. The microorganism must be detectable in the infected host at every stage of the disease.
2. The microorganism must be isolated from the diseased host and grown in pure culture.
3. When susceptible, healthy animals are infected with pathogens from the pure culture. The specific symptoms of the disease must occur.
4. The microorganism must be re-isolated from the diseased animal and correspond to the original microorganism in pure culture.

As for the third of Koch’s Postulates, data support that individuals are more susceptible when infection occurs at certain ages. The result from several studies suggests that individuals who later develop MS have had their primary EBV infection at an older age than referents, while one study proposes a younger age.

In the normal antibody response following EBV-associated infectious mononucleosis (IM), anti-VCA antibodies is seen during the acute phase, while anti-EBNA-1 antibodies emerge first during or after convalescence. Both types of antibodies are elevated during the asymptomatic carrier stage.

Our results show that high activity to EBNA-1 is a significant risk factor for the development of MS. This was true for all the populations studied, and most pronounced <5 years before MS onset. Furthermore, a discrepancy between EBNA-1 and VCA response in MS cases was found. Table 5 aims to clarify this discrepancy by showing the distribution of cases and referents within the three EBNA-1 activity categories by VCA activity category.

<table>
<thead>
<tr>
<th>VCA level</th>
<th>Prospective Cases</th>
<th>Prospective Referents</th>
<th>p-value*</th>
<th>Retrospective Cases</th>
<th>Retrospective Referents</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VCA low</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EBNA low</td>
<td>5</td>
<td>37</td>
<td>&lt;0.001</td>
<td>2</td>
<td>82</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EBNA medium</td>
<td>5</td>
<td>27</td>
<td></td>
<td>21</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>EBNA high</td>
<td>18</td>
<td>20</td>
<td></td>
<td>20</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td><strong>VCA medium</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EBNA low</td>
<td>7</td>
<td>21</td>
<td>0.031</td>
<td>4</td>
<td>44</td>
<td>0.001</td>
</tr>
<tr>
<td>EBNA medium</td>
<td>3</td>
<td>32</td>
<td></td>
<td>26</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>EBNA high</td>
<td>16</td>
<td>32</td>
<td></td>
<td>38</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td><strong>VCA high</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EBNA low</td>
<td>4</td>
<td>15</td>
<td>0.16</td>
<td>4</td>
<td>35</td>
<td>0.014</td>
</tr>
<tr>
<td>EBNA medium</td>
<td>3</td>
<td>16</td>
<td></td>
<td>24</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>EBNA high</td>
<td>12</td>
<td>19</td>
<td></td>
<td>22</td>
<td>42</td>
<td></td>
</tr>
</tbody>
</table>

* Pearson Chi-Square test
This discrepancy was not commented on in the two preceding prospective EBV studies although presented data indicated its presence. The first prospective study showed a significant association with EBNA-1 but not with VCA.\textsuperscript{57} In the second prospective study, high EBNA-1 but not VCA titres were associated with a significant relative risk increase for MS in cases with blood collected ≥5 years before MS onset (data not presented for the <5 year subgroup).\textsuperscript{164} In the present study, however, there was no sign of an MS association with activities to VCA in prospective samples. To our understanding, differences in laboratory methodology are not a plausible explanation for this difference. The non-significant elevation of activities to VCA in the retrospective analysis may be consistent with EBV reactivation after MS onset.\textsuperscript{64, 165} One possible explanation for the diverging VCA results between the studies is that not all prospective cases in the other two prospective studies actually were prospective. Another study supported the findings in the present study by reporting EBNA-1 associated CSF oligoclonal bands in the absence of anti-bodies to VCA.\textsuperscript{71} Lower activities to EBNA-1 in MS cases than in matched referents have also been reported.\textsuperscript{166} One possible explanation of this contrasting finding may be that 57 of 107 cases in that study had exacerbation of disease. MS relapses have been associated with EBV reactivation and lower activities to EBNA-1.\textsuperscript{64}

In our paper we argue against the view that increased activities to EBNA-1 in MS cases just represent a generalized immune dysregulation.\textsuperscript{167} One previous study showed significantly higher levels of auto-antibodies in progressive MS than in relapsing MS.\textsuperscript{168} They also found lower levels in benign MS. In the present study, EBNA-1 activity was instead higher in younger individuals as compared to older individuals (significantly so in prospective individuals).

An important support for the association between EBV and MS comes from studies on child-onset MS. In one case report, MS-onset is described in a 10 month infant. The authors suggested an aetiological role for EBV in MS based on EBV antibody titers during the clinical course of this patient.\textsuperscript{169} In an abstract reported by Dr Banwell, Toronto, Ontario, Canada, at the 2003 American Academy of Neurology meeting, only 12% (3 of 25) of cases with child-onset MS were serologically EBV negative, compared to 69% of referents. This highly significant difference provides important support for the role of EBV in MS pathogenesis. MS in childhood has to some extent different clinical characteristics than adult-onset MS.\textsuperscript{170} It is thus possible that this entity may differ aetiologically from MS in adults, and EBV may not be a prerequisite for all childhood MS cases.

It has previously been shown that EBV needs HLA class II receptors to infect B-cells. This is interesting because the only consistent genetic association for MS identified to this date is for certain HLA class II haplotypes.\textsuperscript{69, 100} However, one study did not find any association between infectious mononucleosis and HLA-DR type.\textsuperscript{171} Another proposed link between EBV and MS is the transactivation of an endogenous retrovirus. This environmental link would still require a genetic predisposition.\textsuperscript{172}

Two antiviral treatment trials have been conducted with acyclovir, both with negative results regarding primary endpoints but with some promising results in subgroup analyses. An inhibitory effect on VCA was not seen in the first study, and only a modest suppressive effect was to be expected from the dosage used in the second study. The question is what efficiency one might expect from this kind of treatment when the effect from EBV on MS starts several years before MS onset. The number of circulating EBV-infected B-cells is not affected by acyclovir treatment.\textsuperscript{62} However, since EBV reactivation has been associated with relapses, an
efficient suppression and prevention of EBV reactivation might be beneficial. Vaccination against EBV during childhood (or early exposure like in the developing countries) might be one way to decrease the risk for MS.62,173

The virus activities in the present study expressed as median values are shown in Figure 7.

![Graph showing activity levels of various viruses in cases and referents](image)

Figure 7. Comparison of median activities to EBV-EBNA-1, HSV, VZV, measles, and HHV-6 in cases and referents. The immunofluorescence grading of activities to EBV-VCA is not shown, but was significantly higher in cases only in the retrospective group (p<0.01). Mann-Whitney test used for test of difference.

The statistical association found in the present study between HHV-6 and MS was weaker than that between EBV and MS. The association was clear in cases with relapsing-remitting onset in samples collected less than five years before MS onset, but it was not significant for those with samples collected earlier. There was not even a trend of an association in retrospective samples. Therefore, we cannot say whether or not there is a biological association between HHV-6 and MS or if this finding is due to residual confounding or an inflammatory response involving the activation of T-cells. However, the fact is striking that two of the viruses (EBV and HHV-6) where MS associations have been found, are resident in two lymphocyte subtypes that are likely to play a key role in the autoimmune reaction in MS.

The Swedish vaccination program for measles, which started on a national basis in 1977, may explain the higher prospective measles activities (median year of collection 1984) compared to the non-prospective measles activities (median year of collection 1992). The
immune response is namely less intensive upon vaccination compared to native infection, which renders gradually decreasing measles activities in the population.

Although non-significant, cases were more often seronegative for HSV and VZV before MS onset and more often seropositive after MS onset, than were the referents. When compared to the prospective results, VZV seronegativity before MS onset (7 out of 73) was significantly more common than VZV seronegativity after MS onset (2 out of 161) (OR=8.4; 95% CI: 1.5-84). The corresponding results for HSV or for referents were not significant. A triggering role for HSV has been suggested previously from the isolation of HSV type 1 during the first attack of MS. Whether HSV or VZV have triggering roles has to be determined in analyses on consecutive samples from prospective cases.
Future prospects

The primary purpose of the present project was primarily to estimate the occurrence of MS in Västerbotten county. The working process needed to achieve this goal opened up the possibility to obtain additional information, enabling further studies. These populations are now well characterized and make further investigations possible. We intend to enlarge the incidence cohort to enable studies on for example seasonal patterns, and birth cohort effects. Furthermore, we will estimate the prevalence of cases with affected relatives. Either Sällström was incorrect, or has this prevalence changed dramatically since, when he estimated that only 17 out of 1010 of his cases had familial related MS. The prevalence of affected relatives is also useful also when comparing epidemiological results on the prevalence or incidence of MS between different areas.

For the virus study, we will analyze consecutive samples from prospective cases. This will show whether or not more cases than expected become seropositive for VZV or HSV. We will compare the serological results for activities to EBNA-1 and VCA from before MS onset, to activities just after MS onset. For many cases serum is available from directly after MS onset, because clinical investigations including the examination of viral activities were performed by then. The influence of HLA-DR type on virus results will be analyzed. Further analysis on the results for different subgroups (for example, clinical course, sex, and disease severity) will also be carried out. Finally, further analyses on serum samples will be performed using, for example, PCR for DNA detection.
Acknowledgements

First I want to thank my supervisors (in order of appearance):
Lars Forsgren, for his insights into scientific thinking, his being available for discussions, and his sound, positive, and pragmatic view on research,
Lennarth Nyström, for providing not only statistical knowledge, but also constructive ideas, and for putting considerable effort in the shaping up manuscripts, and
Anders Sveningsson, for sharing his vast knowledge of the research area, for enabling the production of this thesis by clinical work support, and for conveying an inspired attitude.

I also express gratitude to Sari Wallin and Maria Nyström. Sari Wallin, whose enthusiasm as study nurse was manifested in the high percentage of individuals participating in the follow-up, was devoted to the project 1997-2001. Maria Nyström performed laboratory analyses in the virus study with exceptional swiftness and commitment.

I also thank the co-authors in the fifth paper for fruitful discussions; Martyn Fulford for help with compiling the data on cases in the virus study; Curt Edlund for providing data from the local Social Insurance Board; Hubert Sjödin for providing data from the Medical Biobank; Björn Tafelin for help with LogXact; Margit Lundmark for help with figures in the manuscripts; Martin Gunnarsson for discussions about MS; and Susan Jeglum for English editing.

I must also acknowledge the skilled personal at the neurological department in Umeå and Björkgården, Sävar, for help with blood sample collection, identifying medical records, and several other practicalities. Also I thank Kerstin Gabrielsson for her assistance as clinical MS-nurse.

I am grateful to my colleagues for tolerating my absence from clinical obligations, Peter Andersen who came up with the suggestion to perform an MS prevalence study, and my office roommate for several years Jonas Helander, in memoriam, who encouraged me in the from time to time burdensome compilation of data for the epidemiological study.

I thank Anna for showing unrivalled understanding; and my parents, relatives, and friends for indulgence with a temporary defective social behaviour.

At last, but most importantly, I want to thank all patients for devoted collaboration in the study.

This work was supported by grants from K O Hansson Memorial Fund, Schering Nordiska, NHR, and the Department of Clinical Neuroscience, Umeå University.
References

35. Davenport CB. Multiple sclerosis from the standpoint of geographic distribution and race. Arch Neurol 1922; 8:51-60.


Lycke J, Svennerholm B, Hjelmquist E, et al. Acyclovir treatment of relapsing-


Ahlgren C, Taranger J, Johansson L, Andersen O. Elimination of childhood diseases:
possible influence on the incidence of multiple sclerosis soon detectable.

Bansil S, Trojano R, Dowling PC, Cook SD. Measles vaccination does not prevent

Duke T, Mgone CS. Measles: not just another viral exanthem. Lancet 2003; 361:763-
73.

Ahlgren C, Taranger J, Johansson L, Andersen O. Elimination of childhood diseases:
possible influence on the incidence of multiple sclerosis soon detectable.

Bansil S, Trojano R, Dowling PC, Cook SD. Measles vaccination does not prevent

Duke T, Mgone CS. Measles: not just another viral exanthem. Lancet 2003; 361:763-
73.

Ahlgren C, Taranger J, Johansson L, Andersen O. Elimination of childhood diseases:
possible influence on the incidence of multiple sclerosis soon detectable.

Bansil S, Trojano R, Dowling PC, Cook SD. Measles vaccination does not prevent

Duke T, Mgone CS. Measles: not just another viral exanthem. Lancet 2003; 361:763-
73.

Ahlgren C, Taranger J, Johansson L, Andersen O. Elimination of childhood diseases:
possible influence on the incidence of multiple sclerosis soon detectable.

Bansil S, Trojano R, Dowling PC, Cook SD. Measles vaccination does not prevent

Duke T, Mgone CS. Measles: not just another viral exanthem. Lancet 2003; 361:763-
73.

Ahlgren C, Taranger J, Johansson L, Andersen O. Elimination of childhood diseases:
possible influence on the incidence of multiple sclerosis soon detectable.

Bansil S, Trojano R, Dowling PC, Cook SD. Measles vaccination does not prevent

Duke T, Mgone CS. Measles: not just another viral exanthem. Lancet 2003; 361:763-
73.


