The epidemiology of multiple sclerosis. From southwestern Sweden to northeastern Catalonia.

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From southwestern Sweden to northeastern Catalonia

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LUND
UNIVERSITY

DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine at Lund University to be publicly defended on 17th of January 2025 at 1.00 pm in Lilla Aulan, Jan Waldenströms gata 5, Malmö

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Abstract:

Background: Multiple sclerosis is a chronic autoimmune disease of the central nervous system and the most common cause of permanent disability in young adults after trauma. The current view is that MS develops as a complex interplay between genetic and environmental factors. Higher disease disability has been suggested in certain groups such as African Americans and Hispanic Americans compared to Caucasian Americans, North African immigrants in France and non-western immigrants in Norway.

Aims: The overall aim of the thesis is to advance knowledge regarding vitamin D as one of the putative environmental risk factors for the development of MS and to gain insight into the epidemiology of MS in the City of Malmö and in the county of the Alt Empordà, Catalonia.

Methods: Paper I is a nested case-control study in which six Swedish biobanks, the Swedish MS registry and a local MS/Possible MS database were used as sources. The concentration of 25-hydroxyvitamin D was measured in blood samples drawn before the onset of relapsing-remitting MS and in controls matched by biobank, sex, date of sampling and age. Paper II-III consist of a population-based observational study in the City of Malmö. Case identification was performed through multiple sources and case ascertainment by medical chart review. The 2010 McDonald diagnostic criteria together with the concept of onset-adjusted prevalence and a definition of onset symptoms applied. Cases were classified into Scandinavians, Western and non-Western. Disability progression was assessed with the Multiple Sclerosis Severity Score (MSSS). Paper IV is an observational study based on data derived from a local MS registry and population register data from the Alt Empordà. Onset-adjusted incidence and prevalence together with the 2010 McDonald diagnostic criteria applied.

Results: In Paper I we found that high levels of 5-hydroxyvitamin D (top quintile) were associated with a reduced MS risk (OR 0.68). In Paper II, the incidence of MS in the City of Malmö in 2001–2010 was 5.3/100,000 and the prevalence for December 2010 was 133/100,000. In Paper III, the prevalence of MS in the City of Malmö was 100/100,000 among first-generation immigrants, 154/100,000 among Scandinavians, 123/100,000 in the Western group, and 76/100,000 in the non-Western group. In the adjusted model, the mean MSSS difference between the non-Western and the Scandinavian groups was 1.7 (p= 0.030). No differences in time to diagnosis or time to treatment initiation were found. In Paper IV, the incidence of MS in the Alt Empordà in 2001-2010 was 3.5/100,000 and 1.6/100,000 in 2011-2020. The prevalence in 2010 was 75/100,000 and 81/100,000 in 2020.

Conclusions: This thesis supports the hypothesis that high vitamin D levels reduce the risk of developing MS and the presence of a latitude gradient of MS prevalence in Sweden. Finally, we found that non-Western immigrants had a more rapid disease progression than Scandinavians. It is unclear whether genetic susceptibility and/or environmental factors after migration are responsible for the pattern of disability. Incidence and prevalence figures in the Alt Empordà are concordant with Spain being a medium risk area for MS. The incidence in the second decade of study was lower than expected. It is unclear whether this reflects a true decrease or a consequence of demographical changes or the Covid-19 pandemic.

Key words: Multiple sclerosis, epidemiology, vitamin D, 25-hydroxyvitamin D, prevalence, incidence, immigrant, disability, Sweden, Catalonia.

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To Paul

Caminante, son tus huellas
el camino y nada más;
caminante, no hay camino,
se hace camino al andar.

Al andar se hace camino
y al volver la vista atrás
se ve la senda que nunca
se ha de volver a pisar.

Caminante no hay camino
sino estelas en la mar…

Antonio Machado
- Spanish poet -

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Paper I*

Biström M, Alonso-Magdalena L, Andersen O, Jons D, Gunnarsson, M, Vrethem M, Hultdin J, Sundström P. High serum concentration of vitamin D may protect against multiple sclerosis. Mult Scler J Exp Transl Clin. 2019 Dec 6;5(4):2055217319892291. doi: 10.1177/2055217319892291. eCollection 2019 Oct-Dec. PMID: 31839980

* Included in Martin Byström's PhD "Environmental risk factors for the occurrence of multiple sclerosis".

Paper II

Alonso-Magdalena L, Zia E, Carmona I Codina O, Pessah-Rasmussen H, Sundström P. Incidence and prevalence of multiple sclerosis in Malmö, Southern Sweden. Mult Scler Int. 2022 Mar 19; 2022:5464370. doi: 10.1155/2022/5464370. eCollection 2022.PMID: 35345609

Paper III

Alonso-Magdalena L, Carmona i Codina O, Zia E, Sundström P, Pessah-Rasmussen H. Prevalence and disability in immigrants with multiple sclerosis in Malmö, southern Sweden. Clin Neurol Neurosurg. 2024 May;240:108255. doi: 10.1016/j.clineuro.2024.108255. Epub 2024 Mar 23

Paper IV

Alonso-Magdalena L, Carmona i Codina O, Masuell i Aumatell C, Díaz-Echezarreta L, Pessah-Rasmussen H. Incidence and prevalence of multiple sclerosis in the Alt Empordà, northeast of Catalonia. Manuscript.

Author's contribution to the papers

Paper I

Case ascertainment regarding Region Skåne.

Interpretation of data.

Writing, review and editing

Paper II-III

Conceptualization of the project.

Writing the project and all relevant applications (Ethic Committee, The national Board of Health and Welfare, Statistics Sweden, and the Regional Steering Committee for disclosure of data).

Case ascertainment.

Data processing

Statistics together with a statistician.

Interpretation of data.

Writing, original draft

Writing, review, and editing including all communication with the journals and reviewers.

Paper IV

Conceptualization of the project.

Data processing.

Interpretation of data.

Writing, original draft

Writing, review, and editing including all communication with the journals and reviewers when applicable.

Abbreviations

APC	Antigen presenting cell
BBB	Blood brain barrier
BMI	Body-max index
CI	Confidence interval
CIS	Clinically isolated syndrome
CMV	Cytomegalovirus
CNS	Central nervous system
CLPBA	Automated chemiluminescence protein-binding assay
CLIA	Chemiluminescent immunoassay
CPBA	Competitive protein binding assay
CSF	Cerebrospinal fluid
DMT	Disease modifying treatment
ECTRIMS	European Committee for Treatment and Research in MS
EDSS	Expanded Disability Status Scale
EIA	Enzyme immunoassay
EBV	Epstein-Barr virus
FDA	Federal Drug Administration
HLA	Human leucocyte antigen
HPLC-MS	High-pressure liquid chromatography and spectrometry
HHV	Herpesvirus
ICD	International classification of Diseases
IM	Infectious mononucleosis
IQR	Interquartile range
LC-MS/MS	Liquid chromatography tandem mass spectrometry
MS	Multiple sclerosis
MHC	Major Histocompatibility Complex

MRI	Magnetic resonance imaging
MSIF	Multiple sclerosis international federation
MSSS	Multiple Sclerosis Severity Score
NMSS	National Multiple Sclerosis Society
NMOSD	Neuromyelitis optica spectrum disorder
25 (OH) D	25-hydroxyvitamin
OR	Odds ratio
OCB	Oligoclonal bands
PHAS	Public Health Agency of Sweden
pwMS	Person with MS
RAW	Relapse-associated disability worsening
RRMS	Relapsing-remitting MS
RIA	Radioimmunoassay
RIS	Radiologically isolated syndrome
SPMS	Secondary progressive MS
STROBE	Strengthening the reporting of Observational studies in Epidemiology
PPMS	Primary progressive MS
PRMS	Progressive relapsing MS
PIRA	Progression independent of relapse activity

Introduction

I have my roots in Asturias, nearby Galicia in northwestern Spain, where I moved as a resident in neurology. Later, I joined the MS Unit in Bellvitge, Catalonia, not far from the Alt Empordà. From there I moved to southern Sweden and my MS work traveled with me and continued in Malmö. This thesis offers a similar journey in reverse.

Preface

Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating, and neurodegenerative disease of the central nervous system (CNS). The cause has not yet been established, but the current view is that the disease is a consequence of a complex interplay between genetic and environmental factors (1). It is significantly more common in women, a pattern that has been increasing over time with the female to male ratio now being close to 3:1 in most developed countries (2). MS most commonly begins in early adulthood and, excluding trauma, is the most common cause of permanent disability among young adults. Most patients initially follow a relapsing-remitting course (RRMS), defined by reversible episodes of neurological symptoms (relapses), with periods of relative clinical stability in between (1). Without treatment, secondary progressive MS (SPMS) typically develops 10–15 years after the initial event, with a gradual evolution to slowly progressive disease. A minority of patients (5–15%) have a progressive course from onset, known as primary-progressive MS (PPMS) (2). Furthermore, the disease is responsible for a substantial economic burden due to the negative impact on health, social and employment issues (3). Compared to the general population, the life span in MS is reduced by 7-14 years (4).

Immunology and pathology

The traditional view is that MS is an organ-specific T-cell mediated autoimmune disease in which the potential autoantigen so far remains unclear (5). Two different hypotheses have been suggested to explain the role of the immune system in the early

inflammatory stages of the disease. According to the predominant one, CNS antigen specific immune activation of T cells takes place first in the periphery, and it is then transferred to the CNS through a disturbed blood-brain-barrier (BBB) (5, 6). The underlying mechanism for the activation of T lymphocytes outside the CNS is still unclear, but it may be elicited by molecular mimicry or bystander activation (6, 7). Once in the CNS, T lymphocytes initiate an inflammatory process by recognizing their specific target presented by antigen-presenting cells (APCs) and are then reactivated. Following reactivation, T lymphocytes recruit other immune cells from the periphery, including B lymphocytes and plasma cells which locally produce antibodies against the myelin sheath and enhance immune systems responses leading to loss of myelin (demyelination) (5). According to the other theory, the inflammatory process is instead initiated within the CNS followed by an amplification of the autoimmune response in which the antigens move to the periphery and the adaptive immune system is stimulated (6). Whether the inflammatory process is initiated in the periphery or within the CNS, the common view is that B cells are mainly a passive population waiting for the T cells to differentiate into antibody-secreting plasmablasts and plasma cells. However, the success of antiCD20-mediated B cell depletion therapies in the last decade, has raised the question whether B cells may actually play a more central role in the immunological dysregulation in MS through antibody-independent functions such as antigen presentation and/or cytokine production and T cell regulation (8).

As for the progressive stages of the disease, there are two explanatory models. One proposes that neurodegeneration drives disease progression, i.e. that disturbed homeostasis in the axon-glial unit generates a progressive neurodegenerative disease progress with inflammation being a secondary process. Conversely, the other model postulates that compartmentalised inflammation drives disease progression, with gradual accumulation of inflammatory cells that become trapped within the CNS (6, 9).

The classical pathological feature of MS is the presence of focal areas (plaques or lesions) of inflammation-mediated demyelination with partial axonal preservation and reactive glial scar formation in the white and grey matter of the CNS (9). As the disease progresses, irreversible axonal damage develops (10). In addition to these focal changes, there are substantial alterations in the macroscopically normal white matter (so called normal-appearing white matter), especially in primary or secondary MS as well as diffuse axonal injury in the brain. The inflammatory infiltrates contain mainly lymphocytes, dominated by major histocompatibility complex (MHC) class I restricted CD8+ T cells, and to a lesser extent B lymphocytes and plasma cells (9). It was long believed that remyelination did not occur in MS patients, but indeed it does. Remyelinated lesions (shadow plaques) are sharply demarcated with thin myelin

sheaths throughout. However, the extent of remyelination shows great individual variation and depends in part on lesion location. Moreover, the remyelination process seems to be unstable, with shadow plaques being the target for new demyelination as long as the inflammatory process is still active (9, 11).

Risk factors

While MS is acknowledged to be an autoimmune disease, the definite cause remains unclear. However, current knowledge points towards an interaction between genetic susceptibility and environmental risk factors (1).

Genetic factors

The contribution of genetic factors to MS susceptibility has been extensively investigated (12). Twin studies have shown a higher concordance rate in monozygotic twins (20–30%) than in dizygotic (2–5%) (13-15) and recent population-based studies have estimated that a sibling to an individual with MS has a 7-fold increased risk of the disease than the general population (16, 17) though second- and third-degree relatives only have a slightly increased risk (18). The overall prevalence of familial MS has been estimated to 13% (19).

Genome-wide association studies have been performed in MS revealing its complex genetic architecture, with more than 200 risk loci to date (20). Of note, most of these loci encode molecules involved in the immune system and are also associated with other autoimmune diseases. The MHC on chromosome 6 accounts for the largest contribution to MS susceptibility (12). The presence of the class II HLA-DRB1*15:01 allele has a well-established association with increased risk (odds ratio (OR) ~ 3) whereas the absence of the class I variant HLA-A*02:01 is associated with a protective role (OR 0,6) and the combination of HLA-DRB1*15:01 with the absence of HLA-A*02:01 confers a 5-fold increased risk (21).

Environmental factors

Environmental factors with the strongest current evidence for involvement in MS are briefly reviewed here.

Vitamin D

There are two forms of vitamin D: ergocalciferol (D2) and cholecalciferol (D3). Cholecalciferol is present in fatty fish while plants provide ergocalciferol. Both can be absorbed from the diet, but cholecalciferol can also be produced by the skin´s exposure to ultraviolet B radiation (UVB) (22). In fact, most of the body´s vitamin D reservoir relies on exposure to sunlight, with only 10–20% gained through diet (22, 23). Ergocalciferol and cholecalciferol are then hydroxylated in the liver to 25-hydroxyvitamin D2 (25(OH) D2) and 25-hydroxyvitamin D3 (25(OH) D3, calcidiol) respectively, with calcidiol being the major circulating form of vitamin D (23). In order to become biologically active, calcidiol needs to be further hydroxylated by the enzyme 25 (OH) D3-1α-hydroxylase (CYP27B1) to 1,25-dihydroxyvitamin D3 (calcitriol) (22).

Although calcidiol is considered the main validated surrogate of the body´s vitamin D status there is no consensus about the optimal levels for vitamin D. However, most guidelines agree that serum levels above 50 nmol/L are sufficient for most of the general population while levels under 25–30 nmol/L indicate deficiency and should be avoided (24). To further complicate matters, there are many different available assays for determination of vitamin D status, such as high-pressure liquid chromatography and spectrometry (HPLC-MS), radioimmunoassay (RIA), enzyme immunoassay (EIA), competitive protein binding assay (CPBA), automated chemiluminescence protein-binding assay (CLPBA) and chemiluminescent immunoassay (CLIA). The drawback of so many methods is the inter-assay and laboratory discrepancies which may have implications both in clinical and research settings. In a Swedish study comparing HPLC-MS, RIA and CLIA, 8% of the study population were classified as vitamin D insufficient (<50 nmol/L) with the HPLC-MS method while the same figures for the RIA and the CLIA method were 22% and 43% respectively. The authors concluded that HPLC-MS was more reliable and accurate (25) and liquid chromatography tandem mass spectrometry (LC-MS/MS) is currently considered the gold standard (26).

Calcitriol takes part in the regulation of plasma ionized calcium and phosphate levels due to stimulation of their intestinal absorption, renal excretion, and calcium bone mobilization (27). The vast majority of the effects of calcitriol are mediated by the vitamin D receptor (VDR), which is a member of the nuclear receptor superfamily which is a family of transcriptions factors involved in several physiological processes such as metabolism or reproduction (27, 28) . The VDR is present in many human tissues but primarily in those involved in calcium homeostasis (i.e. intestine, kidney, parathyroid gland, bones) (28).

The essential task of vitamin D in bone metabolism has been known for a long time. It was established in the beginning of the twentieth century that adequate dietary vitamin D supplementation could eliminate vitamin D deficiency childhood rickets (29, 30). However, in the last decades, our knowledge about the role of vitamin D has expanded beyond calcium homeostasis, with growing evidence about its effects in both the innate and the adaptative immune system. The VDR is expressed in many immune cells including macrophages, dendritic cells, and T and B lymphocytes (27, 28). Regarding the innate immune system, vitamin D promotes the differentiation of monocytes into macrophages, enhances the chemotactic and phagocytic properties of macrophages, upregulates cathelicidin antimicrobial peptide levels and alters the function and morphology of dendritic cells into a more tolerogenic state (31). Concerning the adaptative immune system, D vitamin is believed to influence both T and B cell homeostasis. For example, vitamin D exposure leads to supressed secretion of proinflammatory cytokines (Il-2, interferon-ɤ, tumor necrosis factor α, IL-9, IL-22, IL-17), and production of anti-inflammatory cytokines (Il-3, IL-4, IL-5, IL-10). Modulation of T cell homeostasis will necessarily have consequences on B cells, but vitamin D can also target these cells directly, leading to inhibition of their differentiation into memory and plasma cells and reducing antibody production. In summary, vitamin D is thought to have immunomodulatory effects by generating a shift from a proinflammatory to a more tolerogenic immune status (27, 31, 32).

The potential role of vitamin D in the development of MS was already proposed by Goldberg in 1974: "a shortage of vitamin D and calcium for a group of genetically susceptible individuals results in myelin having abnormal composition and diminished structural integrity, with the consequence of a heightened predisposition for MS" (33). According to this hypothesis, increased vitamin D may exert a protective effect. In the following decades, several studies have shown an inverse relationship between vitamin D levels and risk of disease activity (34-38), and between vitamin D levels and risk of MS (39-41). Moreover, studies looking into high consumption of vitamin D (food or supplements) have provided further evidence for a protective effect of vitamin D (42-44).

Three of the above cited studies (39-41) are of particular interest. They were nested case-control studies performed using blood samples from diverse biobanks drawn before disease onset, reducing a potential reverse causation bias. The first study, published in 2006 used prospectively collected serial blood samples among US military personnel. They found that the risk of MS decreased by 41% for every 50 nmol/L increase in 25-hydroxyvitamin D (39). The second one, published in 2012, was conducted in northern Sweden, focused on prospectively collected blood samples from pregnant women during the first trimester. Levels of ≥75 nmol/L were associated with

a 61% decrease in MS risk. No association between 25-hydroxyvitamin D and MS risk in the off-spring was found (40). The third one, published in 2017, was performed in Finland, using prospectively collected serum samples from pregnant women at 10–14 weeks gestation. A 50 nmol/L increase in 25-hydroxyvitamin D was associated with a 39% reduced risk of MS (41).

Ultraviolet radiation

Ultraviolet radiation (UVR) is a type of non-ionizing radiation naturally emitted by the sun, but it can be artificially produced by other sources such as tanning beds. Depending on the wavelength, UVR comprises ultraviolet radiation A, B and C, with the ultraviolet radiation reaching Earth being mainly UVA (315–400 nm) and a small amount of UVB (280–315 nm) (45). The amount of UVR reaching the surface of the Earth depends on different factors such as solar zenith angle, clouds, ozone, surface reflection and altitude. In addition, the amount of UVR reaching the skin is influenced by skin type, clothing, sunscreen, and outdoor activities (46).

Known adverse consequences of UVR include aging and carcinogenesis, and there are solid data linking both melanoma and non-melanoma skin cancer to UVR exposure (47). However, ultraviolet radiation also has immunomodulatory properties leading to tolerogenic and immunosuppressive effects on T cells (48).

The particular latitude dependency in the geographical distribution of MS triggered the idea of an association between sun exposure and MS, with Acheson et al. suggesting a potential protective or preventive roll of sun exposure in the early 1960's (49). The hypothesis was further developed in 1997, highlighting a potential immunomodulatory pathway of UVR (50). Several studies performed in the last decades have shown a decreased risk of MS with increased UVR exposure (51-54).

Vitamin D and/or sunlight?

Keeping in mind that ultraviolet radiation is essential to produce vitamin D, it is not difficult to understand the complexity of trying to distinguish between the effects of UVR and vitamin D and whether they are concurrent. Yet, there are some indications of UVR exerting immunomodulatory effects independently of vitamin D (55-57). Regarding vitamin D, Mendelian randomization studies have found that genetically lowered vitamin D influences the risk of MS providing support for an independent causal role of vitamin D in MS susceptibility (58, 59).

Herpesviruses

A potential role of an unknown infection in the development of MS was postulated already in 1894 by Pierre Marie, a former student of Charcot (60). Many different infectious agents have been suggested over the past century with the current top

candidate being Epstein-Barr virus (EBV). EBV belongs to the herpesvirus family. It is widespread across the world, infecting more than 90% of the healthy adult population (61). EBV is transmitted through saliva, establishing a lifelong latent infection in B cells. Primary infection most often occurs in childhood and is usually asymptomatic, while infection at a later age confers higher risk of developing infectious mononucleosis (IM) (62). The link between MS and EBV was first suggested by Warner and Carp in 1981 (63), followed by several studies in the same decade showing higher levels of antibodies to EBV in persons with MS (pwMS) compared to healthy controls (64-66). A meta-analysis from 2012 found that seronegative EBV persons have a negligible MS odds ratio. Moreover, when looking into studies that had used two independent methods to determine serostatus, EBV turned out to be present in all pwMS (67). There are also studies showing that IM is associated with a two- to threefold increased risk of MS (68). Recently, in the largest biobank survey to date, 10 million persons were prospectively followed up, showing a 32-fold increase in the risk of MS after EBV seroconversion (69). Thus, there is compelling evidence for a role of EBV in the development of MS. However, the exact mechanism behind this interplay remains unclear. Molecular mimicry and an altered immune response to ineffectively controlled EBV infection have been suggested (70, 71).

Other viruses worth mentioning here are herpesvirus 6 (HHV-6) and cytomegalovirus (CMV). Both can establish lifelong infections in the host (72). In 2019, a study using a new serological assay to discriminate serological response to HHV-6A and HHV-6B, high antibody levels against HHV-6A were positively associated with both MS and an increased risk of MS. Further, an additive interaction between EBV and HHV-6A serology was found in the cohort with already established MS (73). A similar association between serological response to HHV-6A and increased MS risk was found in another study from the same authors using enlarged pre-MS material (74). Regarding CMV, a putative protective role has been suggested (75, 76).

Obesity

Overweight and obesity, implying excessive adiposity, are defined in adults as a body-mass index (BMI) of ≥25 Kg/m² and ≥30 Kg/m² respectively. Both are increasing worldwide and have become a major public health problem, with estimations of about one billion obese adults by 2030 (77). Similar to our increased insight into the different properties of vitamin D, our understanding of the adipose tissue has evolved beyond this tissue being just a passive fat depot. Instead, the adipose tissue is now considered a secondary immune organ (78). In addition, increased adiposity induces a chronic low-grade systemic inflammation characterized by elevated serum levels of proinflammatory cytokines (79, 80) and adipokines (81). Leptin, discovered in 1994, is currently one of

the best described adipokines and plays an essential role in energy homeostasis by promoting saturation and stimulating energy consumption (82). Moreover, it also has proinflammatory properties influencing both the innate and the adaptive immune systems (83).

Several recent studies have shown that obesity early in life entails an increased risk of MS. These studies have used BMI or body silhouettes as a proxy of adiposity (84-88). Additional evidence about a potential link between BMI and MS risk has been provided by Mendelian randomization studies (89). Of note, serum levels of leptin have shown good correlation with the percentage of body fat (90). A recent meta-analysis has shown that pwMS have significantly higher circulating levels of leptin than healthy controls, suggesting a relationship between this adipokine and MS. Furthermore, leptin seems to be essential to induce Experimental Autoimmune Encephalomyelitis, the experimental model for MS (91). In addition, a recently nested case-control study using prospectively collected samples before the onset of the disease, found that an increase in leptin concentration was associated with an elevated risk of MS in individuals younger than 20 years. Interestingly, a protective effect of higher leptin levels was found among women aged 30–39 year (92). As mentioned earlier, obesity is increasing worldwide, and a similar trend has been observed for MS (93). However, the underlying mechanisms of the association between obesity and/or leptin levels with MS have not yet been established.

Smoking

Several epidemiological studies, including questionnaire studies (94-97) and a Swedish nested case-control study using prospectively collected biobank samples measuring cotinine levels as a proxy for recent tobacco use (98), have shown an association between exposure to cigarette smoke and the risk of MS. The mechanisms behind this association are still unclear but lung irritation triggering immune responses has been proposed (21, 99). There is also evidence that the risk of developing MS increases with the cumulative dose of smoking (97). Moreover, smoking has also been reported to be a risk factor for faster disease progression (100, 101). Of note, the association between smoking and the risk of MS has been shown mainly in studies performed in White populations. Recently, a nested case-control study among US military personnel failed to find such an association in Black people, raising the question whether there is a differential effect of smoking in different ethnic groups (102).

Gene-environment interactions

Finally, data from interaction studies indicate that MS risk linked to lifestyle and environmental factors can be modified by the HLA genotype. For example, this applies to EBV infection (103, 104), smoking (105), and adolescent obesity (106).

Clinical features

Prodromal phase

The concept of a prodromal phase is now well established in other inflammatory and neurodegenerative diseases such as Parkinson or Alzheimer disease, and even in other immune-mediated inflammatory disorders such as inflammatory bowel disease or rheumatoid arthritis (107). Prodromal Parkinson, for example, implies a variety of nonmotor symptoms and/or subtle motor signs that do not meet current diagnostic criteria (108). The idea of an "incubation" or a "latency" period in MS before classical symptoms are manifest has been largely recognized (109, 110). However, the idea of a prodromal phase in MS has not evolved until the last decade (107, 111). Different studies have assessed the manifestation of signs and symptoms before classical MS onset (112-117). It is now postulated that prodromal MS is noticeable at least 5 years before classical onset symptoms, possibly longer, in persons developing PPMS. When compared to persons without the disease, many different symptoms and signs have been identified as more frequent during the years before the occurrence of MS, including pain, sleep disorders, psychiatric morbidity, fatigue, cognitive deficits, bowel/bladder dysfunction and dermatological issues (118).

Clinical course and symptoms

In most patients the disease is characterized by recurrent periods with neurological symptoms (known as relapses, exacerbations, or attacks), and eventually, the development of disease progression. Yet, a minority of patients will follow a progressive course from onset. Until the mid-nineties, the clinical course was classified into relapsing-remitting, secondary-progressive (relapsing-remitting phase followed by progression with or without relapses) and progressive multiple sclerosis (progressive course from onset with or without superimposed relapses). However, in regard to progressive MS, some authors used different terminology and further distinguished between progressive MS with no superimposed relapses (PPMS) and progressive MS with superimposed relapses (relapsing progressive or progressive relapsing MS, PRMS)

(119). As the first pivotal clinical trials in MS were published, showing promising results, the need for standardization of terminology became evident (120). Soon after, the first standardized classification of the clinical course of MS was published in 1996. It was based on a survey by MS experts on behalf of the National Multiple Sclerosis Society (NMSS) Advisory Committee on Clinical Trials in multiple sclerosis in the USA. The term "relapsing progressive" was eliminated since the panel did not reach consensus (121).

Later, the term "clinically isolated syndrome" (CIS) was introduced, and it is currently widely used to refer to the first clinical episode in which a person develops symptoms and signs suggestive of an inflammatory demyelinating disorder of the CNS. It usually presents in an acute or subacute manner, with no fever, infection, or signs of encephalopathy, lasting for at least 24 hours and reaching a peak within 2–3 weeks. Common presentations include isolated lesions in the optic nerve, brainstem, or cerebellum, with lesions in the cerebral hemispheres being rare. Multifocal presentation is also less common. Paroxysmal symptoms occurring for more than 24 hours can also be the presenting episode (122).

In 2012, the NMSS of the USA, together with the European Committee for Treatment and Research in MS (ECTRIMS) and other experts (the MS Phenotype Group) performed a revision of the former classification leading to the current one. The main phenotypes, relapsing and progressive, were maintained with minor modifications: CIS as the first clinical presentation of the disease was recognised, disease activity and disease progression were incorporated, and the PRMS category eliminated. The original and the revised categories are shown in figures 1 and 2 (123).

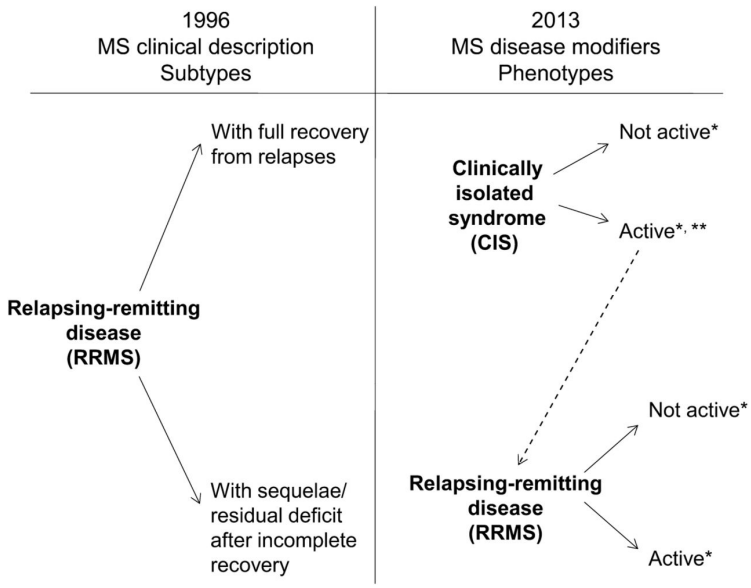

Figure 1. The 1996 vs 2013 multiple sclerosis phenotype descriptions for relapsing disease (reprinted with permission of Wolters Kluwer Health, Inc.)

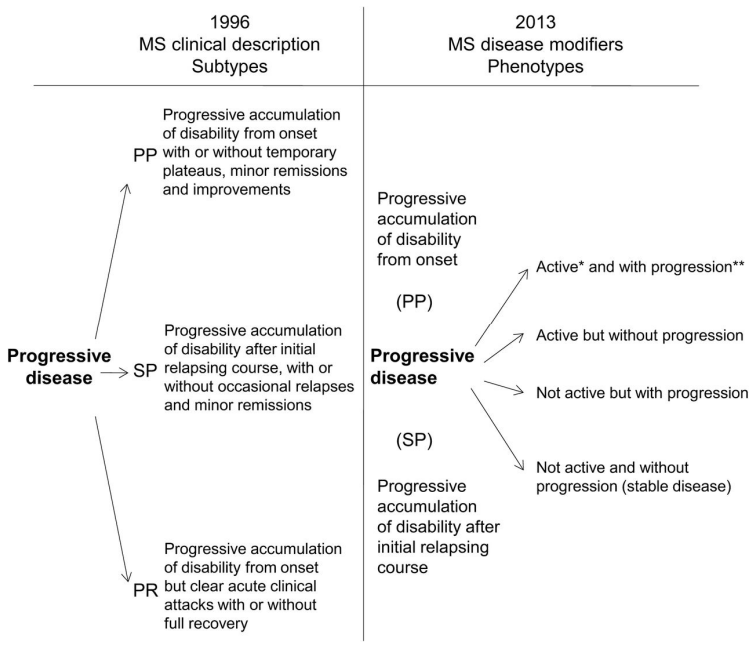

Figure 2. The 1996 vs 2013 multiple sclerosis phenotype descriptions for progressive disease (reprinted with permission of Wolters Kluwer Health, Inc.)

To date, there is no standard definition of SPMS, but it is often referred to as a gradual deterioration independent of relapses for ≥6 months and after a relapsing-remitting course. Most neurologists would also expect a minimum level of disability (124).

A peculiar situation is incidental findings on magnetic resonance imaging (MRI) suggesting inflammatory demyelination but without clinical signs or symptoms. This is referred to as radiologically isolated syndrome (RIS). RIS is not considered an MS phenotype in the above-mentioned classification. However, the aforementioned panel emphasized in 2012 the need for prospective follow-up of this group of patients. The first RIS criteria were published in 2009 (125) and showed a conversion rate to a first clinical attack of 34% and 51% at 5 and 10 years, respectively (126, 127). A proposed revision of these criteria has recently been published (128).

Disability

Traditionally, incomplete recovery from relapses (i.e. relapse-associated disability worsening, RAW) has been considered the hallmark of irreversible disability in RRMS. On the contrary, disability in the progressive forms of the disease has been attributed to relapse-independent mechanisms (123, 124). However, some authors consider that growing evidence in the last decade indicates that there is already a significant amount of disability accumulation early in the disease when the pattern is still classified as relapsing-remitting. Consequently, the novel concept of PIRA (progression independent of relapse activity) has evolved, challenging the classification described above (129-133). A proposed harmonized definition of PIRA has recently been published (134).

The gold standard for assessing clinical disability is the Expanded Disability Status Scale (EDSS) (135) (appendix). The scale was developed by John Kurtzke in 1983, following his former 10 step Disability Status Scale (136). Scoring is based on a standard neurological examination followed by a two-step process. First, a score is applied to eight different functional systems (pyramidal, cerebellar, brainstem, sensory, bowel and bladder, visual, cerebral and others). Finally, each functional system score needs to be combined with the ambulatory function score. The EDSS ranges from 0 (normal neurological examination and no MS-related disability) to 10 (death due to MS) (135). Of note, there is no association between the EDSS and disease duration. To address the important factor of disease duration, the Multiple Sclerosis Severity Score (MSSS) was introduced in 2005 (137) (appendix). The MSSS algorithm is based on the distribution of EDSS scores in relation to disease duration from almost 10,000 patients from eleven countries (10 European and Australia) and has been generally used as a reference standard of disability progression in MS.

Diagnosis

To date, there is no pathognomonic clinical feature, biomarker, or laboratory test to establish the diagnosis of MS. Instead, the diagnosis relies on two principles: to ensure lesions of inflammatory demyelinating origin in different part of the CNS over time (i.e., dissemination in space and time) and to rule out MS-mimicking disorders (i.e., no better explanation) (119).

Until 2001, the diagnosis was based on the Poser criteria, which had been used since 1983 and included clinical history, neurological examination and paraclinical evidence of CNS lesions. Paraclinical evidence could be achieved by examination of the cerebrospinal fluid (CSF), evoked potential studies and tissue imaging techniques (138). In 2001, the International Panel on MS diagnosis developed new diagnostic criteria (McDonald criteria), based on the same principles mentioned above but incorporating MRI and including guidelines for the diagnosis of PPMS (139). The McDonald criteria have since then been regularly revised (140-142) with a new update recently presented in Copenhagen at the 2024 ECTRIMS conference.

Cerebrospinal fluid

The typical CSF in MS is clear with a total leukocyte count greater than 5 cells per mm^3 in about one third of cases and a raised total protein in about one fourth of cases. Cell counts greater than 50/mm^3 and a protein level above 1g/L indicate an alternative diagnosis (143). The same applies for the presence of neutrophils, eosinophils, or atypical cells (144). Other parameters such as CSF pressure, CSF glucose, CSF to plasma glucose ratio and CSF lactate levels are typically normal (143). Intrathecal oligoclonal IgG synthesis (i.e. oligoclonal bands, OCB) is remarkably distinctive of MS and is found in over 95% of patients. However, it is not a specific biomarker for MS and can be present in CNS infections (neurosyphilis, neuroborreliosis, HIV) and other autoimmune and inflammatory conditions (neuro-Behçet, neurosarcoidosis, systemic lupus erythematosus and paraneoplastic conditions) (143, 145).

The gold standard for detecting the presence of oligoclonal bands is isoelectric focusing on agarose gels followed by immunoblotting. The presence of a single band or a negative result suggests the possibility of an alternative diagnosis, but repeating the lumbar puncture and CSF analysis should be considered if clinical suspicion is high (146).

Magnetic resonance imaging

The introduction of MRI in 1981 as a tissue imaging technique in MS became an important milestone in the diagnostic process (147). Basic MRI sequences include T2-weighted and T1 pre- and post-gadolinium images of the brain and the spinal cord. The typical MS lesion is an area of focal hyperintensity on a T2-weighted sequence, round to ovoid in shape with a diameter ranging from a few millimetres to more than one or two centimetres. MS lesions are usually distributed within the periventricular and juxtacortical white matter, the corpus callosum, infratentorial areas (mainly the pons and the cerebellum) and the spinal cord, with a special predisposition for the cervical segment (148). Contrast enhancement is a marker of inflammatory activity and seems to be a consequence of the breakdown of the BBB (119). Enhancement lasts on average 2–8 weeks, often <4 weeks. Enhancing lesions are often nodular, but larger ones can evolve into open-ring enhancement (148).

Visual evoked potentials

Visual evoked potentials (VEP) have been widely used in clinical practice since the 1970s to assess the visual pathways. Increased latency along with preserved waveform have been considered signs of a demyelinating process (149). Increased access to MRI in the last decades has led to VEPs no longer being included in the routine work-up of the disease. However, they are still referred to in the most recent diagnostic criteria, as well as in the upcoming revision, as a means to assess a potential demyelinating process in persons presenting with visual symptoms (140, 142).

Differential diagnosis

As mentioned, a crucial step in the diagnostic approach is to consider other diseases that can mimic MS, i.e. the "no better explanation approach" (119). The spectrum of potential differential diagnosis is wide. Consensus recommendations for the MS differential diagnosis were first published in 2008 (150) and have recently been updated (151).

Prognosis

The clinical course is highly variable between patients, making it difficult in daily clinical practice to predict the long-term prognosis of the individual patient. Classical potential "red flags" for a poorer prognosis are older age at onset (152-157), male sex

(158), tobacco smoking (100), certain ethnicities (159-164), comorbidities (165, 166), progressive course (167, 168), the relapse rate in the first two years (169), incomplete recovery after the first relapse (153, 170), pyramidal, cerebellar, sphincter or visual symptoms at onset (171), polysymptomatic onset (172, 173), early cognitive impairment (174, 175), high number of T2 lesions at baseline (176-181), the presence of Gadolinium-enhancing, spinal cord or infratentorial lesions at diagnosis or early disease (1–3 years) (157), baseline whole brain and grey matter volume measurements (182-184), the presence of OCB bands in the CSF (185), in particular IgM OCB (186, 187), and high neurofilament chain levels in serum (188, 189).

Regarding ethnicity, a pattern of higher disability has been suggested in different studies across the world including African-Americans and Hispanic Americans compared to Caucasian Americans (164), North African immigrants in France compared to Europeans (160, 163), immigrants from the Middle East North Africa (MENA) region compared to those of European descendent (162) or non-western immigrants in Norway compared to native Norwegians and western-immigrants (159).

A practical prognostic tool including different demographic and environmental factors along with clinical, imaging and biomarkers parameter has recently been developed (190).

Treatment

Relapse treatment

Relapses have long been treated with steroids in order to reduce the acute inflammation and hasten recovery. Pulsed intramuscular treatment with corticotropin was extensively used until the 1970's, but was replaced by high dose methylprednisolone (119). The intravenous route of administration has been the gold standard for many years, but today it is established that oral steroids are an equivalent alternative with lower direct and indirect costs (191).

For those who fail to respond to high dose methylprednisolone, plasmapheresis should be considered (192).

Disease modifying treatment

The first disease modifying treatment (DMT) was an injectable medication (interferon beta) approved by the U.S. Food and Drug Administration (FDA) in 1993 (193). Since

then, even though there is still no cure for MS, the treatment landscape has changed dramatically. Today there are many available DMTs with different mechanism of action, routes of administration and safety profiles. Current DMTs target mainly inflammatory activity, which is usually less pronounced in progressive disease, and currently there is only one DMT specifically approved for PPMS. While the benefits and importance of early initiation of DMT are generally well accepted, there is an ongoing debate about which treatment algorithm is best (escalation vs initial high efficacy treatment) (194, 195).

Rehabilitation

Rehabilitation is defined as "a set of interventions designed to optimize functioning and reduce disability in individuals with health conditions in interaction with their environment" (196). While there are different rehabilitation interventions available, there is no universal model of care for MS (197).

In Sweden, the National Board of Health and Welfare has established guidelines for the care of people with MS. The following rehabilitation interventions have been emphasized: multidisciplinary team approach, comprehensive team rehabilitation period and gait rehabilitation (aerobic and resistance training) for those with impaired functional ability, along with fatigue management program and exercise-therapy (aerobic and resistance training) for those with fatigue (198).

Epidemiology

General

Epidemiology is one of the research areas in Public Health Science, referring to the study of the distribution, patterns, and determinants of diseases in populations (199). Much of epidemiological research is based on observational studies (Figure 3) (199, 200).

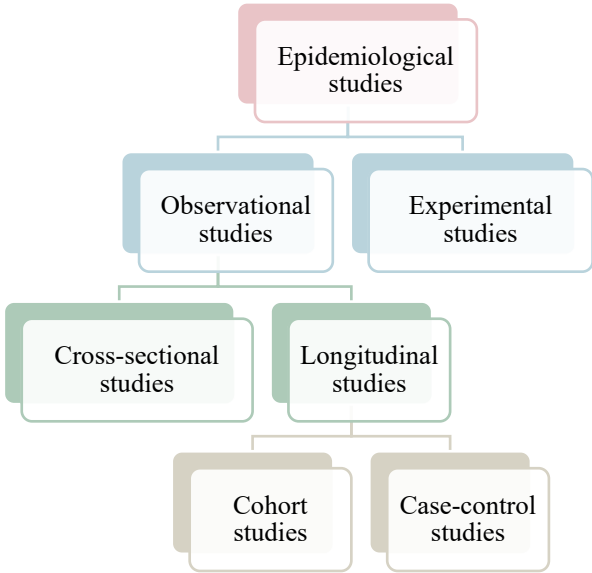

Figure 3. Overview of main subtypes of observational studies
Observational study: the investigator does not intervene, just observes the disease and the associated factors.
Experimental studies: the investigator does intervene, and the effect of the intervention is measured.
Cross-sectional studies: the investigator analyses data from a population at a single time point.
Cohort studies: a group of individuals with predefined common characteristics is followed over time to evaluate the outcome of interest (i.e. develop a disease).
Case-control studies: these studies investigate the relationship between an exposure (i.e. risk factor) and an outcome comparing two group of individuals, those with the outcome of interest (cases) and those without (controls).

Incidence and prevalence are two central measurements in epidemiology. Incidence applies to the number of a new outcome (i.e. develop a disease) in a certain population during a defined time. Prevalence applies to the number of subjects who have a disease at a given time point or during a time period in a certain population (200). Cohort studies are suitable for measuring incidence whereas cross-sectional studies are more appropriate to determine prevalence (199).

A classic cohort study design is to investigate a group of individuals without a certain disease (outcome) from whom baseline data has been collected. The individuals are then subclassified into groups according to an exposure of interest and followed over time to determine who develops the disease and who does not. If the disease is not that common, only a small number of subjects at risk will actually develop the disease. Since many potential exposures are long-term in nature, one disadvantage of such a study design is that it is time-consuming and expensive.

Case-control studies include a group of individuals with a certain disease and another group without the disease. The exposure of interest is then compared between the groups. Thus, case-control studies are often more efficient and with reasonable cost. An adequate selection of controls is a crucial step in these types of studies. The principles are that controls should come from the same population as the cases (i.e. the source population) and that they should be selected independently of exposure status. A particular case-control design is the "nested" case-control study. The design implies that cases of a disease are first identified within a defined cohort and subsequently matched controls are selected among those who have not developed the disease in the same cohort (199, 200).

The quality of reporting observational research is important since poor quality prevents interpretation and generalizability. Strengthening the reporting of Observational Studies in Epidemiology (STROBE) is an initiative from different epidemiology researchers with the ambition of improving this research area. The STROBE checklist includes 22 items that should be addressed when reporting observational studies (201).

The epidemiology of multiple sclerosis

The particular worldwide distribution of MS has been a matter of discussion for many years. The conventional view is that there is a latitudinal gradient with higher latitudes correlating with higher frequency of the disease (119). The latitude gradient theory has been questioned in some studies that either do recognize the gradient in Australia and New Zealand but not in the northern hemisphere (202) or claim no latitude effect as a consequence of age and gender adjustments (203). However, more recent meta-analyses have confirmed the latitude gradient for prevalence globally and the association between incidence and latitude in Europe (204-206). The global distribution of MS is presented in Figure 4 and 5 (207, 208).

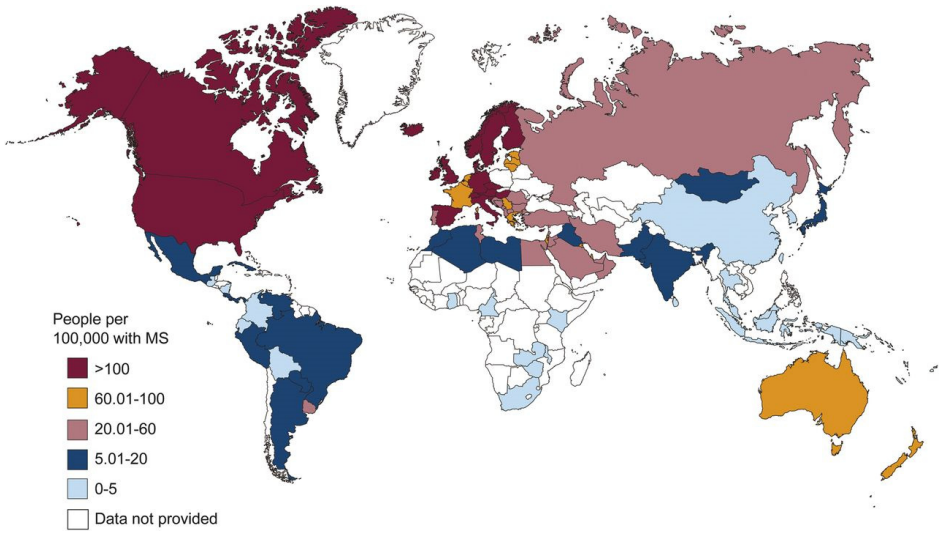

Figure 4. Global prevalence of MS in 2013 (Atlas of MS 2013, with permission of the Multiple Sclerosis International Federation, MSIF).

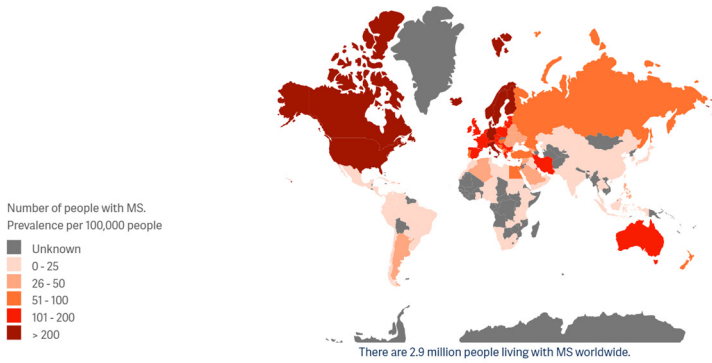

Figure 5. Global prevalence of MS in 2020 (Atlas of MS 2020, with permission of MSIF).

Another controversial topic is whether the incidence of MS has increased in the last decades or not. Overall, the evidence suggests that this is the case during the second half of the 20th century, especially in women and in RRMS, probably as a consequence of better case ascertainment in combination with increased exposure to certain environmental factors (93).

Ethnicity

A remarkable exception to the latitude gradient is a lower prevalence among certain ethnic groups compared to other inhabitants of certain regions, i.e. the Maoris in New Zealand, the Amerindians of North America and the aboriginal population of Australia. These differences may indicate variations in genetic susceptibility, although it is important to keep in mind that these groups usually have a different lifestyle than most of the population in those countries (209).

In addition to studies on different ethnic groups living in the same area, there has been considerable epidemiological research addressing ethnicity in a different manner, i.e. migration. Many studies on migrant populations have been performed over the years leading to the classical dogma stating that migration from a high- to a low-risk area is associated with a risk reduction, while migration in the opposite direction is not associated with substantial change in risk (209). More contemporary studies have nuanced this picture, especially regarding certain groups of immigrants from non-western countries (210-212). Smestad et al. in Oslo, Norway showed a lower prevalence among immigrants from the Middle East than in the reference group (native Norwegians and immigrants from Western countries) but not significantly lower when adjusting for the duration of the residence (212). In a survey from 2010, Ahlgren et al. found that the MS risk in Gothenburg, Sweden was higher among Iranian immigrants than in the general population in Gothenburg as well as higher than that in the country of origin when comparing with available data from Isfahan, Iran (210). A few years later, a nationwide prevalence study from the same group observed lower MS prevalence among migrants from China, India, Iran, and Romania when comparing with the general population in Sweden, although higher than in their country of origin (211).

In this context, age at migration has traditionally been considered a factor that may influence the risk of MS, with the age of 15 being a critical cut-off (213-215). According to this theory, there is a sensitive period during childhood or adolescence when a person is particularly susceptible to environmental factors. However, in a recent and large population-based cohort study conducted in Ontario, Canada, Rotstein et al. found a gradually decreasing risk of MS with increasing age at migration, with no evidence of the presumed cut-off age. In addition, they showed that the risk of MS increased with the duration of time in Canada (216).

MS epidemiology in Sweden

Several epidemiological surveys have been conducted in Sweden over the last century, including Sällström´s dissertation from 1942, which showed a nationwide prevalence of 21.2/100,000 based on data from 87 hospitals in the country collected over a ten-year period. The results led Sällström to raise the question of variation in the geographical distribution of the disease (217). Sällström´s observations, together with other MS studies in Sweden were later included in Kurtzke´s work on the epidemiology of MS in Scandinavia and his theory about the "MS Fennoscandian focus" with Scandinavia being a high-risk region (218, 219). These contributions have been followed by other studies focusing on the distribution of MS in Gothenburg, Värmland and Västerbotten (220-225) and finally, more recent nationwide surveys (226, 227), all confirming the view of Sweden as a high-risk area. Details from selected incidence and/or prevalence studies in Sweden in the last decades are provided in Table 1.

Table 1. Most recent incidence and prevalence studies in Sweden (based on published literature).

Area (ref)	Incidence (CI)	Time period	Prevalence (CI)	Year	Based on
Värmland County (224)	6.5 (5.1– 7.8) 6.4 (5.1 – 7.7)	1991-1995 1996-2000	170.1 (154.5 – 185.5)	2002	Onset
Västerbotten County (223)	5.2 (4.4 – 6.2)	1988-1997	154 (139 – 170)	1997	Onset
Västerbotten County (225)	6 (5.2 – 6.9)	1998-2010	215 (198 – 233)	2010	Onset
Nationwide (226,227)	10.2	2001-2008	188.9 (186.1 – 191.7)	2008	Diagnosis

CI: 95 % confidence interval, ref: Reference number

Regarding MS in immigrant populations, there is only one other survey in addition to the two previously mentioned studies by Ahlgren et al. It is a nationwide incident study among first- and second-generation immigrants in Sweden in which the authors found a lower risk of incident MS among all immigrants combined, and a higher risk among first-generation women from Iran (228).

To date, there are no studies about the pattern of disability in immigrants with MS in Sweden.

Immigration to Sweden

Sweden has a long history of immigration, stretching back to the Viking Age. In the 20th century many migrants from Germany and other Baltic countries came here because of the Second World War. They were followed in the 1950s and 1960s by waves of labour immigrants, mainly from southern Europe and Finland. Refugees and asylum seekers comprised the next waves of immigrants, reflecting ongoing worldwide conflicts, with migrants from Chile and the Middle East dominating in the 1970s and 1980s, and former Yugoslavia in the 1990s. In the first decade of the 21st century, Sweden joined the Schengen co-operation, favouring labour immigration from other European Union countries. There was another wave from the Middle East following the 2003 invasion of Iraq (229).

The City of Malmö

The municipality of Malmö, also known as the City of Malmö, is a Swedish municipality located in the county of Skåne in the southwest of Sweden at 55°36′ N latitude (230) (Figure 6-7). The population of Malmö has been growing since the late 18th century except during a period of significant recession 1970–84. Malmö is the third largest city in Sweden and since the early 20th century it has experienced substantial changes, from being a port city with shipbuilding and textile industry to suffering severe economic decline in the 1970s due to the collapse of both industries, exacerbated in the 1990s by the financial crisis in Sweden (231).

Figure 6. Skåne County, Sweden (https://www.britannica.com/)

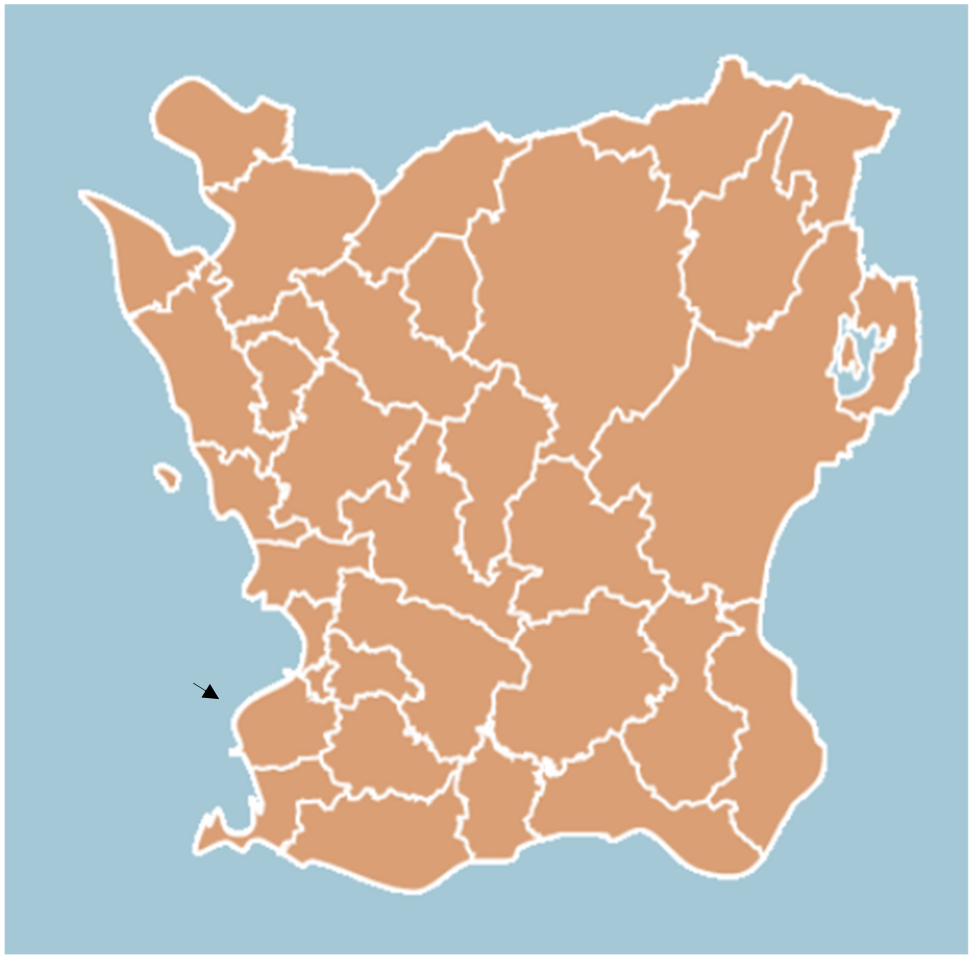

Figure 7. Malmö municipality (arrow) in the County of Skåne (adapted from https://Regionfakta.com).

Malmö is quite often depicted as being highly segregated, with social tensions, violence, poverty, and high rates of unemployment. However, in the last decades, it has emerged as a rapidly developing city, with major changes such as the construction of the Oresund Bridge to Copenhagen, the establishment of the Malmö college (now university) and the ongoing transformation of former industrial areas into areas with environmentally sustainable housing and commerce (231, 232). Malmö has a young population, with 56% under the age of 40 in 2010. The proportion of foreign-born persons is higher than the national average (30% and15% respectively in 2010) (Figure 8-9).

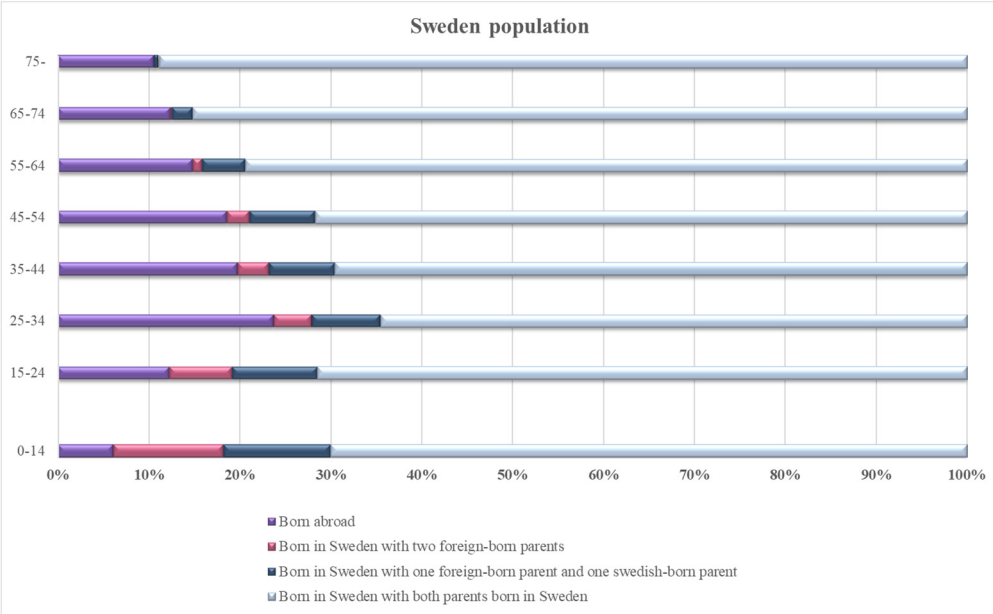

Figure 8. Population in Sweden in 2010 according to background and age (based on data from SCB).

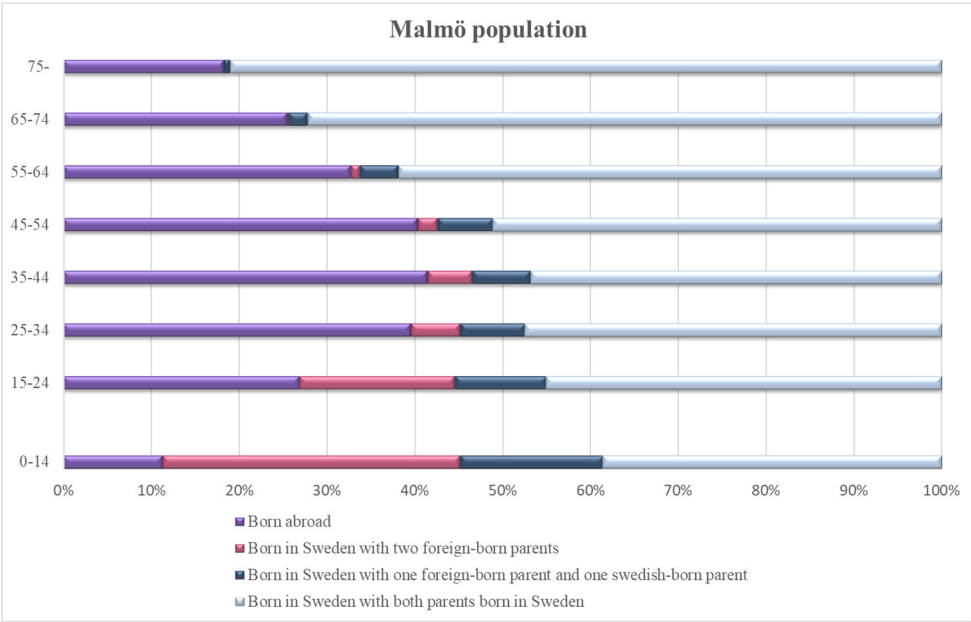

Figure 9. Population in Malmö in 2010 according to background and age (based on data from SCB).

MS epidemiology in Spain

Spain also has a long tradition of epidemiological studies (233-243) but no nationwide MS study has been performed so far. Details from selected incidence and/or prevalence studies in Spain in the last decades are provided in Table 2. In contrast to Sweden, Spain is considered a medium risk region. However, a couple of recent studies from Galicia, in the northwest of the country, have shown considerably higher prevalence figures than expected (242, 243). While case ascertainment in the study from Santiago de Compostela (242) included multiple sources and the validation of the diagnosis by a neurologist, the survey from Ourense (243) was based on primary health care files with no confirmation of the diagnosis by a neurologist.

The Alt Empordà

The Alt Empordà is a county located in the province of Girona, in the northeast of Catalonia at 42° 17′ N latitude (244) (Figure 10-11). The population in 2010 and 2020 was 138,351 and 140,429 respectively in an area of 1,357.4 km^2 (245). Agriculture and fishing have traditionally been the main source of economy in the Alt Empordà, with tourism currently being the major one (246).

Table 2. Most recent incidence and prevalence studies in Spain (based on published literature).

Area (ref)	Incidence (CI)	Time period	Prevalence (CI)	Year	Based on
Santiago de Compostela (233)	5.3 (3.2 –7.5)	1998–2003	78.7 (60.4 – 97)	2003	Diagnosis
Málaga province (234)			125 (102 – 169.5)	2008	Diagnosis
Osona (235)			79.9 (66.3 – 95.6)	2008	Diagnosis
Girona province (238)	3.6 (2.4 – 5.3)	2009			Onset
Murcia (239)			71.9 (60 – 85)	2010	Diagnosis
La Rioja (236)	3.5 (2.8 – 4.2)	2001–2011	65 (56 – 74)	2011	Diagnosis
Seville (237)	4.6 (4.1 – 5.1)	1991–2011	90.2 (75.6 – 104.8)	2011	Diagnosis
Ourense (243)	7.9 (na)	2002–2016	184 (158.5 – 210.5)	2016	Diagnosis
Lanzarote (241)	2.5 (1.1 – 3.8)	2008–2015	50 (44.4 – 56.2)	2015	Onset (I) Diagnosis (P)
Santiago de Compostela (242)	8 (6 – 10)	2010–2015	154 (127 – 176)	2015	Diagnosis
San Vicente del Raspeig (240)	5 (3.6 – 7.1)	2005–2017	111.9 (87.7 – 142.9)	2018	Onset (I) Diagnosis (P)

CI: 95% confidence interval, Ref: reference number, na: not available

Figure 10. Map of Catalonia in Spain and the world (https://www.britannica.com/)

Figure 11. The Alt Empordà (dark blue) in Catalonia (https://www.idescat.cat)

Rationale

A complex interplay between genetic and environmental factors is currently considered to be the underlying cause of MS. There is also evidence for adolescence being a more vulnerable period, while the clinical onset of the disease usually occurs several years later (21). Vitamin D is one of the environmental factors with robust evidence of being a risk factor for the development of MS. When dealing with potential risk factors, it is imperative to limit the risk of reverse causation, i.e. that the outcome causes the exposure. Nested case-control studies based on biobank data such as the one in this thesis provide a good opportunity to minimize that risk.

MS has negative effects on health-, social-, and work-related issues for pwMS and their families, leading to significant socioeconomic burden. Estimates of incidence and prevalence are important for planning in the healthcare system. Moreover, acknowledging potential differences in the pattern of disability in certain groups and/or changes in the epidemiology of the disease over time is crucial for adequate allocation of resources.

Aim of the thesis

The overall aim of the thesis is to gain insight into the epidemiology of MS in the City of Malmö, and in the county of the Alt Empordà, Catalonia, and to advance knowledge regarding vitamin D as one of the putative environmental factors in the etiology of MS.

The specific aims are:

Paper I

To investigate whether high levels of 25-hydroxyvitamin D (25 (OH)D3) have a protective effect against MS and if this putative role is more pronounced among young individuals.

Paper II

To estimate the incidence (2001–2010) and the prevalence (2010) of MS in the City of Malmö.

Paper III

To estimate the prevalence of MS among native Swedes and immigrants in 2010 in the City of Malmö.

To investigate whether there were differences between native Swedes and immigrants in the City of Malmö regarding the prevalence and the clinical characteristics of the disease, including the clinical spectrum of the first attack, clinical course, gender, time to diagnosis, disability progression and access to disease-modifying therapies.

Paper IV

To estimate the incidence (2001–2010 and 2011–2020) and the prevalence (2010 and 2020) of MS in the Alt Empordà, northeast of Catalonia, Spain.

Methods

Paper I

The first study is a nested case-control study, part of a larger national project on risk factors for multiple sclerosis, "Risk factors for MS with focus on glandular fever", led by one of my co-supervisors, Peter Sundström. The project is primarily based on biobank data, the Swedish MS registry, and an MS/possible MS local database.

Case retrieval

We used three sources to identify study subjects:

Biobanks

A biobank is a collection of human biological material collected mainly within routine healthcare. In Sweden, most of the samples belong to biobanks in the administrative "regions" but there are also biobanks available at the universities, at the Public Health Agency of Sweden (PHAS) and in private management. The stored samples can be used within the healthcare system for many purposes such as care and treatment, education, training, and product manufacturing, but also for research (247). Common for the samples in the present study is that they were scraps from analyses performed in daily routine practice in microbiological laboratories at the university hospitals in Umeå, Örebro, Linköping, Gothenburg, and Skåne, and at the Public Health Agency of Sweden (PHAS).

Swedish MS registry

The Swedish MS registry is a national quality registry which aims to promote and improve the quality of MS care and research. It started in 1996 and is part of the Swedish Neuro Registries since 2010. All centres with specialist neurological care are represented in the registry. The current coverage rate is 80% with around 18,000 active patients registered. The registry provides broad data on patients including demographics, diagnosis, treatment, disability and quality of life (248).

The Umeå MS/possible MS database

This database was established in 2009 within another project. Case identification was made through different sources including all the hospitals in the study area, the primary care centres in Västerbotten County, the National Patient Registry (NPR) and the Cause of Death Registry at the National Board of Health and Welfare, and the Swedish MS Registry. They searched the register for MS and nearby diagnosis codes according to the International Classification of Diseases (ICD). An MS diagnosis according to the criteria at the time was assessed by medical chart review (40).

Case retrieval in Paper I was performed through crosslinking between the Swedish MS registry and the microbiological biobanks, and between the local Umeå database and the Umeå biobank.

Case ascertainment

To be included in the study a sample in either of the biobanks, drawn before the age of 40 and before the first MS symptom, and later development of RRMS were mandatory. One control case matched for biobank and sex, together with sampling date and age with decreasing priority was selected. The diagnosis and year of onset were determined by medical chart review when registry data were incomplete. Further details can be found in Table 3.

Table 3. Sample characteristics from cases and controls stratified by biobank (reproduced from "Environmental risk factors for the occurrence of multiple sclerosis" (249) with permission of the author)

Biobank	Years of sample collection	Plasma or serum	N, (%)	Proportion of samples from men/women (%)	Long-term storage temperature
Umeå	1976–2007	Both	204 (15)	21/79	- 20 °C
PHAS	1972–2001	Both	278 (21)	22/78	- 20 °C
Örebro	1994–2008	Serum	58 (4)	0/100	- 20 °C
Gothenburg	1995–2009	Serum	94 (7)	11/89	- 20 °C
Skåne	1977–2007	Both	628 (47)	15/85	- 20 °C
Linköping	1993–2009	Both	78 (6)	10/90	- 20 °C

Laboratory methods

Concentrations of 25(OH) D2 and 25(OH) D3 were measured using LC-MS/MS with a Shimadzu Nexera LC system (Shimadzu CorporatioSerumn, Kyoto, Japan) combined to a Sciex QTrap 5500 MS (Sciex, Framingham, MA, USA).

Statistics

All statistical analyses were performed using IBM SPSS Statistics version 23 (IBM Corporation, New York, NY, USA). All analyses were performed in the whole group and in subgroups stratified by age at sampling (<20, 20–29 and 30–39 years). To increase power in the smaller groups, if a case and a control in a given set were on different sides of an age cut-off, they were allocated to either the youngest or the oldest group containing a case or control. The distribution of vitamin D levels in the controls was used to model vitamin D concentration as quintiles in each biobank separately. In addition, a pooled analysis was performed including all the individuals and their quintiles assignments. The Mann-Whitney test was used to analyse continuous variables. Conditional logistic regression was used to calculate OR and P for trend over quintiles.

Papers II and III

The study area

All studies in Paper II and Paper III are observational studies based on the City of Malmö. The total population was 298,963 on prevalence day, December 31, 2010.

The neurological healthcare map in Malmö and the surrounding area has changed significantly in the last decades. A department of neurology was established in 1984 at Malmö University Hospital (referred to as Malmö General Hospital during the 20th century), staffed by neurologists trained at Lund University Hospital, which was the only centre with neurological care in Skåne until then. In 2010, Skåne University Hospital was established by merging the two centres as well as a rehabilitation hospital (Orup Hospital), 50 km from Malmö. In addition, there is a hospital in the municipality of Trelleborg, 30 km south of Malmö, with neurology care since 1999. Finally, there have been a few private neurologists in the region, with the neurologist based in Malmö mainly focused on movement disorders. Thus, most MS care in the study area is in the public specialized health service.

Case definition

Paper II

Onset symptoms were defined according to a list of symptoms developed by Poser in the 90s and slightly modified, i.e. "transverse myelitis" was replaced by "myelitis" (250).

A prevalent case was defined as a person with onset symptoms before prevalence day, living in the study area on prevalence day (251) and fulfilling the 2010 McDonald diagnostic criteria (140) at the time for case ascertainment while an incident case was defined as a person with onset symptom during the incidence period while living in the study area (251) and fulfilling the above mentioned diagnostic criteria at the time for case ascertainment.

Paper III

The same definition for onset symptoms and prevalent case applied.

The country background was assigned according to the individual´s country of birth, and the definitions were mainly based on those from Statistics Sweden (Table 4) (252). Further details about the classification in Paper III are shown in Table 5.

Table 4. Overview of terms used by Statistics Sweden and in Paper III

Statistics Sweden		**Paper III**
Born in Sweden with both parents born in Sweden.	Swedish background	Native Swedish
Born in Sweden with one parent born in Sweden and one foreign born parent.	Swedish background	Native Swedish
Born abroad with two foreign born parents.	Foreign background	First-generation immigrant
Born in Sweden with two foreign born parents.	Foreign background	Second-generation immigrant

When information about the parents is missing, the same background as their offspring is assumed.

Table 5. Classification according to country background

Country background				
Native Swedish	Born in Sweden with at least one parent born in Sweden			
Immigrant	First generation	Born abroad to two foreign-born parents	Western	Europe, North America, Oceania
			Non-Western	Asia, Africa, the Middle East, South- and Central America
	Second generation	Born in Sweden with two foreign born parents		
Scandinavian	Native Swedish + born in Denmark, Iceland, or Norway			

People born in Denmark, Iceland or Norway were considered closely related to Sweden and included in the native Swedish group.
Middle East included Lebanon, Turkey, Bahrain, Egypt, Kuwait, Syria, Israel, the Palestinian territory, Qatar, Yemen, Iraq, Iran, Jordan, Saudi Arabia, and the United Arab Emirates.

Case retrieval

Paper II

Two registers at the National Board of Health and Welfare (the Swedish NPR and the National Cause of Death register) and the Swedish MS register were used as sources. In addition, cases identified by the author in clinical practice between 2016 and 2019 were included.

The NPR comprises data on all inpatient care since 1987 and outpatient specialist care since 2001. An overview of data available at the NPR is shown in Table 6. The requested data included all individuals attending public or private care diagnosed with MS and/or nearby diagnosis according to ICD 8, 9 and 10 until 2013. The geographical data and selected diagnosis in the search are shown in Table 7.

The Swedish MS register was searched for individuals attending the neurological department at Skåne University Hospital in Malmö.

The National Cause of Death register comprises data on all deaths of people registered in Sweden and it has been digitised since 1961 (253). We requested data on individuals with MS or inflammatory disease of the CNS as the underlying or contributory cause of death who had died in Malmö in the period 2001–2010.

Paper III

For Paper III, we used all pwMS living in Malmö on prevalence day, identified in the previous study (Paper II).

Table 6. Information available in the National Patient Register

Patient data	Personal identity number	
	Sex	
	Age	
	Place of residence	
Geographical data	County council	
	Hospital/clinic	
	Department	
Administrative data	Inpatient	Date of admission and discharge
		Length of stay
		Admitted from
		Discharged to
	Outpatient	Date of admission and discharge
Medical data	Main diagnosis	
	Secondary diagnosis	
	Procedures	
	External cause of injury or poisoning	

The diagnosis is registered according to the ICD.

Table 7. Geographical data and diagnosis requested to the NPR

Geographical data	Public	Malmö University Hospital Lund University Hospital Orup Hospital Trelleborg Hospital Skåne University Hospital
	Private	Malmö Lund Helsingborg
ICD codes	MS	
	Demyelinating disorders in CNS	
	Optic neuritis	
	Spastic paraplegia	
	Myelopathy	
	Ataxia	
	Spinocerebellar disease	
	Myelitis	

Case ascertainment

Case ascertainment was based on medical chart review and the Swedish Total Population register (TPR).

Medical chart review included assessment of the clinical history, neuroradiological examinations (i.e. MRI), CSF analysis and other relevant laboratory tests. The 2010 McDonald criteria applied. The assessment was performed by the author between October 2014 and February 2021, using mainly digital medical records but also paper medical records in the regional Archive in Lund. Originally, there was the possibility to contact individuals for a follow-up interview and/or examination if it was considered necessary to confirm or refute a diagnosis. However, despite approval from the local ethic committee, the national Board of Health and Welfare did not allow this intervention.

In both Paper II and Paper III, the assessment included year of onset, sex, and phenotype. In addition, in Paper III, the assessment comprised year of diagnosis, year of second relapse if appropriate, onset symptoms, MRI (dissemination in time, dissemination in place), oligoclonal bands in the CSF, EDSS, MSSS and disease-modifying therapy. In addition to the previously mentioned Poser`s list of onset symptoms, symptoms were further classified according to anatomical regions: optic nerve, cerebral hemispheres, brainstem/cerebellum, spinal cord, and unclear. Since the EDSS had been performed between 1980 and 2018, the calculation of MSSS was performed only in pwMS with EDSS registered between 2005 and 2015 (n=231). Two-thirds of the EDSS had been performed by an MS specialist, of which 40% by the author. When disease duration was longer than 30 years (n=61), the MSSS was calculated based on 30 years. When year of onset was not available (n=5), the MSSS was calculated based on the year of diagnosis instead.

In 1967, the local population registers in Sweden were digitised, and Statistics Sweden established the TPR. The TPR includes data on date and place of birth, sex, place of residence, country of birth of the parents, immigration, emigration, and death. Since 1947, every person who has resided in Sweden on a permanent basis has been assigned a personal identification number. This number is the unique identifier in the TPR. Since 2000, a coordination number is assigned to individuals who are not registered in the TPR (mainly those staying for <1 year). These individuals are also not registered in the health care registers (254).

In Paper II, the TPR provided information on the place of residence at onset and at prevalence date for each individual. In Paper III, the TPR provided data on country of birth and time of immigration and/or emigration for the MS prevalent population, together with country background, sex, and age for the total Malmö population.

Statistics

All statistical analyses were performed using IBM SPSS Statistics version 25 (IBM Corporation, New York, NY, USA) and Epitools (Sergeant, ESG, 2018. Epitools Epidemiological Calculators. Ausvet.). A significance level of 5% was applied. The Poisson distribution and the Wilson`s score method was used for calculation of 95% CI for the incidence and the prevalence, respectively. In Paper II, incidence and prevalence were calculated stratified by age and sex while in Paper III, together with crude prevalence figures, the adjusted figures were calculated according to the 2013 European Standard population. All clinical characteristics were presented and analysed as proportions, medians, and interquartile range (IQR). A two-sample t-test and a Mann-Whitney test were used to analyse continuous variables. Pearson`s chi-square test or 2-samples Z-test were used to analyse most frequencies and the Fisher´s test in case of small samples (n<5). Multiple linear regression was used to analyse differences in MSSS, adjusted for sex, age at onset, and phenotype.

Paper IV

The study included in Paper IV is an observational study primarily based on data derived from the Figueres MS registry and population register data from the Alt Empordà, Catalonia. The total population on December 31, 2010 was 138,351 and 140,429 on December 31, 2020.

The Figueres MS registry

All neurological care in the Alt Empordà is referred to the Figueres Hospital, which is the largest in the county, with an established MS Unit since 2002. The healthcare system in the area also includes eight public Primary Care Centres, a public intermediate care centre and a private hospital. In addition, there is a referral hospital in the provincial capital.

The Figueres MS registry was established in 2008 at the Figueres Hospital in collaboration with the Primary Healthcare Centres and other neurologists in the province of Girona. Case identification was made through different sources including the electronic medical records system from the Primary Healthcare network in the Alt Empordà and from relevant departments at the Figueres Hospital (ophthalmology, urology, rehabilitation of medicine and neurology). The diagnosis of MS according to the criteria at that time was assessed by medical chart review. The registry has since then

prospectively registered individuals attending the neurological department at the Figueres Hospital presenting with symptoms suggestive of MS.

Case definition

The same definition for incidence and prevalence as in Paper II and Paper III applied (251), as well as the classification of the course of the disease (123). The 2010 McDonald diagnostic criteria applied (140).

Case retrieval

Case retrieval from the Figueres MS registry was performed in December 2022.

Case ascertainment

Case ascertainment was based on medical chart review and the Population and Housing Census at the Statistical Institute of Catalonia (245).

The medical chart review included assessment of the date of onset and diagnosis, phenotype, EDSS and DMT. The population register data included the place of residence for the year of disease onset and both prevalence dates.

Statistics

All statistical analyses were performed using IBM SPSS Statistics version 29 (IBM Corporation, New York, Ny, USA). A significant level of 5 % was applied. As in paper II, the Poisson distribution and the Wilson´s score method were used for calculation of 95% CI of the incidence and the prevalence, respectively. The clinical characteristics were presented and analysed as proportions, medians, and interquartile range (IQR).

Results

Paper I

A total of 670 case-control sets qualified for inclusion in the study. One set was excluded due to a disturbance in the internal standard, and four additional sets were excluded due to low sample volumes, leaving 665 case-control sets for the final analysis. Quantifiable levels of 25 (OH) D2 were available in 60 individuals (37 cases and 23 controls). Therefore, most analyses were performed using 25 (OH) D3. Sufficient levels of vitamin D (above 50 nmol/L) were present in 54.6 % of cases and 56.1% of controls. No differences in median 25 (OH) D3 were found between cases and controls (Table 8). However, being in the top quintile was significantly associated with a decreased risk of developing MS (OR 0.68, 95% confidence interval (CI) 0.50–0.93). Yet, when looking into the different age groups, the results yield no significant findings. In the total cohort, no trend over quintiles was found (Table 9).

Table 8. Characteristics of cases and controls (adapted from Paper I).

	n	Cases	n	Controls	*p*
Sex (M/F)	665	16.2/83.8 %	665	16.2/83.8 %	
Age at sampling, y	665	25 (21–29)	665	25 (21–29)	
Age at disease onset, y	665	33 (28–40)	n.a.		
Time from sampling until disease onset, y	665	8 (4–13)	n.a.		
Biobank – latitude					
Umeå – 63°N	102	15.3 %	102	15.3 %	
Vitamin D3		47 (37–61)		53 (39–68)	0.07
Samples collected between, y		1976–2007		1976–2007	
PHAS – n.a.	137	20.6 %	137	20.6 %	
Vitamin D3		59 (43–75)		56 (39–77)	0.64
Samples collected between, y		1972–2001		1972–2001	
Örebro – 59°N	29	4.3 %	29	4.3 %	
Vitamin D3		52 (41–63)		50 (36–70)	0.96
Samples collected between, y		1994–2008		1994–2008	
Göteborg – 57°N	47	7.1 %	47	7.1 %	
Vitamin D3		56 (41–65)		55 (44–67)	0.97
Samples collected between, y		1995–2009		1995–2009	
Skåne – 55°N	311	46.8 %	311	46.8 %	
Vitamin D3		52 (41–68)		52 (40–70)	0.93
Samples collected between, y		1977–2007		1977–2007	
Linköping – 58°N	39	5.9 %	39	5.9 %	
Vitamin D3		43 (30–57)		51 (32–61)	0.33
Samples collected between, y		1993–2009		1993–2009	
All subjects	665		665		
Vitamin D3		53 (40–67)		53 (39–70)	0.50
Age group <20	142		142		
Vitamin D3		49 (38–64)		51 (39–67)	0.47
Age group 20-29	374		374		
Vitamin D3		53 (41–67)		53 (39–71)	0.73
Age group 30-39	149		149		
Vitamin D3		55 (41–72)		56 (42–73)	0.83

M: Male, F: Female. Y: years.
PHAS: Public Health Agency of Sweden.
Median (25th–75th percentiles) for continuous variables and percent for proportions.
Vitamin D concentrations expressed as nmol/L.

Table 9. Associations of vitamin D_3 concentration and MS stratified by biobank and age (reprinted from Paper I).

	Vitamin D Categories	Cut-off nmol/L	Number of (%)		OR	95% CI
Biobank			Cases	Controls		
Umeå	Quintile 1–4	≥38, 48, 59	90 (88.2)	81 (79.4)	ref	
	Quintile 5	≥73	12 (11.8)	21 (20.6)	0.47	0.20–1.1
PHAS	Quintile 1–4	≥37, 50, 63	114 (83.2)	109 (79.6)	ref	
	Quintile 5	≥82	23 (16.8)	28 (20.4)	0.75	0.38–1.5
Örebro	Quintile 1–4	≥35, 48, 60	26 (89.7)	23 (79.3)	ref	
	Quintile 5	≥72	3 (10.3)	6 (20.7)	0.40	0.08–2.1
Gothenburg	Quintile 1–4	≥41, 50, 59	41 (87.2)	37 (78.7)	ref	
	Quintile 5	≥71	6 (12.8)	10 (21.3)	0.43	0.11–1.7
Skåne	Quintile 1–4	≥38, 47, 58	252 (81.0)	248 (79.7)	ref	
	Quintile 5	≥73	59 (19.0)	63 (20.3)	0.90	0.58–1.4
Linköping	Quintile 1–4	≥27, 42, 55	37 (94.9)	31 (79.5)	ref	
	Quintile 5	≥70	2 (5.1)	8 (20.5)	0.25	0.05–1.2
All	Quintile 1–4		560 (84.2)	529 (79.5)	ref	
	Quintile 5		105 (15.8)	136 (20.5)	0.68	0.50–0.93
All[a]	Quintile 1		134 (20.1)	133 (20.0)	ref	
	Quintile 2		142 (21.4)	133 (20.0)	1.1	0.77–1.5
	Quintile 3		131 (19.7)	130 (19.5)	0.99	0.71–1.4
	Quintile 4		153 (23.0)	133 (20.0)	1.1	0.78–1.6
	Quintile 5		105 (15.8)	136 (20.5)	0.72	0.49–1.1
Age group						
<20	Quintile 1–4		126 (88.7)	118 (83.1)	ref	
	Quintile 5		16 (11.3)	24 (16.9)	0.60	0.29–1.2
20-29	Quintile 1–4		313 (83.7)	297 (79.4)	ref	
	Quintile 5		61 (16.3)	77 (20.6)	0.70	0.46–1.1
30-39	Quintile 1–4		121 (81.2)	114 (76.5)	ref	
	Quintile 5		28 (18.8)	35 (23.5)	0.72	0.39–1.3

OR: odds ratio; CI: confidence interval; PHAS: Public Health Agency of Sweden.

[a]In the total cohort, *p* for trend over quintiles was 0.24.

Since the PHAS had higher 25 (OH) D3 levels than the other biobanks (cut-off for the top quintile at 82 nmol/L), a sensitivity analysis excluding the PHAS was performed. This analysis yielded significant results both when using the median cut-off for the other biobanks (72 nmol/L) and a higher one (100 nmol/L): OR= 0.69; CI: 0.49–0.97 and OR= 0.30; CI: 0.13–0.71, respectively.

Paper II

A total of 726 individuals fulfilling the 2010 McDonald diagnostic criteria were found. The NPR was the main source of identification (87.5 % of cases) (Figure 12).

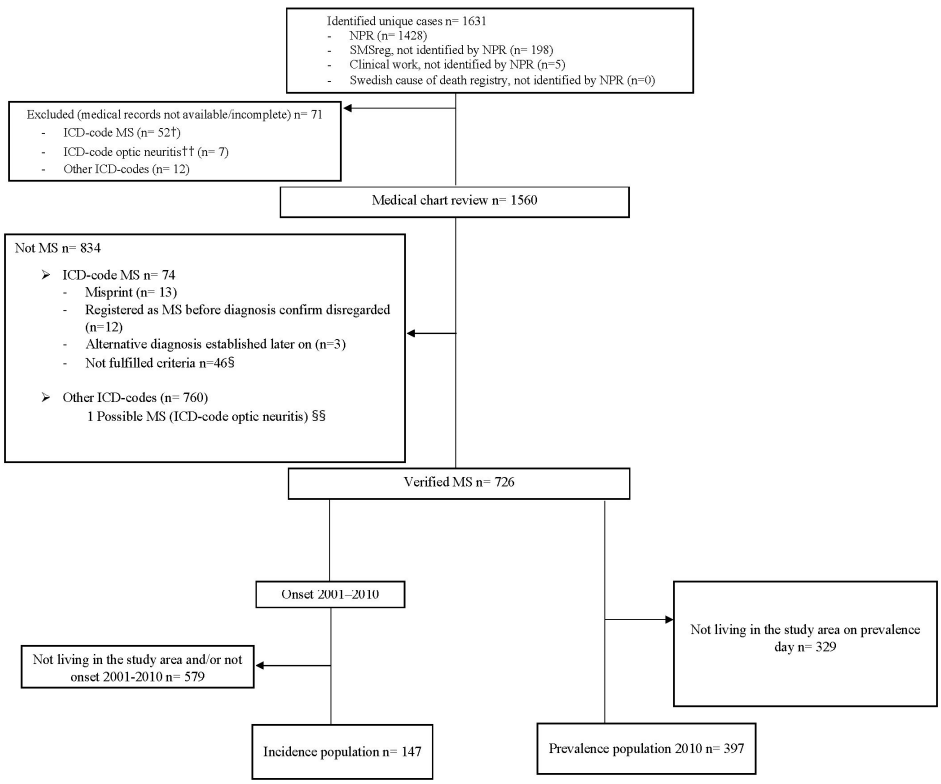

Figure 12. Flowchart of case retrieval and ascertainment
NPR: National patient register, MS: multiple sclerosis, SMSreg: Swedish MS registry, PPMS: primary progressive MS
† Twenty-five out of 52 resident in the study area on the prevalence day, 12 out of 52 with PPMS diagnos (all of them with onset before the incidence study period, 5 out of 12 living in the study area on prevalence day).
†† All with onset within the incidence study period and living in the study area at that time. All living in the study area on prevalence day.
§ Thirty out of 46 had Possible MS, 2 out of 30 with onset within the incidence study period and living in the study area at that time.
§§ Onset within the incidence study period and living in the study area at that time.

Incidence

We identified a total of 147 pwMS with onset in the period 2001–2010, giving a crude incidence rate of 5.3/100,000 (95% CI, 4.5 – 6.2). The age-specific incidence rates are displayed in Table 10. The vast majority (90.5%) had a relapsing onset, and the median age at disease onset was 31 (IQR 27–38) for both sexes.

Table 10. Annual incidence 2001–2010 of multiple sclerosis in the City of Malmö per 100,000 population by age and sex (reprinted from Paper II).

	Males		Females		Total	
Age group	**Number**	**Rate**	**Number**	**Rate**	**Number**	**Rate**
0–14	0	0	1	0.5	1	0.2
15–24	7	4.1	17	9.2	24	6.8
25–34	28	11.1	43	17.5	71	14.2
35–44	14	7.1	20	11.0	34	8.9
45–54	3	1.8	10	6.1	13	3.9
55–64	1	0.7	3	2	4	1.3
65–74	0	0	0	0	0	0
75–	0	0	0	0	0	0
Total	53	3.9	94	6.6	147	5.3
95 % CI		2.9 – 5		5.3 – 8		4.5 – 6.2

Prevalence

We identified a total of 397 individuals fulfilling the case definition, giving a crude onset adjusted prevalence of MS in the City of Malmö on December 31, 2010, of 133/100,000 (95% CI, 120 – 146). The stratified figures are displayed in Table 11. The demographic and clinical data are shown in Table 12. Most of the pwMS in the incident group (84%) were residents in the City of Malmö on prevalence day.

Table 11. Prevalence of multiple sclerosis on December 31, 2010, in the City of Malmö per 100,000 population by age and sex (reprinted from Paper II).

	Males		Females		Total	
Age group	**Number**	**Prevalence**	**Number**	**Prevalence**	**Number**	**Prevalence**
0–14	0	0	0	0	0	0
15–24	9	48	9	44	18	46
25–34	25	87	53	186	78	136
35–44	34	158	53	273	87	212
45–54	27	149	58	337	85	240
55–64	16	105	55	347	71	228
65–74	14	131	26	213	40	175
75-	5	58	13	86	18	76
Total	130	89	267	175	397	133
95 % CI		73 – 104		154 – 196		120 – 146

Table 12. Overview of clinical characteristics of multiple sclerosis in the prevalent MS population.

Females, n (%)	267 (67.3)
Age at prevalence day (years), median (IQR)	46 (35–59)
Age at onset (years), median (IQR)	30 (24–38.5)
Disease duration (years), median (IQR)	13 (5.5–24)
Clinical phenotype, n (%) **Relapsing**	235 (59.2)

Paper III

A total of 371 pwMS were eligible for the study (Figure 13).

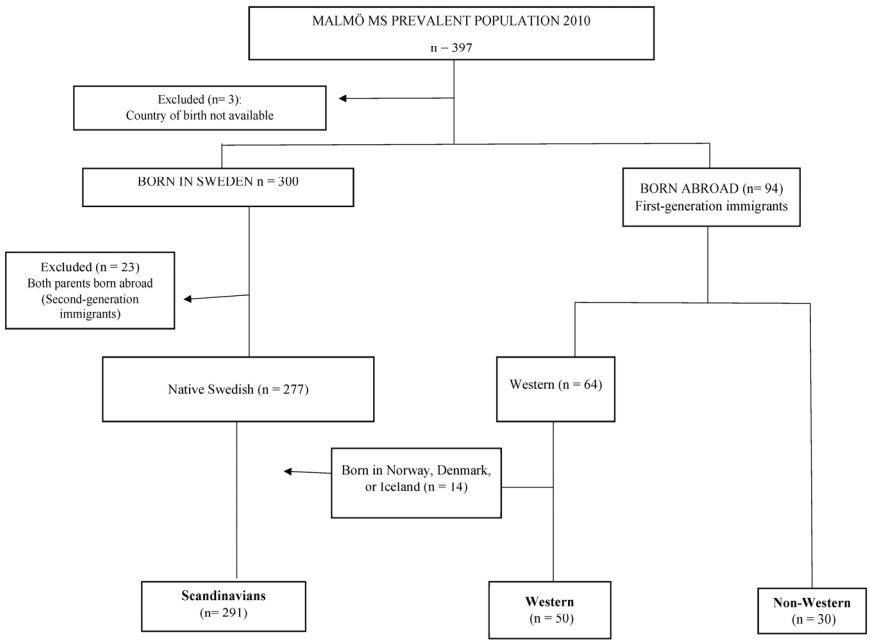

Figure 13. Flowchart of the distribution of the study population according to country background (reprinted from Paper IV).

Non-Western: Asia (n= 4), Africa (n= 3), Middle East (n=22) and Central and South America (n=1).

Middle East: Lebanon, Turkey, Bahrain, Egypt, Kuwait, Syria, Israel, the Palestinian territory, Qatar, Yemen, Iraq, Iran, Jordan, Saudi Arabia, and the United Arab Emirates.

Prevalence

We identified a total of 291 pwMS with Scandinavian background yielding a crude prevalence of 154/100,000 (95% CI, 137–173). As for first-generation immigrants, we identified 80 pwMS, of which 50 were of Western background and 30 of non-Western background. In the Western group, the majority (86%) came from Central or South Europe. Most (73%) pwMS in the non-Western group came from the Middle East, mainly Iran (n=10) or Iraq (n=6).

The crude prevalence among first-generation immigrants was lower compared to the Scandinavian population ($p<0.010$ for both crude and adjusted prevalence). The crude and adjusted figures are shown in Table 13. The stratified figures are displayed in Table 14.

Table 13. Crude and adjusted prevalence of multiple sclerosis in Scandinavians and immigrants on December 31, 2010, in the City of Malmö (reprinted from Paper III).

Prevalence	**Scandinavian (n=291)**	**First-generation immigrant (n= 80)**	**Western (n= 50)**	**Non-Western (n= 30)**	**p***	**p****	**p*****
Crude (95% CI)	154 (137 – 173)	100 (80 – 124)	123 (94 – 162)	76 (53 – 108)	< 0.010	0.14	< 0.010
Adjusted† (95% CI)	161 (144 – 180)	79 (62 – 101)	104 (77 – 140)	58 (39 – 87)	< 0.010	0.010	< 0.010

†: Adjusted to the 2013 European Standard Population; CI: Confidence Interval; p*: Scandinavian versus First-generation immigrants; p**: Scandinavian versus Western immigrants;
p***: Scandinavian versus non-Western immigrants

Table 14. Prevalence of multiple sclerosis on December 31, 2010, in the City of Malmö per 100,000 population by age, sex, and country background (reprinted from Paper III).

	Scandinavians				Western				Non-Western			
	Male		Female		Male		Female		Male		Female	
Age group	Number	Prevalence	Number	Prevalence	Number	Prevalence	Number	Prevalence	Number	Prevalence	Number	Prevalence
0–14	0	0	0	0	0	0	0	0	0	0	0	0
15–24	4	38	3	25	3	170	2	101	0	0	1	33
25–34	20	117	30	177	0	0	9	213	3	50	10	175
35–44	24	187	33	297	4	118	10	304	3	71	7	174
45–54	18	165	47	451	4	121	7	195	1	28	2	72
55–64	14	132	45	414	2	71	7	200	0	0	2	150
65–74	11	136	24	252	2	102	0	0	1	166	0	0
75–	5	69	13	102	0	0	0	0	0	0	0	0
Total	96	105	195	201	15	78	35	164	8	39	22	115
95 % CI	86 – 128		174 – 231		47 – 129		118 – 228		20 – 77		76 – 174	

Clinical characteristics

The demographic and clinical data are summarized in tables 15 and 16. Both immigrant groups were younger and had a shorter disease duration than the Scandinavian population at prevalence day. The age of onset was younger among non-Western immigrants compared to Scandinavians even when looking into those with onset after migration (median age 26, IQR 21–31, $p<0.010$). No diagnosis or treatment delay was identified when comparing Scandinavians with both Western and non-Western immigrants.

The distribution of onset symptoms among those with relapsing onset is shown in Table 17. Most pwMS had a monofocal onset and no statistically significant differences between the groups were found. Regarding anatomical regions, brainstem/cerebellar were the most common among non-Western immigrants (48%, $p=0.010$ when compared to Scandinavians) whereas the optic nerve was the most frequent region in the other groups. The second most common anatomical region in all groups was the spinal cord.

Non-Western immigrants displayed an increased disability progression compared to Scandinavians (Table 18).

Table 15. Clinical characteristics of multiple sclerosis in Scandinavians and immigrants (reprinted from Paper III).

	Scandinavian n = 291	Western n = 50	Non-Western n =30	p*	p**	p***
Females, n (%) †	195 (67)	35 (70)	22 (73)	0.68	0.48	
Age at prevalence day (years), median (IQR) ¶	49 (37 to 61)	42 (32 – 54)	36 (30 –43)	< 0.010	< 0.010	
Age at migration (years), median (IQR) ¶		21 (11 – 25)	12 (3 –24)			0.18
Time from migration to onset (years), median (IQR) ¶		10 (5 – 21)	12 (7 –16)			0.89
Migration before 15 yo n (%) †		11/37 (30)	10/18 (56)			0.06
Age at onset (years), median (IQR) ¶	31 (25 – 40)	28 (26 – 35)	27 (22 – 31)	0.49	< 0.010	
Clinical Phenotype, n (%) † Relapsing	169/291 (58)	32/50 (64)	16/30 (53)	0.43	0.62	
OCB-positive, n (%) ‡	218/231 (94)	37/39 (95)	24/24 (100)	1	0.62	
DIS MRI, n (%) §	236/271 (87)	41/48 (85)	28/30 (93)	0.75	0.32	
DIT MRI, n (%) §	191/271 (71)	37/48 (77)	24/30 (80)	0.35	0.27	
EDSS, median (IQR) ¶	3.3 (1 – 6.5)	3.5 (1 – 6.5)	4 (1 – 7.5)	0.86	0.59	
Disease duration (years), median (IQR) ¶	15 (6.4 – 27)	9.4 (2.9 –16.4)	9.9 (5.4 – 14)	< 0.010	0.010	
Time from onset to diagnosis (years), median (IQR) ¶	2.3 (0.5 – 7.5)	1.6 (0.3 –5.2)	1.8 (0.4 – 5.8)	0.14	0.28	
Time to CD, median (IQR) ¶	2.1 (1 – 6)	2.3 (1 – 7)	1.8 (0.5 – 4)	0.83	0.19	
Any DMT ever, n (%) †	142/289 (49)	27/50 (54)	21/30 (70)	0.53	0.03	
Time from diagnosis to DMT (years), median (IQR) ¶	0.4 (0.1 – 3.8)	0.3 (0.1 – 2.1)	0.4 (0 – 3.6)	0.56	0.43	
Time on first DMT (years), median (IQR) ¶	2.6 (0.9 – 5.4)	1.5 (0.8 –2.2)	1.3 (0.6 – 2.3)	0.05	0.010	
Current first-line DMT, n (%) †	72/289 (25)	12/50 (24)	13/30 (43)	0.89	0.030	
Current second-line DMT, n (%) ‡	20/289 (6.9)	4/50 (8)	1/30 (3.3)	0.77	0.71	
Second-line DMT as first choice, n (%) ‡	3/289 (1)	0/50 (0)	1/30 (3.3)	1	0.33	
Second-line DMT ever, n (%) ‡	25/289 (8.6)	4/50 (8)	2/30 (6.7)	1	1	

MS: multiple sclerosis; IQR: interquartile range; yo: years old; OCB: oligoclonal bands; DIS MRI: dissemination in space on magnetic resonance imaging according to 2010 McDonald´s criteria; DIT MRI: dissemination in time on magnetic resonance imaging according to 2010 McDonald´s criteria; EDSS: expanded disability status scale; CD: Clinically definite; DMT: disease modifying therapies including beta interferon, glatiramer acetate, natalizumab and other treatments (laquinimod, linomide, azathioprine, mitoxantrone); current first-line DMT: patients on first-line DMT (beta interferon or glatiramer acetate) at prevalence day; current second-line DMT: patients on second-line DMT (natalizumab) at prevalence day.

p*: Scandinavian versus Western immigrants; p**: Scandinavian versus non-Western immigrants; p***: Western versus non-Western immigrants.

†: Chi-square test; ‡: Fisher test; §: 2-samples Z-test; ¶: Mann-Whitney

EDSS: Individuals with EDSS performed between 2005 and 2015 (186/291 Scandinavian individuals, 26/50 Western immigrants and 19/30 non-Western immigrants).

Classification according to Lublin 2013 (relapsing vs progressive).

Age at onset: Data available for 284/291 Scandinavian individuals, 47/50 Western immigrants and all non-Western immigrants.

Age at migration, time from migration to onset and migration before 15 yo: data available for 48/50 Western immigrants and all non-Western patients. Data shown from individuals with onset after migration (37/48 Western immigrants and 18/30 non-Western immigrants).

OCB: Data available for 231/291 Scandinavian individuals, 39/50 Western immigrants and 24 non-Western immigrants

MRI available in 271/291 Scandinavian individuals, 48/50 Western immigrants and all non-Western immigrants

Disease duration: Data available for 285/291 Scandinavian individuals, 47/50 Western immigrants and all non-Western immigrants Time from onset to dx: Data available for 272/291 Scandinavian individuals, 44/50 Western immigrants and 29/30 non-Western immigrants

Time to CD: Data available for 228/259 Scandinavian individuals, 36/42 Western immigrants and 21/25 non-Western immigrants

DMT: data available for 289/291 Scandinavian individuals and all immigrants

Table 16. Female to male ratio by onset and country background.

	Sex ratio (female to male)		
Onset	**Scandinavians**	**Western**	**Non-Western**
Relapsing	2.4	3.2	3.2
Progressive	0.5	0.7	0.5

Table 17. Onset symptoms in pwMS with relapsing onset (reprinted from Paper III).

Poser onset symptoms n (%)	**Scandinavian N= 252**	**Western N = 41**	**Non-Western N= 25**
Unilateral optic retrobulbar neuritis	68 (27)	13 (31.7)	3 (12)
Oscillopsia	1 (0.4)	----	----
True binocular diplopia	32 (12.7)	2 (4.8)	5 (20)
Acute unilateral diminution of hearing (< 40 yo)	2 (0.8)	----	----
Transient acute non-positional vertigo (< 40 yo)	27 (10.8)	1 (2.4)	3 (12)
Myelitis	63 (25)	7 (17.1)	5 (20)
Lhermitte symptom	3 (1.2)	1 (2.4)	----
Unilateral dysmetria/intention tremor/incoordination	4 (1.6)	1 (2.4)	----
Sensory useless hand syndrome	1 (0,4)	----	----
Transient weakness/paresthesia of one limb	78 (30.6)	18 (43.9)	11 (44)
Unilateral facial palsy	3 (1.2)	----	2 (8)
Transient hemiparesis (< 40 yo)	2 (0.8)	----	----

Scandinavian group: 169/259 with RRMS, 90/259 with SPMS, 7 individuals with missing data

Western immigrants: 32/42 with RRMS, 10/42 with SPMS, 1 individual with missing data.

Non-Western immigrants: 16/25 with RRMS, 9/25 with SPMS.

Yo: years old.

Onset symptoms according to a list of onset symptoms by Poser (1995). The original list was slightly modified (“myelitis” instead of “transverse myelitis”).

Onset symptoms with no cases at all: Acquired monocular colour blindness, trigeminus neuralgia (< 40 yo), hemifacial spasm (< 40 yo), transient scanning speech, gait ataxia, transient painless urinary retention (< 40 yo), transient painless urinary urgency/incontinence in men (< 40 yo), organic erectile dysfunction and painful tonic seizure.

Table 18. Multiple Sclerosis Severity Score: results from the multiple linear regression analysis (reprinted from Paper III).

	Unadjusted			Adjusted†		
	β	**95% CI**	**p**	**β**	**95% CI**	**p**
Scandinavians	0 (ref)			0 (ref)		
Western immigrants	0.38	-1.1 – 1.8	0.60	0.22	-1.2 – 1.6	0.75
Non-Western immigrants	0.98	-0.67 – 2.6	0.24	1.7	0.18 – 3.3	0.030

† Covariates: sex, age at onset, phenotype (relapsing/progressive onset).

Paper IV

Incidence

We identified a total of 71 pwMS with onset between 2001 and 2020, giving a crude incidence rate of 3.5/100,000 (95% CI 2.6–4.6) during 2001–2010 and 1.6/100,000 (95% CI 1.1–2.5) during 2011-2020. The age-specific incidence rates are shown in tables 19-20. The majority had a relapsing onset (91.7 and 87% respectively). Median age at onset was similar in both periods: 36 (IQR 21) in 2001–2010 and 37 (IQR 13) in 2011–2010. Diagnosis delay was also similar in both decades: 12.4 months (IQR 39) and 11.1 (IQR 20) respectively.

Table 19. Annual incidence of multiple sclerosis 2001–2010 in the Alt Empordà per 100,000 population by age.

Age group	Number	Rate
0–15	0	0
16–24	3	2.4
25–44	24	5.3
45–64	18	5.2
65–	3	1.3
Total	48	3.5
95% CI		2.6 – 4.6

CI: Confidence interval

Table 20. Annual incidence of multiple sclerosis 2011–2020 in the Alt Empordà per 100,000 population by age.

Age group	Number	Rate
0–15	1	0.4
16–24	2	1.6
25–44	13	3.3
45–64	7	1.7
65–	0	0
Total	23	1.6
95 % CI		1.1 – 2.5

CI: Confidence interval

Prevalence

We identified a total of 103 individuals fulfilling the case definition in 2010 and 114 in 2020, giving a crude onset adjusted prevalence of MS in the Alt Empordà on December 31, 2010, of 75/100,000 (95% CI 61– 90) and 81/100,000 (95% CI 68–98) on December 31, 2020. The stratified figures are presented in Table 21 and 22.

Table 21. Prevalence of multiple sclerosis on December 31, 2010, in the Alt Empordà per 100,000 population by age.

Age group	Number	Prevalence
0–15	0	0
16–24	3	24
25–44	37	82
45–64	54	156
65–	9	40
Total	103	75
95% CI		61 – 90

CI: Confidence interval

Table 22. Prevalence of multiple sclerosis on December 31, 2020, in the Alt Empordà per 100,000 population by age.

Age group	Number	Prevalence
0–15	1	4
16–24	2	16
25–44	31	85
45–64	58	144
65–	22	82
Total	114	81
95% CI		68 – 98

CI: Confidence interval

Ethical considerations

Paper I

The study was approved by the local review board in Umeå (Dnr 2011-198-31 M), the regional steering committee for each biobank, and the Swedish MS Society research board. Handling of sensitive data was approved by the Swedish Authority for Privacy Protection. Written informed consent was not required. An information letter was sent to potential study participants outlining the project and including information about how to opt out.

The collection of human biological material is not a new phenomenon in Sweden, i.e. pathological samples have been stored within the healthcare system since the 1920s. Today, there are about 450 biobanks with more than 150 million stored samples, an invaluable resource for both healthcare and research. This entails great responsibility for the healthcare system and the research community to ensure that ethical principles are applied. The first biobank law was enacted in 2003 with one of the aims being to protect the integrity of the individuals who either already had stored samples or were potential donors. According to this law, "a sample stored in a biobank may be used for a purpose other than covered by previous information and consent only if the person who provided the consent has been informed of and consented with the new purpose" (255). Concerning the biobanks used in this study, some of them were established within prospective population-based studies (The Malmö Diet and Cancer study, The Malmö Preventive Project and The Västerbotten project) whereas others (Malmö Microbiological biobank and the biobank at the Public Health Agency of Sweden) were established along with routine healthcare. Informed consent was required to participate in the above studies, but it is uncertain whether individuals leaving blood samples many decades ago were actually informed either about long-term storage or the potential research use in the future. This raised an important ethical issue in which the potential benefits of this kind of research for the general population (but probably not for current pwMS) on one hand, must be weighed against the potential breach of privacy for the individual on the other. In this context, the information letter with the option to opt-out was considered a reasonable compromise and only 10 individuals (5 cases and 5 controls) decided not to participate.

Paper II–III

Both studies were approved by the local ethics committee of Lund University, Lund (Dnr 2013/890) and the National Board of Health and Welfare (Dnr 10576/2014).

The risk for individuals to be harmed or wronged due to the medical chart review was judged as negligible for most of those with an established MS diagnosis. Still, the risk of psychological harm for individuals registered in the NPR with other ICD codes had to be considered. These two assumptions were the rationale behind informed consent not being required. However, an advertisement in a regional newspaper was published with information about the project including the option to opt-out. The information was written in general terms, i.e. using the phrase "inflammatory diseases of the nervous system" instead of multiple sclerosis. This was a carefully selected strategy based on the risks, but it should be acknowledged that an announcement in daily press does not ensure that the information reaches the whole population. No individuals declined to participate.

Paper III deserves certain ethical consideration, not least given the current debate in Sweden about immigrants, which was not as present when the project started. According to the 19th principle in the Declaration of Helsinki (World Medical Association, 2013), "Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm. All vulnerable groups and individuals should receive specifically considered protection" (256). Since migration is one of the topics indirectly addressed in Paper III, concerns about processing this kind of sensitive data must be raised. All published data is anonymised, but we were not allowed to do prevalence estimations for individual countries in order to respect the immigrant population´s right to privacy. Moreover, the 20th principle states that "Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of the group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research." (256). Considering that estimates of disease prevalence are important for forward planning in the healthcare system and that potential differences in disability progression in certain groups can lead to a revision of current care, it was considered plausible to perform the study even though there is some potential for stigmatization.

Paper IV

The local MS registry of the Alt Empordà was launched in 2008 after approval from the local ethics committee of Doctor Josep Trueta University Hospital (Dnr 2008/013). The main objectives at that time were to perform a field survey and establish the incidence in the years 2001–2006, and to provide a base for further follow-up epidemiological studies. All individuals are informed about the register, and that data may be used for continuous disease monitoring and research purposes. Informed written consent is required to be included in the register. Consent is not required for specific research projects. Participation in this register implies no direct risk for the pwMS.

Discussion

Methodological considerations

All the studies in this thesis are observational and we have attempted to comply with the STROBE statement (201).

Paper I

One of the advantages of a cohort study is the possibility to register exposure information at baseline and then subsequently follow-up for the disease incidence (outcome). Another advantage, provided that the study is prospective (i.e. the exposure information is collected at the beginning of the follow-up), is that the risk for recall or reporting bias decreases. However, considering that MS is not that common, this approach is less cost-effective when studying environmental exposures as potential risk factors for MS.

In this context we found a nested case-control design to be more suitable in Paper I, given the invaluable resource that the biobanks represent.

Case retrieval

Selection bias occurs when there is a systematic difference between the features of the subjects included in a study and those who are not, with the consequence that the study will not be representative of the population it aims to describe. We cannot exclude the possibility that there have been such systematic differences between individuals with and without stored samples.

Another concern regarding the biobanks is that they had different catchment areas and demographic characteristics along with variations in the procedures used to handle and store the samples. The samples used in this study were not primarily intended to be stored for research purposes, and much information about the circumstances and the process itself is unfortunately lacking. We do know that all the biobanks share long-term storage temperature at -20°C and that most of the samples (except the PHAS)

were analysed for hepatitis or HIV screening, pregnancy screening and unspecified serological analysis (38%, 30% and 24%, respectively). In addition to samples collected for diagnostic microbiology the PHAS also contains samples related to surveillance studies linked to vaccination programs or pandemics, but it is more likely that the samples in our study were collected for diagnostic purposes (249).

Case ascertainment

Reverse causation refers to a situation when the outcome precedes and causes the exposure instead of the opposite. One of the inclusion criteria in the study was "to have a sample drawn before the first MS symptom", which reduces the possibility of reverse causation. However, we cannot exclude the presence of individuals with earlier neurological symptoms that had not been recorded. Moreover, in view of the current idea of a prodromal phase in MS (118) and a median of 8 years prior to the onset for the samples to be collected, reverse causation might be present.

Confounding occurs when an exposure-outcome association is obscured by the presence of other factors also affecting the outcome. Confounding can be prevented in the study design by using matching. In case-control studies, matching is a method used to achieve a similar distribution in the control group and the cases with respect to specific characteristics of interest (i.e. potential confounders). One of the benefits is a uniform number of cases and controls across the levels of the selected variables which leads to better statistical efficiency. In Paper I, the selected matched variables were biobank and sex, along with date of sampling and age at sampling with decreasing priority. The biobank matching was made with consideration given to the previously discussed heterogeneity among the different biobanks. As expected, median Vitamin D concentrations were lower during the winter compared to the summer (Figure 14). This seasonal variation was the rationale behind giving the date of sampling higher matching priority than age at sampling. Moreover, storage time is important with respect to old samples, even if vitamin D is considered quite stable under many conditions (257, 258). Mean difference between cases and controls for the date of sampling was 6 days. Hence, specific correction for seasonal variation was deemed not necessary. By using this strategy, we might have mitigated confounding. However, residual confounding may still been present since there are other factors, both recognized (i.e. sun exposure, obesity, smoking and ethnicity) and unrecognized that have not been addressed.

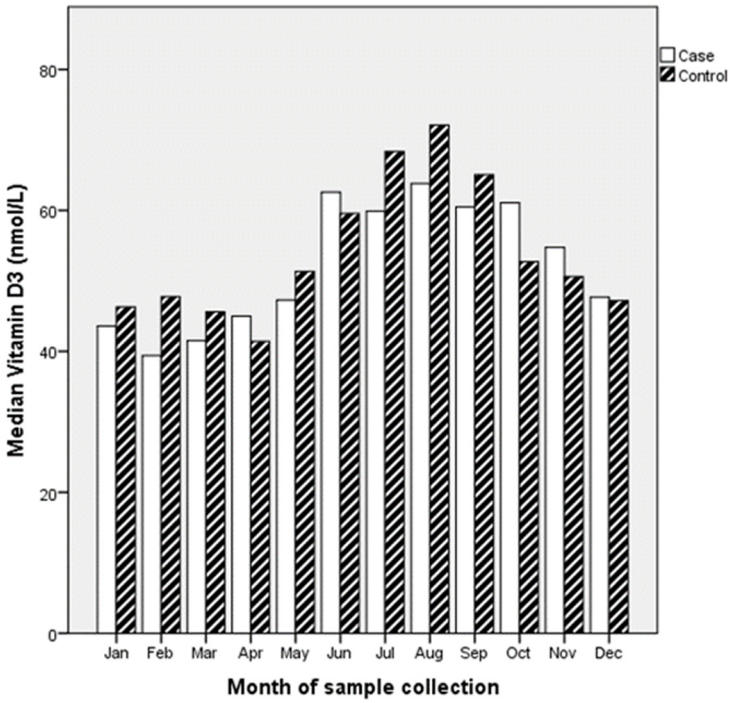

Figure 14. Median 25 (OH)D3 concentrations by month of sample collection. Data from 1 330 samples with 48% (n=633) collected in the winter months November–April (reproduced from "Environmental risk factors for the occurrence of multiple sclerosis" (249) with permission of the author).

The matching ratio was set to 1:1, mainly because the restricted availability to stored samples in the biobanks that tend to lessen the volume of the released samples. Although a reasonable policy from the biobanks, the drawback is that we only had a single baseline vitamin D measurement which may not reflect the long-term status.

Statistics

Serum concentrations of vitamin D differed between the biobanks, with the PHAS displaying the highest levels (12% more). To compensate for this disparity and for the potential variations in pre-analysis conditions between biobanks, the distribution of vitamin D levels in the controls was used to model vitamin D concentration as quintiles in each of the biobanks. The same quintiles assignments were used when performing the pooled analysis with all the individuals.

Sensitivity analyses are suitable when investigating the robustness of the obtained results. Thus, in view of the divergent vitamin D concentrations in the PHAS biobank,

we performed sensitivity analysis excluding samples from that biobank. The analysis was performed using the median for top quintile cut-offs for the remaining biobanks (72 nmol/L) as well as a higher one (100 nmol/L). Both procedures yielded significant findings (OR=0.69, 95% CI: 0.49–0.97 and OR=0.30, 95% CI: 0.13–0.71).

Paper II-IV

Study design

In Paper II–III, the study area was chosen in view of the lack of epidemiological studies in southwestern Sweden and with the intention of providing a base for further follow-up studies. The geographical location was judged particularly suitable considering the latitudinal gradient theory and the fact that previous surveys were conducted further north. Further, the demographic features of Malmö with potential differences between groups with different country background suggested the area as a natural study target.

In Paper IV, the base for further follow-up studies had already been established in 2008, so the design was specifically chosen with the intention of continuing the previous work in northeastern Catalonia while attempting to harmonize with the methodology in Paper II–III.

Case definition

Two different approaches can be used to measure incidence: cumulative incidence and incidence rate. The first, also known as "risk or incidence proportion", is a measure of the probability of an individual developing a certain disease during a specified time span. In turn, incidence rate is a measure of how fast the disease occurs in a population. In both cases, it is assumed that all the individuals are disease-free at the beginning of the study and the numerator is the number of new cases developing the disease during the observation time. However, while incidence proportion does not take into consideration dropouts (lost to follow-up), the key principle in incidence rate is that only the actual time at risk is considered. Time at risk for each subject is defined as the time from the beginning of the observation period until the development of the disease, lost to follow-up (i.e. moving out the study area) or death. Incidence rate is then usually expressed as annual incidence and displayed as "n cases per 100,000, i.e. n cases per 100,000 person-years" (199). In Paper II and IV, we used the incidence rate since it is a more solid measure when dealing with long-term studies.

Regarding prevalence, there are two options: point prevalence and period prevalence. The former is the proportion of individuals with a given disease on a particular date while period prevalence refers to the proportion of individuals with the disease at any time during the defined interval (200). In Paper II–IV we used point prevalence since it was deemed more suitable for addressing the research questions of this thesis.

Another issue of interest here is how to deal with the definition of "having a certain disease", i.e. whether to use the date of diagnosis or, as we actually did, the onset date (onset-adjusted prevalence) (259). Applying the date of diagnosis implies that there would be individuals with symptoms of the disease but not diagnosed yet. Thus, the onset-adjusted strategy is far more relevant from a biological perspective. As for incidence, diagnosis-based estimations imply risk for bias (under and/or overestimates rates). Since time to diagnosis can differ depending on many factors such as awareness of the disease or access to neurological expertise, an observation time is mandatory.

Embracing the onset-adjusted concept implies the need to define the onset. In contrast to the diagnostic criteria which have been updated on a regular basis, especially in the last two decades, the definition of onset has not drawn as much attention. The current criteria emphasize the importance of limiting the application of the criteria to individuals presenting with a typical CIS. The criteria specifically mention that "CIS presentations typically involve the optic nerve, brainstem/cerebellum, spinal cord, or cerebral hemispheres" (140). However, no detailed definition about these presentations per se is provided. The lack of guidelines was pointed out already in the 90s by Poser, who proposed a list med onset symptoms based on his own experience and consensus with other experts in the field (250). Yet, the list has remained quite unnoticed. Consequently, most epidemiological studies do not define this topic, hampering the comparability of different studies. In Sweden, Poser´s list was used in the first MS surveys from Västerbotten County although with a minor modification ("myelitis" applied instead of "transverse myelitis") (222, 223). Because there has been no tradition of MS epidemiological research in Malmö and our purpose was to create a base for further follow-up studies, it was important to perform this study using clear definitions on as many items as possible. Therefore, the same list (including the aforementioned modification) was used in Paper II. In addition, the STROBE statement supports this rationale.

In Paper IV, a predetermined onset definition was not used since the material was primarily derived from the Figueres MS registry which did not include a predefined list med onset symptoms. One approach that was considered when discussing the current project was to reassess all the medical charts using the Poser´s list. However, such reassessment had not been reviewed by the Ethical approval board and implied the need to initiate a new ethical revision which was deemed too time consuming.

One of the aims of this thesis was to investigate potential differences in the burden of the disease between the native population and immigrants. Following the previous argument, it was essential to further define those terms in Paper III. First, we classified the population according to Statistics Sweden, whose definitions were developed in 2002 in cooperation with the Swedish Integration Agency (252). These definitions are based on the country of birth of the individual and their parents but does not perform any other group allocation. In a second step, we classified first-generation immigrants according to previous studies in Northern Europe with similar aims (159, 212) to be able to make comparisons. An alternative would have been to request this information from the study population itself which was not feasible due to the study design.

Race and ethnicity are frequently recorded in medical research but how the research community approaches this issue has been questioned (260) and highlighted (261). One of the reasons is probably the lack of an exact definition of these designations. As for ethnicity, one definition could be "the fact of belonging to a particular ethnic group, i.e. a group of people who have a shared sense of identity because they have their own cultural background, traditions, history, language, etc" (262). Translation into a comprehensive checklist with different items is obviously difficult. In Paper III, the focus has been on the country background without the intention of assimilating country of birth and ethnicity even if to some extent, and especially when comparing with similar studies, the country background seems to be used as a proxy for ethnicity.

Case retrieval

Selection bias occurs when the study population does not represent the target population (199). In Paper II, we used multiple sources for case identification including the NPR, the Swedish MS register, and the Swedish Cause of Death register together with a few cases gathered by the author in clinical work. As explained before, applying the concept of onset-adjusted prevalence implies a need for observation time. The awareness about MS has increased over time and an observation period up to 3 years from prevalence date was considered sufficient.

The NPR does not contain data on primary care which might have prevented us from identifying individuals with mild symptoms with or without established diagnosis who had never been referred to or followed up in specialized care. In addition, there might have been individuals with suspected or even known MS attending specialized care before the establishment of the NPR in 1987, but without actual contact after that and therefore not registered in the NPR.

We were also aware of a few Danish pwMS living and working in Malmö but attending the Danish MS Center and therefore registered in the TPR but not in the Swedish healthcare system.

Thus, both Paper II and Paper III might have been subject to selection bias which may have resulted in incidence and prevalence figures being underestimated.

In contrast to the Malmö based studies, data from the Primary Healthcare System in the Alt Empordà was included as one of multiple sources when establishing the Figueres MS registry. However, even Paper IV might have been subject to selection bias due to potential variations in the awareness of the disease among primary care physicians.

Information bias occurs when there are errors in the information collected about or from the study subjects leading to individuals being placed in the wrong category (199). This can be exemplified by the previously mentioned Danish pwMS since they were classified as not having MS, subsequently contributing to the denominator when calculating incidence and prevalence, instead for the numerator. However, the number of pwMS attending the Danish MS Centre was so small that the consequences of such bias are considered negligible.

Case ascertainment

Medical chart review for research purpose implies dependence on data that were originally collected for clinical purposes (263). This method has certain challenges; it is time consuming, there may be difficulties in the interpretation of the information, variability in the quality of the records, incomplete records, missing charts, and inconsistency between reviewers (264). The last issue does not apply to this thesis since the author was the only reviewer in Paper II–III and one of the co-supervisors the only one in Paper IV. Incomplete and missing charts accounted for less than 5% of all the charts which normally can be disregarded in terms of bias (265). To address the remaining pitfalls, an approach based on predefined research questions, together with a protocol with clear definitions of all the items and systematic data collection was used.

To assess the neuroradiological examinations the corresponding reports from the neuroradiologist were evaluated. In general, these reports describe the findings in the scans and suggest potential diagnosis. Since the introduction of the McDonald diagnostic criteria, it has become more common to specify whether dissemination in time and/or place is fulfilled, but there is no standard protocol followed by the neuroradiologist.

We assessed disability progression using the MSSS, which relies on disease duration and the EDSS (137). In Paper III–IV, most EDSS assessments were performed by an

MS-specialist. Measuring disability with the EDSS is an internationally accepted method, especially in clinical trials settings. However, it has been questioned due to weaknesses in sensitivity to change and reliability. Inter-rater kappa values between 0.32 and 0.76 have been reported, with the intra-rater figures being somewhat higher (266). Moreover, while specific training as an EDSS rater is mandatory in randomized clinical trials, there is no such requirement in daily clinical practice. Yet, MS-specialists are usually confident with the scale. Consequently, some degree of information bias must be acknowledged.

Regarding the MSSS, it is relevant to highlight that it was originally developed based on measurements in pwMS mainly from European countries (137). The algorithm was later validated in a cohort with American patients (267) and more recently, in a large international dataset with pwMS (268). The latest such dataset included pwMS across the world but not all countries were represented. Thus, the MSSS has not been specifically validated in certain populations (i.e. Asia, Africa, or Middle East) which may affect its external validity. Another caveat to consider when using the MSSS is potential fluctuations in the EDSS. To address this issue, we excluded EDSS registered within 6 months of a relapse in Paper III.

Discussion of results

Paper I

In this nested case-control study we found that high levels of 25 (OH) D3 (i.e. top quintile among controls) were associated with a decreased risk of developing MS (OR 0.68, 95% CI 0.50–0.93) which is in line with previous studies (39-41). However, we could not duplicate the results from one of these studies showing a significantly larger effect size among young individuals (<20 yo) (39).

As already mentioned, common to all studies is the design with blood samples collected before the onset of the disease which presumably reduced the risk for reverse causation. In addition, all studies had specified diagnostic criteria for the subjects to be included (Poser or Mc Donald) and no major differences in vitamin D levels between cases and controls were found. Still, a few differences deserve to be mentioned. For relevant details about our study the reader is referred to the "Methods" sections. The first study from Munger et al. (39) was performed among US military personnel, using up to 4 samples per subject (3 before and the fourth after the onset), a matching ratio of 1:2 with the majority of cases being men (68%). Matching was done for age, sex, sampling

date, branch of military service and race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, or other). In addition, adjustments for seasonal variation of vitamin D and latitude of residence at entry into the military were done. Measurements of 25 (OH) D were performed by RIA. Mean 25 (OH) D level was 78.2 nmol/L (standard deviation (SD) 28.1) among non-Hispanic whites, 45.5 (SD 21.2) in Blacks and 66.6 (SD 25.4) in Hispanic/other. Among non-Hispanic whites, the study found a 41% decreased risk for every 50 nmol/L increase in 25 (OH) D (OR 0.59, 95% CI 0.36–0.97). Moreover, being in the top (cut-off 99.2) versus (vs) the bottom quintile (cut-off <63.2) was also associated with a decreased risk (OR 0.38, 95% CI 0.19–0.75), with the overall trend across quintiles being significant. When looking into younger individuals (<20 yo), with 25 (OH) D levels of 100 nmol/L or higher, the effect was stronger (OR 0.09, 95% CI 0.01–0.75). Among Blacks, Hispanics and those of other race/ethnicity, the overall association between vitamin D levels and MS risk was not statistically significant.

The study from northern Sweden (40) was performed among pregnant women, with one available sample collected during the first trimester and a matching ratio of 1:2. Matching was performed by sex, biobank, sampling date and age. Measurements of 25 (OH) D were performed with ELISA. There were 56 individuals among cases and 112 among controls (29.2 % in both groups) with 25 (OH) D levels ≥50 nmol/L (269). The study showed that levels of 25 (OH) D ≥75 /vs <75) nmol/L were associated with a 61% decreased risk of developing MS (OR 0.39, 95% CI, 0.16–0.98). The effect was more pronounced in younger individuals (median age at sampling <26.4 yo) but not statistically significant (OR 0.16, 95% CI, 0.02–01.3). Notably, this subgroup was small (1/96 and 11/192 with 25 (OH) D levels ≥75 among cases and controls respectively).

Finally, the study by Munger et al. (41) was performed among women in Finland, with 1–3 samples collected between 10 and 14 weeks gestation and a matching ratio of 1:3. This was a larger study, with 1,092 cases and 2,123 controls. When medical records were not available (n=640), the date of diagnosis applied instead of onset date. Matching was performed by birth date and place of residence. Measurements of 25 (OH) D were performed with CLIA and seasonal adjustments were done. There were only 65 individuals among pwMS and 160 among the controls (6 and 7.5% in each group respectively) with 25 (OH) D levels ≥50 nmol/L. The study found a 39% reduced risk for every 50 nmol/L increase in 25 (OH) D (adjusted RR 0.61, 95% CI, 0.44–0.85). In addition, women with 25 (OH) D levels <30 nmol/L (deficient) had a 43% increased risk of MS compared to those with ≥50 nmol/L, while comparison to women with 25 (OH) D levels of 30– 50 nmol/L yielded a 27% increased MS risk. The effect when comparing women with deficient vs sufficient levels was stronger in

analyses restricted to the subgroup of cases/controls with 2 or more samples (2-fold increase). In quintiles analysis, being in the bottom 2 quintiles (cut-off <26.8) was associated with a 37% to 87% increased risk of MS compared to being in the top quintile (cut-off ≥40.7).

A striking feature of these studies is the variety in the vitamin levels among both cases and controls. A reasonable explanation is the use of different methods to assess vitamin D status. As mentioned earlier, such methodological differences have implications both in clinical practice and research with the drawback being that it is not suitable to compare absolute vitamin D levels between these studies. However, given that the results point in the same direction and that they are performed in different geographical areas including both males and females, they could be said to complement each other. Taking all this together and considering the results from previous and more classical observational studies (34-38, 42-44), it is fair to claim that there is evidence for the association between vitamin D and MS risk, with Paper I being an additional piece in this puzzle.

To our knowledge, the study in Paper I is the first MS survey using the LC-MS/MS method, i.e. the gold standard procedure, which we consider one of the strengths of the study. Another important aspect is the relatively large number of young individuals (<20 yo) even if we could not reproduce the findings by Munger et al. among US veterans (39). Nevertheless, the effect size was in line with two of the studies (39, 40).

There are several limitations in our study, some of them already presented in the previous section ("Methodological considerations"). First, the heterogeneity between biobanks. Second, we had only one available sample per subject which, unlike both studies by Munger et al., (39, 41) implies that the measurements in our study might have been affected by incidental variations and consequently do not reflect long-term status of vitamin D. Third, the lack of data on comorbidities and sociodemographic factors other than sex and age might reduce the external validity of the results. Finally, we cannot exclude the presence of residual confounding.

In this framework, it should be emphasized that case-control studies are appropriate when trying to establish associations between exposure and disease but not causality. In addition, reverse causation can be a concern. A causal relationship requires that the exposure precedes the disease. Nested case-control studies with samples prospectively collected before the clinical onset of the disease potentially reduce reverse causation. However, considering the prevailing idea of a prodromal phase in MS, and that it is not evident when the inflammatory process actually begins, reverse causation might still be a concern. A different approach to causality assessment in order to overcome reverse causation and confounders is Mendelian randomization. Recent such studies have

shown that genetically lowered vitamin D levels increase the risk of MS (58, 59). However, this approach has its own challenges and limitations (270).

One of the central topics in the geoepidemiology of MS is the latitude gradient theory, i.e. higher latitude correlating with higher frequency of the disease. This peculiar latitude dependency prompted the hypothesis of an association between sun exposure and MS (49). Sun exposure is crucial to the production of vitamin D, with only 10–20% of the body´s reserve coming from diet (22, 23). The latitude gradient theory has been questioned over the years (202, 203), but recent meta-analyses have provided evidence of the gradient for prevalence globally as well as for incidence in Europe (204-206). Two of these meta-analyses are of particular interest since they include both global and regional analyses (205, 206). Regarding Scandinavia, the first study found an inverse gradient within this region (205). A plausible explanation was proposed by Kampman et. al stating that vitamin D intake, especially at northern latitudes, would be responsible for this phenomenon (271). Higher latitude implies reduced sun exposure, and thus lower vitamin production should be expected. However, this is not the case for Scandinavia because the dietary consumption here is higher than in other European populations (271-275). In Paper I, we found higher vitamin D levels than expected by latitude alone in both cases and controls which is in concordance with results from a previous population-based study from northern Sweden (276). Moreover, our results further support the former interpretation.

The more recent meta-analysis is an update of the preceding one by the same authors, showing a remarkable attenuation of the inverse gradient in the Scandinavian region (206). Nationwide studies from Sweden and Norway (226, 277) were included in this meta-analysis which may have contributed to mitigating the gradient, but it also raises the question whether there have been behavioural changes in the population leading to less dietary intake of vitamin D (206).

Paper II–III

Prevalence and incidence in the general population

This is the first population-based study of MS in the City of Malmö, southern Sweden, which is of particular interest due to the lack of MS epidemiology research in this geographical area compared to the rest of the country.

We found an incidence of 5.3/100,000 (95% CI, 4.5–6.2) and an onset-adjusted prevalence of 133/100,000 (95% CI, 121–147), with a female to male ratio of 1.8 and 2.1 respectively.

Three contemporary observational studies in Sweden are of relevance when discussing our results: a regional survey in Västerbotten (225) and two nationwide surveys (226, 227).

The incidence found in Paper II is in concordance with the study from Västerbotten, which applied a similar methodology, i.e. multiple sources, and validation of the diagnosis by a neurologist. However, the prevalence in Västerbotten in 2010 was higher, namely 215/100,000 (95% CI, 198–233) (225), a finding that might support the theory of a north-south latitude gradient of MS prevalence in Sweden.

The nationwide studies (226, 227), reported an average MS incidence in Sweden from 2001 to 2008 of 10/100,000 person-years and a prevalence of 188.9/100,000 (95% CI, 186.1–191.7). The prevalence for Skåne was 176/100,000 but no specific data for the City of Malmö were available (Cecilia Ahlgren, personal communication). Potential pwMS in these studies were identified by linking data from the NPR, the TPR, and the Swedish Neuroregister. No specific validation of the diagnosis was performed, and cases with Possible MS diagnosis were included. Incidence estimates were calculated with a novel method based on logistic regression to calculate the probability of the date of a diagnosis being within the incidence period for a subset of individuals. This probability applied then to other subjects and finally, hazard functions of MS diagnosis were estimated. In turn, the prevalence was calculated based on the total number of individuals with a registered diagnosis of MS on December 31, 2008, and the total population in Sweden at that point.

The figures in our study were clearly lower than the national estimates. While it is tempting to make comparisons, it may not be appropriate for several reasons. First, onset-adjusted incidence applied in our study, which is more appropriate from a biological and epidemiological perspective, whereas date of diagnosis was used by Ahlgren et al. Second, we did perform a validation of the MS diagnosis thorough systematic case ascertainment with predefined criteria for both diagnosis and what should be considered onset of the disease. Third, we did not include Possible MS cases. Finally, the specific demographics of the population in Malmö differ from the national average both in terms of age distribution and background.

In addition to prevalence estimates, the above nationwide study also investigated the presumed presence of a latitude gradient of MS prevalence in Sweden. It found that the prevalence significantly increased for each degree of north latitude with 1% and 1.5% in women and men respectively. This is in contrast to results from a Norwegian nationwide study that could not find clear associations between latitude and prevalence in 2012. In that survey, the prevalence was significantly higher in the Middle region of the country, but there was no difference between the Northern and Southern regions,

and potential changes in prevalence with each degree of latitude were not explored (278).

Thus, with reservations for methodological differences, together with the previous studies in Sweden, our results support the presence of a latitude gradient of MS prevalence in Sweden.

One of the key features of Paper II was the rigorous case ascertainment. There are many clinical and paraclinical features typical for MS but so far none are considered pathognomonic. Rather, the diagnosis is based on two essential principles: dissemination in space and time along with exclusion of a more probable explanation for the clinical picture. According to the McDonald criteria, if the criteria are fulfilled and there is no better explanation, the diagnosis will be "MS", while if the diagnosis is suspected but the criteria are not completely met, the diagnosis will be "Possible MS" (140).We found 46 individuals with MS diagnosis who did not fulfil the McDonald criteria. Many of them (65%) could be considered Possible MS.

The "better explanation" principle is challenging in real life since many other neurological diseases may present as "MS mimics". Moreover, some of these, i.e. neuromyelitis optica spectrum disorders (NMOSD) or Myelin oligodendrocyte glycoprotein antibody disease have been better described in the last decades. Among individuals with incomplete or not available medical records we found 52 individuals registered as MS. In this group, there were at least two with old medical records and a clinical picture resembling NMOSD. Also, it can be particularly demanding to establish a diagnosis of PPMS, a phenotype that was not specifically included in the diagnostic criteria until the arrival of the McDonald epoch (139). We found 12/52 individuals registered in the medical charts as PPMS. All had a clinical course suggestive of progressive disease and some of them had oligoclonal band in CSF, but none had had an MRI. This was not unexpected since the majority were older patients who had received their diagnosis before MRI became routine procedure. We cannot exclude that some or all of them would have been classified as MS if they had undergone an MRI. Unfortunately, it was not possible for us to complete the diagnostic workup for reasons already explained.

When analysing the findings in a research project based on real life data, it is worth mentioning that the diagnostic criteria by Poser (138) were developed to facilitate the inclusion of individuals in clinical trials and other research protocols, and that the McDonald criteria (139-141) were specifically developed to predict the development of MS in individuals presenting with a typical CIS. In daily clinical practice each neurologist faces a broader spectrum of symptoms.

Prevalence according to country background

Estimates of prevalence and incidence among immigrants have been performed in Sweden before, either at a national level or with focus on a specific group (Iranian immigrants in Gothenburg). Thus, Paper III is the first population-based study addressing this topic in the City of Malmö.

The onset-adjusted prevalence was lower among immigrants compared to Scandinavians, both in first-generation immigrants and in the predefined subgroups, Western and non-Western. One striking feature of our material was the subgroup with country background in the Middle East which accounted for most (73.3%) pwMS among non-Western immigrants. According to the Atlas of MS from 2013, most countries in that area had prevalence estimates clearly lower than the one in our study (207). This is in line with a previous study from Oslo, Norway (212). Of note, most of the Middle East pwMS in the Norwegian study came from Iran, which was also the case in our population. A potentially higher susceptibility in the Iranian-born population has been previously suggested in a survey from Gothenburg (210) and two Swedish nationwide studies (211, 228). As outlined in the introduction, presence of the class II HLA-DRB1*15:01 allele has a well-established association with increased MS risk (OR~3) (21) and this association has been described in the Iranian population in some studies (279, 280). Moreover, in a case-control study from 2017 investigating the relationship between different environmental factors and MS in Iran, the authors found that sun exposure was significantly associated with a decreased risk of MS, OR 0.09 (95% CI 0.02–0.38) (281). Together, our results suggest that a presumed genetic predisposition in the Iranian population combined with environmental factors in the host country may contribute to their susceptibility.

Common also with the study in Oslo was the small number of pwMS from Asia, Africa, and Central and South America which precludes further interpretations.

As already outlined in the previous section (Methodological considerations), there were some shortcomings in Paper II-III such as possible selection bias due to the lack of data from the primary health care services and potential older cases before the establishment of the NPR, which might account for underestimation of our incidence and prevalence figures. Moreover, incomplete or missing medical charts, but given the small numbers it should be negligible. In addition, as for Paper III, we cannot preclude a different threshold for seeking medical attention in immigrants with mild symptoms, which might bias the prevalence figures.

Clinical characteristics

To our knowledge Paper III is the first study in Sweden investigating disease severity in immigrants with MS. We found a pattern of higher disability among non-Western immigrants compared to Scandinavians which is in line with previous studies from the US (164), Canada (162), France (160, 163) and Norway (159).

Older age at onset, time to clinically definitive disease (CD), and polysymptomatic onset are some of the classical potential "red flags" indicating poorer long-term prognosis (282). Interestingly, we found increased disability progression among non-Western immigrants even though all these characteristics were exactly the opposite of what would be expected based on the classical view. The anatomical region of the first relapse has also been suggested as a prognostic marker, with some studies showing a more favourable prognosis for onset with ON (153, 181). This was the most frequent localization at onset for both Scandinavians and Western immigrants. The most common among the non-Western group was brainstem/cerebellum, which has been associated with a less favourable outcome (153). A natural question in this context is whether all individuals had equal health care system access or not. As to the previous studies from France and Norway (159, 160, 163) we found no differences in time to diagnosis or time to treatment which might indicate equal access to the health care system. The proportion of pwMS with any DMT ever was higher among non-Westerners compared to Scandinavians which confirms equal availability of medical services and might indicate a more severe disease. However, considering that the first DMT were not available until 1993, it could also reflect a longer disease duration among Scandinavians, with a higher proportion of pwMS in this group not suitable for treatment when this became available.

An additional concern when looking into DMT is our finding of a negligible use of second-line therapy (both ever and as first choice) among non-Western immigrants. For natural reasons, the only second-line treatment in our study was natalizumab. The introduction of natalizumab in our department was delayed due to economic restrictions which could explain the limited number of pwMS on this therapy. However, it raises the question whether the threshold for switching therapy was different due to factors such as different coping strategies among groups when dealing with information on potential severe side-effects or the challenge of sharing complex information through a translator.

As outlined in the previous section, genetic and environmental factors may interact and contribute to differences in disease frequency among groups. Thus, assuming equal access to adequate medical services, differences in severe disease between groups raise the question whether such factors contribute to differences in disease disability. Moving

from geographical areas with low/medium MS risk to a high-risk area may suggest a crucial role for environmental factors. Moving to Norway or Sweden from more southern countries implies changing to higher latitude and reduced sun exposure, which may be one of the unknown environmental factors. On the other hand, little is known about the clinical characteristics of the disease in non-Western countries. In a contemporary study from the MENA region, the median time to reach an EDSS score of 6.0 was 15.7 years, which is shorter than reported in Western countries. EDSS 6 corresponds to substantial ambulatory disability and the need of ambulatory devices. This survey was a registry study establish in 2016 by the Committee for the treatment of MS in the region (MENACTRIMS) in order to register individuals with MS from the area. Given that all data were retrospectively collected with no predefined criteria for clinical parameters, and the big differences in access to DMT within the region and probably also to the health care system, it is not possible to draw firm conclusions (283). Nevertheless, a recent review of available literature including clinical and immunological studies comparing outcomes among "Whites", "Black/African Americans" and "Hispanic/Latinx" pwMS established the presence of heightened humoral responses in the two last groups which may be associated with disease severity (284). Thus, a pattern of disability connate to certain groups may explain the findings in our study, but further research is needed.

Some limitations have already been presented, but two important limitations common to many of the published studies should be mentioned. The first is the lack of data on socioeconomic status. The second is the "migration bias", i.e. immigrants are usually healthier, younger and of higher socioeconomic status than those who do not emigrate, possibly making them less representative of their country of origin.

Paper IV

Incidence

We found an incidence of 3.5/100,000 (95% CI 2.6–4.6) during 2001–2010 which is similar to the estimate from a regional study in the province of Girona in 2009, which also applied the concept of onset-adjusted incidence (238). Notably, the incidence was lower than in the former study in the Alt Empordà (285) which may be explained by methodological differences since diagnosis in the pilot study relied on date of diagnosis, and only immigrants that had moved to the area before the age of 15 had been included.

An increase in incidence rates over time in Spain has recently been reported in a systematic review of published literature between 1968 and 2018 (286). Interestingly,

that was not the case in our study in which we found an incidence of 1.6/100,000 (95% CI 1.1–2.5) during 2011–2020. Generally, a decrease in incidence may reflect a real decline in the frequency of a disease or be a false decrease. Different factors, such as a higher threshold among the population for seeking medical attention, restrictions in the availability of medical services, or less awareness among primary health care physicians, may lead to lower figures. Considering the impact of the Covid-19 pandemic in Catalonia, some of these factors may have been present at the end of the study period, favouring the idea of a false decrease. On the other hand, current knowledge indicates that there has been an increase in incidence during the last century followed by a stabilization or maybe a decrease (93), which may support the idea of a true decrease in incidence.

Prevalence

This is the first epidemiological study in Spain using the concept of onset-adjusted prevalence. We found a prevalence of 75/100,000 (95% CI 61–90) in 2010, followed by slightly higher figures in 2020: 81/100,000 (95% CI 68–98). In the previous survey in the Alt Empordà, the prevalence in 2006 was 63/100,000 (95% CI 50– 79), which is difficult to compare with the current figures due to the previously mentioned differences in methodology. Demographical changes and the decrease in incidence may explain the modest increase in prevalence between decades.

Overall, it is difficult to make comparisons with previous studies on MS prevalence in Spain since all were based on date of diagnosis. There is only one other contemporary study in Catalonia, showing a prevalence of 80/100,000 (95% CI 66– 96) in 2008 in Osona, central Catalonia.

Our results are in agreement with the geographical distribution model of Kurtzke, which placed Catalonia and the rest of Spain in the medium-prevalence band. However, other studies in the last decades have shown higher prevalence figures, suggesting that Spain should be considered a medium to high-risk area. Excluding the study from Ourense, the highest MS prevalence in Spain so far was reported in 2015 in Santiago de Compostela, in the northwest part of the country. The authors found a prevalence of 152/100,000 (95% CI 127–176) which is in contrast with the rest of the country, especially the Mediterranean coast. Different genetic ancestors (i.e. Celtic in the northwest) together with more sun exposure (essential to D vitamin production) in the Mediterranean, have been proposed as potential explanations (Figure 15). Prevalence figures in our study were both lower than in Santiago de Compostela, which may support these explanations as well as the proposed interplay between genetic susceptibility and environmental factors.

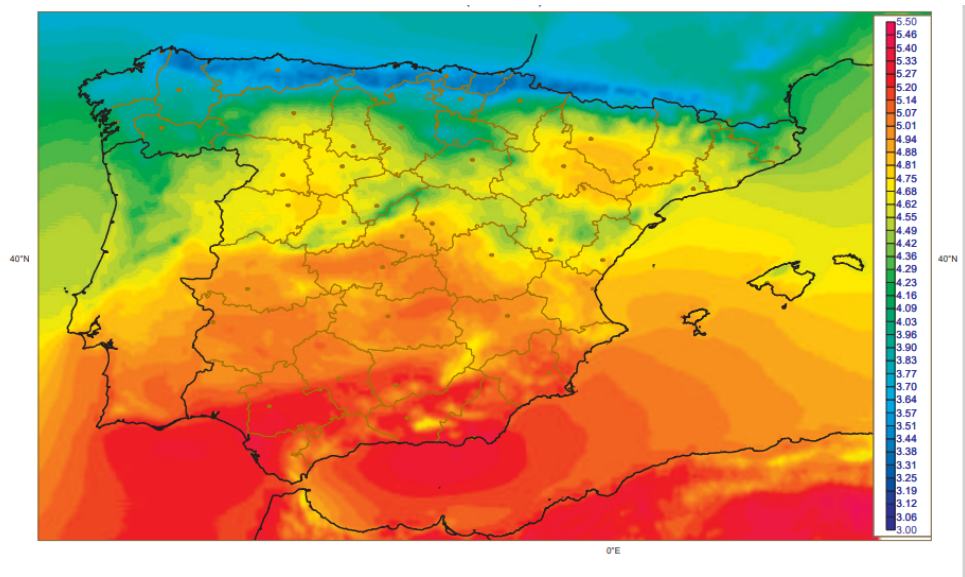

Figure 15. Map representing the mean sun irradiation in Spain, created by AEMET, the State Meteorological Agency of Spain with data from EUMESAT (European Organisation for the Exploitation of Meteorological Satellites) (287).

Future perspectives

Paper I is part of a larger national project focusing on different environmental exposures and the risk for MS. The project has already resulted in several publications, with contributions to several of them by this author (73-75, 92, 288, 289). Further research with focus on interactions between different exposures is much needed.

Regarding vitamin D specifically, given that all published nested case-control studies so far used different methods to measure vitamin D, and did not include data on socioeconomic status, a multinational study including sociodemographic factors and applying the gold standard method LC-MS/MS for measuring vitamin D would have the additional strength of a large study population and uniform analyses methods to increase generalisability.

Considering that the overall aim of this thesis was to gain insight into the epidemiology of MS in the City of Malmö, future research will include an extension study in the same geographical area. Such a study will focus on incidence 2010–2020 and point prevalence 2020 as well as essential sociodemographic factors. In addition, other variables such as estimations of incidence and prevalence among second-generation immigrants and the economic burden of the disease would be of interest.

In view of the results in Paper IV, an overview of the healthcare network in the Alt Empordà is already ongoing. In addition, an extension study with focus on incidence 2011–2025 and point prevalence 2025 could be considered, in order to specifically address temporal trends.

Finally, it is important to acknowledge that the upcoming update of the diagnostic criteria for MS will probably increase the number of cases detected. It will be essential to address this question when interpreting future study results.

Conclusions

The present work provides further support for the association between high serum vitamin D concentrations and a reduced risk of developing MS. In addition, this thesis constitutes the first population-based epidemiology study on MS in the City of Malmö and the first study in Sweden looking at disability progression among immigrants and the native population. Moreover, we have established a very valuable foundation for future follow-up studies. Finally, our results are in agreement with previous work suggesting that the northwest of Spain is a higher risk area for MS compared to the Mediterranean coast.

The conclusions are:

Paper I

Relatively higher 25-hydroxyvitamin D concentrations were associated with a reduced risk of developing MS. This protective effect was not more pronounced in young individuals.

High serum vitamin D concentrations may have a protective role against MS.

Paper II

The first population-based observational study on MS in the City of Malmö, southern Sweden, showed high incidence and prevalence figures, although lower than expected, possibly due to methodological differences between our study and previous nationwide surveys.

Together with previous reports, our results support the presence of a latitude gradient of MS prevalence in Sweden.

Paper III

First-generation immigrants (Western and non-Western) had lower MS prevalence than the Scandinavian population.

No diagnosis or treatment delayed was identified across the groups.

Non-Western immigrants had a more rapid disease progression than the Scandinavians. It is unclear whether genetic susceptibility and/or environmental factors after migration are responsible for the pattern of disability.

Paper IV

Incidence and prevalence figures in the Alt Empordà are consistent with Kurtzke´s distribution model, i.e. Spain being located in the medium risk area.

The incidence in the second decade of study was lower than expected. It is unclear whether this reflects a true decrease in incidence or a consequence of demographical changes and the Covid-19 pandemic.

The prevalence in the Alt Empordà was lower than in the northeast of Spain, possibly due to different genetic ancestors and more sun exposure.

Populärvetenskaplig sammanfattning

Bakgrund

Multipel skleros (MS) är en kronisk neurologisk sjukdom som drabbar ungefär dubbelt så många kvinnor som män. Sjukdomen påverkar det centrala nervsystemet vilket innebär att symtomen kommer från hjärnan och/eller ryggmärgen. Vanliga symtom är balans- och koordinationsstörningar, pareser, domningar, stickningar, suddig syn eller dubbelseende, men även ”dolda symtom” såsom trötthet, smärta, påverkan på arbetsminne och blås- samt tarmrubbningar förekommer. Vanligast är att insjukna mellan 20 och 40 års ålder. Utan behandling leder MS oftast till någon form av neurologisk funktionsnedsättning och efter 15–20 år med sjukdomen behöver ungefär hälften av de drabbade någon form av gånghjälpmedel.

Ingen vet den exakta orsaken till MS men det finns idag en bred enighet bland forskare om att MS är en autoimmun sjukdom vilket innebär att kroppens immunsystem angriper den egna kroppen. I MS fall betyder detta att immunförsvaret ger sig på nervtrådar i hjärnan och ryggmärgen och förstör myelinet, en fettliknande substans som omger och stödjer nervtrådar samt gör att dessa kan leda impulser snabbt. Vad som orsakar denna felaktiga reaktion är okänt, men den rådande hypotesen är att det är ett komplext samspel mellan ärftliga och miljömässiga faktorer såsom Epstein-barr virus, låga nivåer av D-vitamin, rökning och övervikt i ungdomen.

Förekomsten av MS är inte jämnt fördelad över världen utan blir högre på både norra och södra halvklotet ju längre man kommer från ekvatorn. Dessa skillnader har länge misstänkts vara kopplade till solljusexponering som för många är den viktigaste källan till D-vitamin. Vissa folkgrupper med lägre förekomst av MS verkar utveckla en allvarligare sjukdom. Anledning till detta är okänd.

Målsättning

Delarbete I handlar om D-vitamins roll för uppkomsten av MS medan Delarbete II och III är en kartläggning och beskrivning av MS i Malmö Stad med särskilt fokus på skillnader mellan individer av olika ursprung. Delarbete IV är en kartläggning av MS i L`Alt Empordà i nordöstra Catalonien med fokus på ändringar över tid.

Frågeställningar

Delarbete I:

Har höga nivåer av D-vitamin en skyddande effekt mot uppkomsten av MS? I förekommande fall, är effekten mest framträdande bland yngre individer?

Delarbete II:

Vilken var incidensen (antal nya fall) av MS i Malmö Stad under perioden 2001–2010?

Vilken var prevalensen (andel individer) av MS i Malmö Stad den 31 december 2010?

Delarbete III:

Vilken var prevalensen av MS i Malmö Stad den 31 december 2010 bland individer med svensk bakgrund respektive invandrare?

Fanns det skillnader i sjukdomens uttryckt bland individer med svensk bakgrund respektive invandrare?

Delarbete IV:

Vilken var incidensen (antal nya fall) av MS i L´Alt Empordà under perioden 2001–2010 och 2011–2020?

Vilken var prevalensen (andel individer) av MS i L´Alt Empordà den 31 december 2010 och 2020?

Metod

Delarbete I: Blodprover från individer som senare utvecklat MS identifierades genom samkörning av sex svenska biobanker, svenska MS-registret eller en lokal MS-databas i Umeå. D-vitaminnivåer mättes med hjälp av vätskekromatografi-tandemmasspektrometri som anses vara standardmetod. Sammanlagt analyserades biobanksprover från 665 individer med MS och lika många matchade friska kontroller. Matchning gjordes för biobank, kön, provtagningsdatum samt provtagningsålder. Matchning innebär att kontroller väljs ut så att fall och kontroller blir mer jämförbara avseende vissa viktiga egenskaper för det utfall som studeras.

Delarbete II och III: I ett första steg identifierades potentiella MS-fall genom samkörning av olika register (Patientregistret och Dödsorsaksregistret från Socialstyrelsen, svenska MS-registret och Registret över totalbefolkningen från SCB). I ett andra steg genomfördes en strukturerad journalgranskning för att fastställa vilka individer som uppfyllde gängse diagnoskriteria för MS och relevanta data (datum för

första symtom, datum för diagnos, kön, typ av förlopp, karaktär av första symtom, förskrivning av bromsmedicin, grad av funktionsnedsättning) registreras.

Delarbete IV: I ett förta steg identifierades potentiella MS-fall genom en lokal MS-databas i Figueres och registret över totalbefolkningen från Katalonien. I ett andra steg genomfördes en strukturerad journalgranskning för att fastställa vilka individer som uppfyllde gängse diagnoskriteria för MS och relevanta data (datum för första symtom, kön, typ av förlopp, förskrivning av bromsmedicin och grad av funktionsnedsättning) registreras.

Resultat

Delarbete I: Höga nivåer av D-vitamin var associerat med minskad MS-risk.

Delarbete II:

Incidensen av MS i Malmö Stad 2001–2010 var 5.3/100 000.

Prevalensen av MS i Malmö Stad 2010 var 133/100 000 vilket innebär sammanlagt 397 individer med MS.

Delarbete III:

Förekomsten av MS bland första generationens invandrare var lägre än för individer med svensk bakgrund.

Icke-västerländska invandrare hade en snabbare grad av sjukdomsprogression än skandinaver trots likvärdig tillgång till specialistsjukvården.

Delarbete IV:

Incidensen av MS i L´Alt Empordà var 3.5/100 000 under perioden 2001–2010 och 1.6/100 000 under perioden 2011–2020.

MS prevalens i L´Alt Empordà var 75/100 000 2010 och 81/100 000 2020 vilket innebär sammanlagt 103 respektive 114 individer med MS.

Betydelse

Detta arbete ger ytterligare stöd till sambandet mellan höga D-vitaminnivåer i serum och en minskad risk att utveckla MS. Dessutom utgör denna avhandling den första populationsbaserade epidemiologiska studien om MS i Malmö stad och den första studien i Sverige som studerar utvecklingen av funktionshinder utifrån individens ursprung. Därutöver har vi skapat en värdefull grund för ytterligare uppföljningsstudier såväl i Malmö som i L´Alt Empordà.

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Appendix

Functional systems

Pyramidal functions

0. Normal.
1. Abnormal signs without disability
2. Minimal disability.
3. Mild or moderate paraparesis or hemiparesis; severe monoparesis.
4. Marked paraparesis or hemiparesis; moderate quadriparesis; or monoplegia.
5. Quadriplegia.

Cerebellar functions

0. Normal.
1. Abnormal signs without disability.
2. Mild ataxia.
3. Moderate truncal or limb ataxia.
4. Severe ataxia, all limbs.
5. Unable to perform coordinated movements due to ataxia.

Brains Stem functions

0. Normal.
1. Signs only.
2. Moderate nystagmus or other mild disability.
3. Severe nystagmus, marked extraocular weakness, or moderate disability of other cranial nerves.
4. Marked dysarthria or other marked disability.
5. Inability to swallow or speak.

Sensory functions

0. Normal.
1. Vibration or figure-writing decrease only, in one or two limbs.
2. Mild decrease in touch or pain or position sense, and/or moderate decrease in vibration in one or two limbs; or vibratory (c/s figure writing) decrease alone in three or four limbs.
3. Moderate decrease in touch or pain or position sense, and/or essentially loss of vibration in one or two limbs; or mild decrease in touch or pain and/or moderate decrease in all proprioceptive tests in three or four limbs.

4. Marked decrease in touch or pain or loss of proprioception, alone or combined, in one or two limbs; or moderate decrease in touch or pain and/or severe proprioceptive decrease in more than two limbs.
5. Loss (essentially) of sensation in one or two limbs; or moderate decrease in touch or pain and/or loss of proprioception for most of the body below the head.
6. Sensation essentially lost below the head.

Bowel and Bladder functions

0. Normal.
1. Mild urinary hesitancy, urgency, or retention.
2. Moderate hesitancy, urgency, retention of bowel or bladder, or rare urinary incontinence.
3. Frequent urinary incontinence.
4. In need of almost constant catheterization.
5. Loss of bladder function.
6. Loss of bowel and bladder function.

Visual (or Optic) functions

0. Normal
1. Scotoma with visual acuity (corrected) better than 20/30.
2. Worse eye with scotoma with maximal visual acuity (corrected) of 20/30 to 20/59.
3. Worse eye with large scotoma, or moderate decrease in fields, but with maximal visual
   acuity (corrected) of 20/60 to 20/99.
4. Worse eye with marked decrease of fields and maximal visual acuity (corrected) of 20/100 to 20/200; grade 3 plus maximal acuity of better eye of 20/60 or less.
5. Worse eye with maximal visual acuity (corrected) less than 20/200; grade 4 plus maximal acuity of better eye of 20/60 or less.
6. Grade 5 plus maximal visual acuity of better eye of 20/60 or less.

Cerebral (or Mental) functions

0. Normal.
1. Mood alteration only (Does not affect DSS score).
2. Mild decrease in mentation.
3. Moderate decrease in mentation.
4. Marked decreased in mentation (chronic brain syndrome-moderate).
5. Dementia or chronic brain syndrome-severe or incompetent.

Expanded Disability Status Scale (EDSS)

0 = Normal neurologic exam (all grade 0 in Functional Systems [FS]; Cerebral grade 1 acceptable).

1.0 = No disability, minimal signs in one FS (i.e., grade 1 excluding Cerebral grade 1).

1.5 = No disability minimal signs in more than one FS (more than one grade 1 excluding Cerebral

grade 1).

2.0 = Minimal disability in one FS (one FS grade 2, others 0 or 1).

2.5 = Minimal disability in two FS (two FS grade 2, others 0 or 1).

3.0 = Moderate disability in one FS (one FS grade 3, others 0 or l), or mild disability in three or four FS (three/four FS grade 2, others 0 or 1) though fully ambulatory.

3.5 = Fully ambulatory but with moderate disability in one FS (one grade 3) and one or two FS grade 2; or two FS grade 3; or five FS grade 2 (others 0 or 1).

4.0 = Fully ambulatory without aid, self-sufficient, up and about some 12 hours a day despite relatively severe disability consisting of one FS grade 4 (others 0 or l), or combinations of lesser grades exceeding limits of previous steps. Able to walk without aid or rest some 500 meters.

4.5 = Fully ambulatory without aid, up and about much of the day, able to work a full day, may otherwise have some limitation of full activity or require minimal assistance; characterized by relatively severe disability, usually consisting of one FS grade 4 (others 0 or 1) or combinations of lesser grades exceeding limits of previous steps. Able to walk without aid or rest for some 300 meters.

5.0 = Ambulatory without aid or rest for about 200 meters; disability severe enough to impair full daily activities (e.g., to work full day without special provisions). (Usual FS equivalents are one grade 5 alone, others 0 or 1; or combinations of lesser grades usually exceeding specifications for step 4.0.).

5.5 = Ambulatory without aid or rest for about 100 meters; disability severe enough to preclude full daily activities. (Usual FS equivalents are one grade 5 alone, others 0 or 1; or combinations of lesser grades usually exceeding those for step 4.0.)

6.0 = Intermittent or unilateral constant assistance (cane, crutch, or brace) required to walk about 100 meters with or without resting. (Usual FS equivalents are combinations with more than two FS grade 3+.)

6.5 = Constant bilateral assistance (canes, crutches, or braces) required to walk about 20 meters without resting. (Usual FS equivalents are combinations with more than two FS grade 3+.)

7.0 = Unable to walk beyond about 5 meters even with aid, essentially restricted to wheelchair wheels self in standard wheelchair and transfers alone; up and about in w/c some **12** hours a day. (Usual FS equivalents are combinations with more than one FS grade **4** + ; very rarely, pyramidal grade 5 alone.)

7.5 = Unable to take more than a few steps; restricted to wheelchair; may need aid in transfer; wheels self but cannot carry on in standard wheelchair a full day; may require motorized wheelchair. (Usual FS equivalents are combinations with more than one FS grade **4+** .)

8.0 = Essentially restricted to bed **or** chair or perambulated in wheelchair, but may be out of bed itself much of the day; retains many self-care functions; generally has effective use of arms. (Usual FS equivalents are combinations, generally grade **4** + in several systems.)

8.5 = Essentially restricted to bed much of the day; has some effective use of arm(s); retains some self-care functions. (Usual FS equivalents are combinations, generally **4** + in several systems.)

9.0 = Helpless bed patient; can communicate and eat. (Usual FS equivalents are combinations, mostly grade **4** + .)

9.5 = Totally helpless bed patient; unable to communicate effectively **or** eat/swallow. (Usual FS equivalents are combinations, almost all grade **4** + .)

10. = Death due to MS.

Multiple Sclerosis Severity Scale

	0	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5	EDSS
1	0.67	2.44	4.30	5.87	7.08	7.93	8.64	9.09	9.35	9.50	9.63	9.74	9.84	9.90	9.94	9.97	9.98	9.98	9.99	
2	0.53	2.01	3.69	5.24	6.46	7.27	7.98	8.58	8.95	9.18	9.38	9.59	9.79	9.88	9.93	9.97	9.99	9.99	9.99	
3	0.45	1.77	3.34	4.82	6.00	6.81	7.54	8.14	8.55	8.83	9.07	9.35	9.63	9.77	9.86	9.92	9.97	9.98	9.99	
4	0.35	1.45	2.87	4.27	5.41	6.24	6.98	7.65	8.12	8.42	8.70	9.08	9.47	9.68	9.80	9.88	9.95	9.98	9.99	
5	0.30	1.28	2.60	3.90	4.95	5.79	6.58	7.26	7.75	8.08	8.38	8.83	9.32	9.60	9.76	9.86	9.95	9.98	9.99	
6	0.25	1.13	2.33	3.54	4.55	5.38	6.14	6.81	7.33	7.66	7.98	8.50	9.08	9.45	9.68	9.81	9.93	9.97	9.99	
7	0.24	1.04	2.10	3.17	4.13	4.96	5.75	6.46	6.98	7.32	7.65	8.24	8.91	9.33	9.59	9.76	9.90	9.95	9.99	
8	0.21	0.94	1.92	2.93	3.81	4.57	5.36	6.10	6.61	6.95	7.32	7.97	8.71	9.21	9.55	9.74	9.89	9.96	9.99	
9	0.21	0.88	1.76	2.65	3.45	4.17	4.93	5.64	6.14	6.50	6.90	7.65	8.53	9.09	9.47	9.70	9.87	9.95	9.99	
10	0.19	0.78	1.53	2.34	3.10	3.79	4.55	5.28	5.77	6.14	6.58	7.39	8.31	8.92	9.34	9.61	9.83	9.94	9.99	
11	0.17	0.71	1.40	2.13	2.82	3.46	4.21	4.94	5.42	5.82	6.30	7.18	8.15	8.79	9.24	9.52	9.78	9.92	9.98	
12	0.16	0.64	1.28	1.98	2.64	3.25	3.94	4.63	5.13	5.54	6.03	6.92	7.93	8.63	9.13	9.43	9.71	9.88	9.97	
13	0.13	0.57	1.14	1.80	2.44	3.05	3.70	4.38	4.91	5.32	5.80	6.74	7.83	8.55	9.03	9.34	9.65	9.85	9.96	
14	0.11	0.49	1.03	1.70	2.33	2.91	3.55	4.26	4.82	5.23	5.70	6.56	7.59	8.34	8.86	9.20	9.57	9.82	9.95	
15	0.10	0.45	0.99	1.64	2.26	2.82	3.44	4.14	4.68	5.09	5.51	6.33	7.41	8.17	8.70	9.11	9.51	9.78	9.94	
16	0.09	0.38	0.85	1.42	1.99	2.56	3.17	3.86	4.41	4.81	5.18	6.00	7.14	7.97	8.54	9.04	9.49	9.75	9.94	
17	0.05	0.32	0.76	1.28	1.77	2.30	2.95	3.65	4.17	4.55	4.94	5.74	6.89	7.77	8.38	8.99	9.52	9.79	9.96	
18	0.04	0.26	0.66	1.12	1.57	2.09	2.70	3.37	3.89	4.27	4.62	5.43	6.62	7.54	8.23	8.94	9.51	9.78	9.96	
19	0.05	0.28	0.63	1.00	1.39	1.89	2.50	3.19	3.72	4.12	4.49	5.35	6.59	7.51	8.22	8.98	9.57	9.81	9.96	
20	0.05	0.26	0.59	0.94	1.29	1.71	2.29	2.99	3.51	3.93	4.30	5.15	6.43	7.45	8.23	8.98	9.58	9.80	9.95	
21	0.05	0.30	0.66	1.02	1.39	1.77	2.34	2.97	3.43	3.83	4.21	5.09	6.35	7.33	8.08	8.87	9.49	9.77	9.96	
22	0.04	0.23	0.54	0.90	1.28	1.66	2.20	2.82	3.29	3.69	4.09	5.04	6.35	7.35	8.10	8.84	9.42	9.73	9.95	
23	0.05	0.27	0.58	0.91	1.26	1.64	2.19	2.78	3.21	3.69	4.19	5.16	6.47	7.46	8.20	8.87	9.43	9.75	9.95	
24	0.05	0.24	0.52	0.86	1.25	1.63	2.15	2.71	3.09	3.52	4.01	5.03	6.36	7.38	8.15	8.81	9.39	9.74	9.96	
25	0.05	0.23	0.47	0.77	1.15	1.56	2.05	2.53	2.84	3.21	3.74	4.88	6.26	7.24	8.00	8.73	9.35	9.75	9.98	
26	0.05	0.20	0.45	0.78	1.17	1.58	2.08	2.63	2.99	3.40	3.95	5.02	6.39	7.44	8.21	8.89	9.48	9.80	9.96	
27	0.05	0.22	0.48	0.78	1.15	1.56	2.03	2.56	2.91	3.29	3.86	4.93	6.33	7.38	8.14	8.91	9.56	9.85	9.98	
28	0.04	0.17	0.40	0.74	1.16	1.52	1.88	2.39	2.76	3.04	3.46	4.54	5.99	7.07	7.90	8.75	9.45	9.80	9.98	
29	0.03	0.18	0.47	0.80	1.19	1.51	1.79	2.27	2.68	3.01	3.41	4.35	5.68	6.76	7.66	8.62	9.38	9.75	9.96	
30	0.01	0.13	0.45	0.82	1.19	1.45	1.69	2.23	2.75	3.13	3.50	4.35	5.61	6.66	7.54	8.47	9.27	9.67	9.91	
Years																				

=1st Decile
=2nd Decile
=3rd Decile
= 4th Decile
= 5th Decile
= 6th Decile
= 7th Decile
= 8th Decile
= 9th Decile
= 10th Decile

The 2010 McDonald MRI criteria for DIS and DIT

DIS Can Be Demonstrated by ≥1 T2 Lesion[a] in at Least 2 of 4 Areas of the CNS:
Periventricular
Juxtacortical
Infratentorial
Spinal cord[b]

Based on Swanton et al 2006, 2007.[22,27]
[a]Gadolinium enhancement of lesions is not required for DIS.
[b]If a subject has a brainstem or spinal cord syndrome, the symptomatic lesions are excluded from the Criteria and do not contribute to lesion count.
MRI = magnetic resonance imaging; DIS = lesion dissemination in space; CNS = central nervous system.

DIT Can Be Demonstrated by:
1. A new T2 and/or gadolinium-enhancing lesion(s) on follow-up MRI, with reference to a baseline scan, irrespective of the timing of the baseline MRI
2. Simultaneous presence of asymptomatic gadolinium-enhancing and nonenhancing lesions at any time

Based on Montalban et al 2010.[24]
MRI = magnetic resonance imaging; DIT = lesion dissemination in time.

The 2010 McDonald MRI criteria for diagnosis of MS in disease with progression from onset

PPMS May Be Diagnosed in Subjects With:
1. One year of disease progression (retrospectively or prospectively determined)
2. Plus 2 of the 3 following criteria[a]:
A. Evidence for DIS in the brain based on ≥1 T2[b] lesions in at least 1 area characteristic for MS (periventricular, juxtacortical, or infratentorial)
B. Evidence for DIS in the spinal cord based on ≥2 T2[b] lesions in the cord
C. Positive CSF (isoelectric focusing evidence of oligoclonal bands and/or elevated IgG index)

[a]If a subject has a brainstem or spinal cord syndrome, all symptomatic lesions are excluded from the Criteria.
[b]Gadolinium enhancement of lesions is not required.
MS = multiple sclerosis; PPMS = primary progressive MS; DIS = lesion dissemination in space; CSF = cerebrospinal fluid; IgG = immunoglobulin G.

The 2010 McDonald criteria for diagnosis of MS

Clinical Presentation	Additional Data Needed for MS Diagnosis
≥2 attacks[a]; objective clinical evidence of ≥2 lesions or objective clinical evidence of 1 lesion with reasonable historical evidence of a prior attack[b]	None[c]
≥2 attacks[a]; objective clinical evidence of 1 lesion	Dissemination in space, demonstrated by: ≥1 T2 lesion in at least 2 of 4 MS-typical regions of the CNS (periventricular, juxtacortical, infratentorial, or spinal cord)[d]; or Await a further clinical attack[a] implicating a different CNS site
1 attack[a]; objective clinical evidence of ≥2 lesions	Dissemination in time, demonstrated by: Simultaneous presence of asymptomatic gadolinium-enhancing and nonenhancing lesions at any time; or A new T2 and/or gadolinium-enhancing lesion(s) on follow-up MRI, irrespective of its timing with reference to a baseline scan; or Await a second clinical attack[a]
1 attack[a]; objective clinical evidence of 1 lesion (clinically isolated syndrome)	Dissemination in space and time, demonstrated by: For DIS: ≥1 T2 lesion in at least 2 of 4 MS-typical regions of the CNS (periventricular, juxtacortical, infratentorial, or spinal cord)[d]; or Await a second clinical attack[a] implicating a different CNS site; and For DIT: Simultaneous presence of asymptomatic gadolinium-enhancing and nonenhancing lesions at any time; or A new T2 and/or gadolinium-enhancing lesion(s) on follow-up MRI, irrespective of its timing with reference to a baseline scan; or Await a second clinical attack[a]
Insidious neurological progression suggestive of MS (PPMS)	1 year of disease progression (retrospectively or prospectively determined) plus 2 of 3 of the following criteria[d]: 1. Evidence for DIS in the brain based on ≥1 T2 lesions in the MS-characteristic (periventricular, juxtacortical, or infratentorial) regions 2. Evidence for DIS in the spinal cord based on ≥2 T2 lesions in the cord 3. Positive CSF (isoelectric focusing evidence of oligoclonal bands and/or elevated IgG index)

If the Criteria are fulfilled and there is no better explanation for the clinical presentation, the diagnosis is "MS"; if suspicious, but the Criteria are not completely met, the diagnosis is "possible MS"; if another diagnosis arises during the evaluation that better explains the clinical presentation, then the diagnosis is "not MS."

[a]An attack (relapse; exacerbation) is defined as patient-reported or objectively observed events typical of an acute inflammatory demyelinating event in the CNS, current or historical, with duration of at least 24 hours, in the absence of fever or infection. It should be documented by contemporaneous neurological examination, but some historical events with symptoms and evolution characteristic for MS, but for which no objective neurological findings are documented, can provide reasonable evidence of a prior demyelinating event. Reports of paroxysmal symptoms (historical or current) should, however, consist of multiple episodes occurring over not less than 24 hours. Before a definite diagnosis of MS can be made, at least 1 attack must be corroborated by findings on neurological examination, visual evoked potential response in patients reporting prior visual disturbance, or MRI consistent with demyelination in the area of the CNS implicated in the historical report of neurological symptoms.

[b]Clinical diagnosis based on objective clinical findings for 2 attacks is most secure. Reasonable historical evidence for 1 past attack, in the absence of documented objective neurological findings, can include historical events with symptoms and evolution characteristics for a prior inflammatory demyelinating event; at least 1 attack, however, must be supported by objective findings.

[c]No additional tests are required. However, it is desirable that any diagnosis of MS be made with access to imaging based on these Criteria. If imaging or other tests (for instance, CSF) are undertaken and are negative, extreme caution needs to be taken before making a diagnosis of MS, and alternative diagnoses must be considered. There must be no better explanation for the clinical presentation, and objective evidence must be present to support a diagnosis of MS.

[d]Gadolinium-enhancing lesions are not required; symptomatic lesions are excluded from consideration in subjects with brainstem or spinal cord syndromes.

MS = multiple sclerosis; CNS = central nervous system; MRI = magnetic resonance imaging; DIS = dissemination in space; DIT = dissemination in time; PPMS = primary progressive multiple sclerosis; CSF = cerebrospinal fluid; IgG = immunoglobulin G.

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Lucía Alonso Magdalena is a consultant physician in neurology and rehabilitation medicine at Skåne University Hospital in Sweden. This thesis aims to advance knowledge regarding vitamin D as one of the putative environmental factors in the etiology of MS and to gain insight into the epidemiology of MS in the City of Malmö and in the county of the Alt Empordà, Catalonia.

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