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Olga Krökki

MULTIPLE SCLEROSIS IN NORTHERN FINLAND

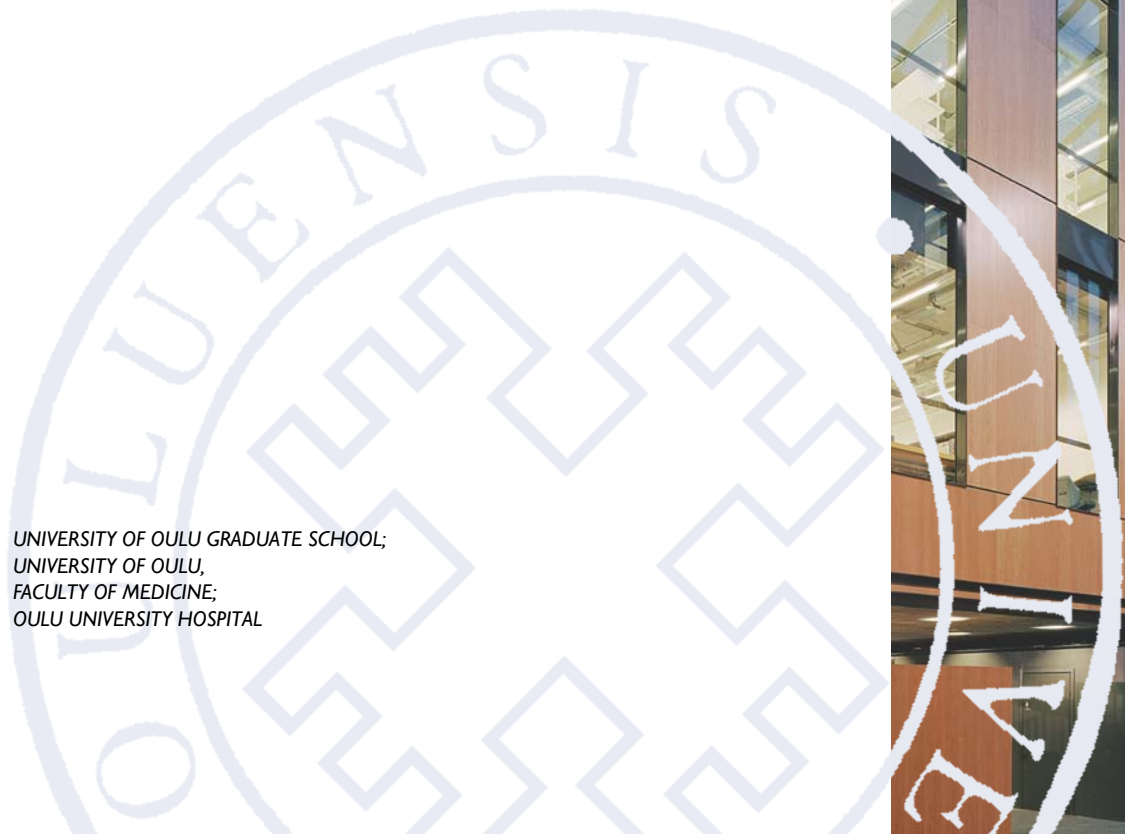
**EPIDEMIOLOGICAL CHARACTERISTICS AND
COMORBIDITIES**

UNIVERSITY OF OULU GRADUATE SCHOOL;
UNIVERSITY OF OULU,
FACULTY OF MEDICINE;
OULU UNIVERSITY HOSPITAL



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OLGA KRÖKKI

**MULTIPLE SCLEROSIS IN
NORTHERN FINLAND**

Epidemiological characteristics and comorbidities

Academic dissertation to be presented with the assent of the Doctoral Training Committee of Health and Biosciences of the University of Oulu for public defence in Auditorium 8 of Oulu University Hospital (Kajaanintie 50), on 10 June 2016, at 12 noon

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Abstract

Multiple sclerosis (MS) is the most frequent chronic inflammatory demyelinating disease of the central nervous system leading to neurological disability in young adults. Finland belongs to the high-risk areas of MS with continuously increasing of prevalence rates. In comparison to the general population, patients with MS have an increased risk of premature mortality. The extent of comorbidity rates and their role in the outcomes of MS have not been fully investigated.

The aim of this thesis was to evaluate the epidemiology and survival of MS in Northern Ostrobothnia. The neurological comorbidity and frequency of fractures in patients with MS were also investigated. All MS patients diagnosed in Oulu University Hospital during 1990–2010 were included in this study.

The prevalence of MS was $103/10^5$; the mean overall incidence was $6.3/10^5$. Women had a 2.3-fold higher MS incidence than men. The overall incidence figures were clearly affected by a pronounced increase among females. Neurological comorbidities were common in MS patients with 17.1 % of patients suffering from some other neurological diseases in addition with MS. The most common diseases were migraine (10.4 %) and epilepsy (4.7 %). The prevalence of stroke was similar in MS patients compared to the general population, but the 21-year survival rate was significantly lower in MS patients who had suffered a stroke compared with those MS patients without stroke. MS patients experienced a high risk for fragility fractures especially vertebral fractures. The possibility of osteoporosis had been examined only 26 % patients with fragility fractures and osteoporosis was detected in the majority.

The prevalence of MS in our cohort was similar to the Finnish average, but in the Seinäjoki area, the prevalence was double the national average. Although the increasing incidence of female MS has been confirmed also in other studies, the reasons for this phenomenon are unknown. The increased risk for fragility fractures may be associated with low D vitamin levels or pathogenesis of MS. These findings emphasize the role of comorbid diseases in disability and survival of MS patients and attention should be paid to these conditions also in clinical practice.

Keywords: comorbidity, disability, epidemiology, fracture, multiple sclerosis, neuroimmunology, osteoporosis

Krökki, Olga, MS-tauti pohjoissuomalaisessa väestössä. Epidemiologiset erityispiirteet sekä liitännäissairaudet

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Tiivistelmä

MS-tauti on nuorten aikuisten yleisin etenevä ja usein työkyvyttömyyteen johtava neuroimmunologinen sairaus. Suomi kuuluu korkean riskin alueeseen ja maassamme on havaittu alueellisia eroja MS-taudin esiintyvyydessä. MS-taudin ennusteeseen vaikuttavia tekijöitä ei täysin tunneta, mutta luonnollisen kulun tutkimuksissa on havaittu, että MS-tauti lyhentää eliniän ennustetta useita vuosia muuhun väestöön verrattuna. MS-tautia sairastavien potilaiden toimintakyvyn ja eliniän ennusteeseen vaikuttavista liitännäissairauksista on hyvin vähän tietoa.

Tutkimuksen tarkoituksena oli selvittää MS-taudin epidemiologiaa sekä eliniän ennustetta Pohjois-Pohjanmaalla. Lisäksi tutkittiin MS-tautia sairastavien muuta neurologista sairastavuutta sekä luun murtumien esiintyvyyttä. Aineistona oli Oulun yliopistollisessa sairaalassa MS-taudin diagnoosin saaneet potilaat vuosilta 1990–2010.

MS-taudin esiintyvyys oli $103/10^5$ ja ilmaantuvuus keskimäärin $6.3/10^5$. Naisilla MS-taudin ilmaantuvuus oli 2,3-kertainen miehiin verrattuna. MS-taudin ilmaantuvuus lisääntyi 15 vuoden seurannassa, mikä selittyi MS-taudin ilmaantuvuuden kasvulla naisten keskuudessa. MS-taudin lisäksi 17,1 %:lla potilaista oli jokin muu neurologinen sairaus. Yleisimmät sairaudet olivat migreeni (10,4 %) ja epilepsia (4,7 %). Neurologisista liitännäissairauksista aivoverenkiertohäiriöiden esiintyvyys oli yhtä suuri kuin väestössä yleensä, mutta sairastettu aivoverenkiertohäiriö lisäsi merkittävästi MS-potilaiden kuolleisuutta. MS-tautia sairastavilla havaittiin kohonnut riski etenkin matalaenergiisiin murtumiin, joista merkittävä osuus oli selkärangan nikamamurtumia. Matalaenergisien murtuman saaneista potilaista luuntiheyden mittausta oli tehty vain 26 %:lle, joista suurimmalla osalla todettiin osteoporoosi.

Tutkimuksen perusteella MS-taudin esiintyvyys vastaa Suomen keskimääristä esiintyvyyttä ja on noin puolet pienempi kuin Seinäjoen alueella, missä MS-taudin esiintyvyys on Suomen korkein. MS-taudin ilmaantuvuuden lisääntyminen naisten keskuudessa on havaittu myös muissa tutkimuksissa, mutta syy tähän ilmiöön on epäselvä. MS-tautia sairastavilla kohonnut murtumariski saattaa liittyä mataliin D-vitamiinipitoisuuksiin tai MS-taudin patogeneesiin. Koska liitännäissairastavuus sekä luunmurtumat heikentävät MS-tautia sairastavan toimintakykyä ja lisäävät kuolleisuutta, tulisi näihin kiinnittää enemmän huomiota myös kliinisessä työssä.

Asiasanat: epidemiologia, liitännäissairaus, luun murtuma, MS-tauti, neuroimmunologia, osteoporoosi, toimintakyky

To my family

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30.04.2016

Olga Krökki

Abbreviations

ATP	adenosine triphosphate
BMD	bone mineral density
CI	confidence interval
CIS	clinically isolated syndrome
CNS	central nervous system
CSF	cerebrospinal fluid
DIS	dissemination in space
DIT	dissemination in time
DXA	dual-energy x-ray absorptiometry
EAE	experimental autoimmune encephalomyelitis
EBV	Epstein-Barr virus
EDSS	expanded disability status scale
EEG	electroencephalography
ENMG	electroneuromyography
GIO	glucocorticoid induced osteoporosis
HLA	human leukocyte antigen
HTLV-1	human T-cell lymphotropic virus type 1
IL	interleukin
ILAE	International League Against Epilepsy
MRI	magnetic resonance imaging
MS	multiple sclerosis
NARCOMS	North American Research Committee on Multiple Sclerosis
NMO	neuromyelitis optica
OUH	Oulu University Hospital
PML	progressive multifocal leukoencephalopathy
PPMS	primary progressive multiple sclerosis
RR	risk ratio
RRMS	relapsing remitting multiple sclerosis
SPMS	secondary progressive multiple sclerosis
UV	ultraviolet
WHO	World Health Organization

List of original publications

This thesis is based on the following publications, which are referred to throughout the text by their Roman numerals:

- I Krökki O, Bloigu R, Reunanen M & Remes AM (2011) Increasing incidence of multiple sclerosis in women in Northern Finland. *Mult Scler* 17 (2):133–8.
- II Krökki O, Bloigu R, Ansakorpi H, Reunanen M & Remes AM (2014) Neurological comorbidity and survival in multiple sclerosis. *Mult Scler Relat Disord* 3(1):72–7.
- III Krökki O, Bloigu R & Remes AM (2014) High frequency of fractures in an early stage of MS. *J Mult Scler* 1:109. doi: 10.4172/jmso.1000109.

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1 Introduction

Multiple sclerosis (MS) is the most common chronic inflammatory demyelinating disease of the central nervous system (CNS) leading to a neurological disability in young adults. The societal lifetime costs of MS per patient are higher than those associated with stroke or Alzheimer's disease because of the long duration of the disease, the incapacitating effect on the working abilities of young adults. These are attributable to the patient's physical disability, fatigue and comorbidity, the need for assistance in daily living, the expensive disease-modifying therapies and multidisciplinary health care (Pugliatti *et al.* 2006). In Finland, the mean total annual cost of MS was 46 994€; in fact, this value increases almost ten-fold as the disease progresses from an initial annual cost of 10 835€ (EDSS score = 0) up to 109 901€ (EDSS score = 8–9) (Ruutiainen *et al.* 2016).

MS affects more than 2.5 million persons worldwide and there are about 7000 MS patients in Finland. The geographical distribution of MS patients has been the subject of numerous studies originating from different countries. Finland is one of the high-risk areas of MS and furthermore the nation has a continuously increasing prevalence rate. The epidemiology of MS has been studied in southern, central and western parts of Finland. However, there is extensive variation in these epidemiological values between different parts of the country, though they do seem to be highest in the Seinäjoki area (Kinnunen *et al.* 1983, Sarasoja *et al.* 2004, Sumelahti *et al.* 2001, Tienari *et al.* 2004).

MS is a complex disease that is characterized by a high degree of heterogeneity in its clinical, radiological and pathological features as well as in the therapeutic response. The central pathological findings of MS are inflammation, demyelination, remyelination and neurodegeneration. However, the exact pathogenesis and aetiology of MS are also not fully understood. According to current understanding, both genes and environmental factors are involved in the etiology of MS. The majority of patients have relapsing-remitting disease course (RRMS); only about 10–15 % of patients will experience the primary progressive form of MS (PPMS) at onset (Miller *et al.* 2007). The typical course for RRMS includes the initial stages of relapse with either a full or partial recovery, followed by a relapse leaving a persistent deficit, and eventually secondary progressive disease (SPMS). Today there are about ten different licensed disease modifying agents for RRMS. All of these drugs reduce the frequency of new episodes but do not reverse already present deficits and have

debatable effects on the long-term development of disability and disease progression.

There are significant variations in the prognosis of MS. Approximately 50 % of individuals diagnosed with RRMS will enter a progressive phase with increasing disability after 10 years and the majority of them will need help in walking 15 years after the onset of disease. However, 10 % of patients are able to function well for 20 years or even more (Noseworthy *et al.* 2000). Patients with MS seem to have a lifetime expectancy which is 10 years lower than the general population (Bronnum-Hansen *et al.* 1994, Bronnum-Hansen *et al.* 2004b, Compston & Coles 2008, Grytten Torkildsen *et al.* 2008, Kingwell *et al.* 2012, Ragonese *et al.* 2010, Sadovnick *et al.* 1992). Although the reason for the variation in the prognosis and decreased survival may be due to MS itself, the presence of other comorbid diseases may also influence their survival time. There is increasing interest in elucidating the roles of co-morbidity with cancer, psychiatric, cardiovascular and neurological diseases in MS. The data which have been mainly based on national registers and findings are partly contradictory.

There is no previous epidemiological data from the northern part of Finland. The aim of the present work was to investigate the epidemiology of MS in Northern Finland and evaluate the neurological comorbidity of these patients. In addition, a further aim was to estimate the frequency of fractures in patients with MS.

2 Review of the literature

2.1 Etiology and pathogenesis of multiple sclerosis

The etiology of MS is still unknown. Based on epidemiological and genetic data and the immune pathogenesis of MS, three main theories i.e. the autoimmune, infective (viral) or neurodegenerative theories have been proposed as underpinning the etiology of MS (Milo & Kahana 2010, Gildea 2005, Kurtzke 2013, Stadelmann 2011).

The pathogenesis of MS is characterised by a cascade of pathobiological events, starting from focal lymphocytic infiltration and microglial activation, ultimately culminating in demyelination and axonal degeneration. Demyelination is a hallmark of MS; it occurs in white and grey matter lesions. Myelin-specific autoreactive lymphocytes (mainly CD4+T cells) from outside the CNS cross the blood-brain barrier, leading to the formation of new inflammatory demyelinating lesions. The activation of autoreactive CD4+T cells may require additional environmental triggers in genetically susceptible individuals. The inflammatory process finally leads to tissue damage within the plaque. However, some degree of remyelination is possible and this can repair damaged tissue. The cortex is also affected in the early stages of the disease, which leads to cortical demyelination, neurodegeneration and finally to cortical atrophy (Ciccarelli *et al.* 2014, Kamm *et al.* 2014, Lucchinetti *et al.* 2011).

In addition to T-cells, B cells are involved in the pathogenesis of MS (Krumbholz *et al.* 2012). In patients with MS, the proinflammatory effects of B cells are particularly predominant. A B cell follicle-like aggregation in the meninges of SPMS has been observed, evidence in support of the central role of B cells in damaging the grey matter. The activated microglia and macrophages are also involved in the formation of MS lesions since they release myeloperoxidase, which maintains an inflammatory cascade and causes tissue damage (van der Veen *et al.* 2009).

The production of reactive-oxygen species and nitric oxide from activated microglia and infiltrated macrophage could induce mitochondrial dysfunction within neurons and a glutamate –mediated excitotoxicity plays also a role in the pathogenesis of MS. (Campbell *et al.* 2011, van Horssen *et al.* 2012, Ciccarelli *et al.* 2014).

2.2 Clinical course and diagnostics of multiple sclerosis

2.2.1 Clinical course

Usually, MS patients have multiple neurological symptoms related to the localisation of demyelinating lesions in CNS. There are sensory symptoms (numbness and paraesthesia), optic neuritis and other visual symptoms, motor symptoms (pyramidal weakness in limbs, including an acute transverse myelitis), spasticity, problems with bladder control and sexual dysfunction, bowel symptoms, fatigue, cognitive impairment, dysarthria, dysphagia, action or intention tremor, ataxia of gait and trunk, neuropathic and musculoskeletal pain.

Two initial clinical subtypes of MS are distinguishable. Approximately 85 % of patients have the relapsing-remitting MS (RRMS) form and the remaining 10–15 % of patients suffer from primary progressive MS (PPMS).

A relapse defined as patient-reported or objectively observed acute symptoms of neurological disturbance in the CNS of the kind seen in MS has a duration of at least 24 hours and it is not associated with fever or infection, and it is then followed by a partial or complete recovery (McDonald *et al.* 2001a). A repeat relapse has to occur at least 30 days after the initial relapse to be considered as a separate event (McDonald *et al.* 2001a). The first relapse is usually termed as a “clinically isolated syndrome” (CIS). CIS is the first clinical presentation of inflammatory demyelination that could be MS, but it does not fulfill the criteria of dissemination in time (Miller *et al.* 2005).

RRMS is characterized by clearly defined relapses with full recovery or with sequelae and a residual deficit after the recovery. The periods between disease relapses (remissions) are characterized by a lack of disease progression. Secondary-progressive MS (SPMS) develops when RRMS is followed by a progression with or without occasional relapses, minor remissions and plateaus. In contrast, PPMS is characterized by disease showing progression or disability from onset, without plateaus or remissions or with occasional plateaus and temporary minor improvements.

Benign MS has been defined as RRMS, in which an Expanded Disability Status Scale (EDSS) score is less or equal to 3.0 at least 10 years after the disease onset (Weinshenker 1995b). However, only about 52 % of patients continue to remain benign after 20 years of follow-up after MS diagnosis and about 23 % have progressed to EDSS score ≥ 6 (Sayao *et al.* 2007).

2.2.2 Diagnostics of multiple sclerosis

The ability to have an accurate diagnosis as early as possible is important for patient management, counseling and optimal treatment. The diagnosis of MS is currently based on both clinical parameters, such as medical history and neurological examination, and paraclinical measures such as magnetic resonance imaging (MRI), cerebrospinal fluid (CSF) examination and evoked potential testing. A definite diagnosis of MS requires evidence of dissemination in time (DIT) and dissemination in space (DIS), i.e. the presence of symptoms affecting more than one discrete CNS region and at more than one time point during the course of the disease.

In 1983, the new diagnostic criteria were proposed by Poser *et al.* (Poser *et al.* 1983), and these criteria were expanded to include the use of paraclinical parameters, such that they became the standard MS diagnostic criteria for about 20 years. In 2001, an international panel revised the Poser criteria in order to highlight the significance of MRI information: early McDonald criteria of 2001 (McDonald *et al.* 2001a), the McDonald criteria of 2005 (Berg *et al.* 2010, Polman *et al.* 2005).

Table 1. Poser criteria 1983 (Modified from Milo *et al.* 2014).

Diagnostic	Attacks	Clinical lesions	and/or	Paraclinical lesions	CSF Bands or elevated IgG index
Clinically definite MS	2	2			
	2	1	and	1	
Laboratory-supported definite MS	2	1	or	1	+
	1	2			+
	1	1	and	1	+
Clinically probable MS	2	1			
	1	2			
	1	1	and	1	
Laboratory-supported probable MS	2				+

Table 2. McDonald updates from 2001 to 2010 (Modified from Milo *et al.* 2014).

Criteria	2001	2005	2010
MRI DIS ¹	<p>≥3 of:</p> <ul style="list-style-type: none"> • ≥9 T2 lesions or ≥1 enhancing lesion • ≥3 periventricular lesions • ≥1 juxtacortical lesion • ≥1 infratentorial lesion <p>1 cord lesion can replace 1 brain lesion</p>	<p>≥3 of:</p> <ul style="list-style-type: none"> • ≥9 T2 lesions or ≥1 enhancing lesion • ≥3 periventricular lesions • ≥1 juxtacortical lesion • ≥1 infratentorial lesion <p>Any number of cord lesions can be included</p>	<p>≥1 T2 lesions in ≥2 of the following areas:</p> <ul style="list-style-type: none"> • periventricular • juxtacortical • infratentorial • spinal cord
MRI DIT ²	<p>≥1 enhancing asymptomatic lesion</p> <p>≥3 months after CIS⁴ onset</p> <p>≥1 new T2 lesion on a scan obtained</p> <p>≥3 months after CIS</p>	<p>≥1 enhancing asymptomatic lesion</p> <p>≥3 months after CIS onset</p> <p>≥1 new T2 lesion on a scan obtained</p> <p>≥30 days after CIS</p>	<p>Asymptomatic enhancing and nonenhancing lesions simultaneously present at any time</p> <p>≥1 new T2 or enhancing lesion on follow-up MRI⁵ at any time</p>
CSF ³ to support?	Yes	Yes — in RRMS ⁶	No

¹ DIS — Dissemination in space, ² DIT — Dissemination in time, ³ CSF — Cerebrospinal fluid,

⁴ CIS — Clinically isolated syndrome, ⁵ MRI — Magnetic resonance imaging, ⁶ RRMS — Relapsing–remitting multiple sclerosis

The recent diagnostic criteria for relapsing-remitting MS

The most recent criteria are those published in 2010, the so-called revised McDonald criteria (Polman *et al.* 2011).

With the 2010 McDonald criteria, the diagnosis can be established with a single MRI scan in a patient with a clinically silent gadolinium-enhancing lesion and another clinically silent T2 lesion and after only one clinical event suggestive of MS (Polman *et al.* 2011). Dissemination in time can be also demonstrated should a second clinical attack involve a different site.

Dissemination in time can be demonstrated on MRI: 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 non-enhancing lesions at any time.

Dissemination in space can be demonstrated on MRI by ≥ 1 T2 lesion in at least two out of four areas of the CNS: periventricular, juxtacortical, infratentorial, spinal cord. Gadolinium enhancement of lesions is not required for

dissemination in space. If a subject has a brainstem or spinal cord syndrome, the symptomatic lesions are excluded from the criteria.

A CSF examination is not required for MS diagnosis according to the 2010 criteria. However, many experts have emphasized the importance of CSF studies as potential supporting evidence for the diagnosis of MS as well as in evaluating for other potential etiologies in the case of differential diagnostics.

The recent diagnostic criteria for primary progressive MS

One year of disease progression (retrospectively or prospectively determined) and two or three of the following:

1. Evidence of DIS in the brain based on one or more T2 lesions in the MS-characteristic regions (periventricular, juxtacortical or infratentorial).
2. Evidence of DIS in the spinal cord based on two or more T2 lesions in the cord.
3. Positive CSF (isoelectric focusing evidence of oligoclonal bands and/or elevated IgG index).

2.2.3 Differential diagnosis of multiple sclerosis

The presentation of MS can be monosymptomatic or to have multifocal signs and symptoms, and many neurological disorders of young adults can be similar to MS in their initial presentation. However, other potential explanations for the symptoms need to be ruled out, for example similar presentations can occur in patients who have infectious, neoplastic, congenital, metabolic, systemic disorders, vascular disease or non-MS inflammatory demyelinating disease. Table 3 summarizes the most important disorders for differential diagnostic with MS.

Table 3. Differential diagnosis of MS.

Metabolic disorders	Disorders of vitamin B12 metabolism ¹ Lysosomal leukodystrophies (metachromatic leukodystrophy and Krabbe's disease)
Autoimmune diseases	Sjögren's syndrome, systemic lupus erythematosus, Behçet's disease, sarcoidosis, chronic inflammatory demyelinating polyradiculopathy associated with central nervous system demyelination, antiphospholipid-antibody syndrome antibody mediated encephalopathias: N-methyl-D-aspartate receptor encephalitis, PREM, acute disseminate encephalomyelitis with MOG antibodies, limbic encephalitis
Infections	HIV-associated myelopathy ¹ and HTLV-1-associated myelopathy ¹ , Lyme disease ¹ , meningovascular syphilis, Eales' disease (angiopathia retinae juvenilis), tropical spastic paraparesis
Vascular disorders	Spinal dural arteriovenous fistula ¹ Cavernous hemangiomas Central nervous system vasculitis, including retinocochlear cerebral vasculitis Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL)
Genetic syndromes	Hereditary ataxias and hereditary paraplegias ¹ Leber's optic atrophy (LHON) and other mitochondrial cytopathies (MELAS)
Lesions of the posterior fossa and spinal cord	Arnold–Chiari malformation, nonhereditary ataxias Spondylotic and other myelopathies ¹
Psychiatric disorders	Conversion reaction, malingering
Neoplastic diseases	Spinal cord tumors, ¹ central nervous system lymphoma Paraneoplastic disorders
Other demyelinating diseases	ADEM, NMO/NMO spectrum disorders, idiopathic transverse myelitis

¹ These disorders are of particular relevance in the differential diagnosis of progressive myelopathy and primary progressive multiple sclerosis (Noseworthy *et al.* 2000, Miller 2008).

Neuromyelitis optica and neuromyelitis optica spectrum disorders

One of the most important differential diagnostic diseases for MS is neuromyelitis optica (NMO, Devic's syndrome) and neuromyelitis optica spectrum diseases (NMOSD). Myelitis and optic neuritis are clinical presentations of both MS and NMO. The presence of longitudinally extensive T2 hyperintense and T1 hypointense spinal cord lesions extending over three vertebral segments of the

spinal cord that preferentially involve spinal central gray matter is a characteristic of NMO, while MS-typical spinal lesions are shorter extending one to two vertebral segments. By using FLAIR and diffusion-weighted MRI, it has been possible to demonstrate hypothalamic, corpus callosum and brainstem lesions in the periependymal regions surrounding the third ventricle, cerebral aqueduct and fourth ventricle in as many as 60 % of NMO patients (Wingerchuk *et al.* 2006). Nonspecific small lesions in deep or subcortical white matter are frequent, but only occasionally fulfill the Barkhof criteria for the diagnosis of MS (Barnett *et al.* 2014).

Most patients with NMO have detectable serum antibodies that target the water channel aquaporin-4 (AQP4-immunoglobulin G, AQP4-Ab); these antibodies are highly specific and beneficial in the clinical diagnosis of NMO (Wingerchuk *et al.* 2006, Wingerchuk *et al.* 2015). Almost all AQP4-IgG-seronegative, have been reported to have detectable serum myelin-oligodendrocyte glycoprotein (MOG) antibodies (Kitley *et al.* 2012). An examination of CSF specimens from NMO and NMOSD patients revealed marked pleocytosis and neutrophilia as well as the absence of oligoclonal IgG bands (Wingerchuk *et al.* 2006).

2.3 Epidemiology of multiple sclerosis

MS is one of the best studied neurological diseases in terms of epidemiology (Koch-Henriksen & Sorensen 2010). Epidemiological analyses of MS are usually based on retrospectively investigating historical cohorts which include patients receiving health care rather than being based on surveying the general population. In addition, population-based registries provide relevant nationwide information on the epidemiology of MS. For example, the Danish Multiple sclerosis register which was established in 1956, has collected MS cases since 1948 (Koch-Henriksen *et al.* 2001). The European Database for Multiple Sclerosis (EDMUS) has been available since 1992 (Confavreux 1994). The Norwegian National Multiple Sclerosis Registry was established in 2001 (Myhr *et al.* 2006).

Recently, some main features have emerged from the epidemiology of MS: first that the disease has a familial recurrence, second that the disease varies greatly in frequency in different countries but that there is a trend to growth (Sawcer *et al.* 2014). Understanding the geoepidemiology of MS can be a valuable research resource in clarifying the environmental and genetic risk factors.

2.3.1 The geographical distribution of multiple sclerosis

If one examines the worldwide geographical distribution of MS, it is clear that latitude is significantly associated with the prevalence of the disease. The prevalence increases with increasing latitude up to the temperate climate regions. This north-to-south latitudinal gradient is observed in both hemispheres. The extensive studies by Kurtzke conducted in 1970s showed that MS was a geographically-related disease, with a high frequency zone in the northern hemisphere with a prevalence of 30–80 per 100 000 population (or more) extending from about 43° to 65° north latitude and medium frequency zones where the prevalence was in the range 5–25 per 100 000 from about 38° to 46° north latitude. The low frequency zones were defined as those with a prevalence less than 5 per 100 000 from 12°–19° and from 63°–67° north latitude. In Australia and New Zealand, higher southern latitude zones were found out as high-frequency regions. The prevalence rates of MS from Asia and Africa in both hemispheres were low, except for English-speaking native-born whites of South Africa (Kurtzke 1977, Kurtzke 1980).

The strong and statistically significant latitudinal gradient of MS prevalence has been confirmed globally in the recent most comprehensive meta-analysis of MS prevalence which examined 59 countries between 1923 and 2009 (Simpson *et al.* 2011). The main result of this study was that the gradient increases steadily with increasing latitude, reaching a peak at around 55°, before changing to a significant inverse gradient above 60°; this is true even for northern Scandinavia. Explanations of these phenomena are still lacking although there has been speculation of gene-environmental interactions in the populations of northern Scandinavia. Some possible explanations for the inverse gradient in this region have been previously suggested, i.e. that there are several ethnic groups that make up the genetic structure of northern Scandinavia, including the Swedes, the Finns, the Norwegians and ancestral Sami with a low prevalence of MS (Einarsdottir *et al.* 2007, Gronlie *et al.* 2000, Harbo *et al.* 2007). Another hypothesis was that the very high dietary vitamin D intake may explain the absence of the north-to-south gradient in Northern Norway (Kampman & Brustad 2008).

2.3.2 Sex ratio, prevalence and incidence

The most commonly used measurements in MS epidemiology are sex ratio, the incidence rate, the point of prevalence ratio, the survival and mortality

Sex ratio

MS is more common in women. One possible explanation of this phenomenon is that MS, is similar to the other autoimmune diseases in displaying a female predominance.

In Europe, the female to male ratio is around 2.0 (Pugliatti *et al.* 2006). The female to male ratio has been shown to have increased from 1.9 to 3.2 for people with MS and birth year from 1930s to the 1970s in a recent Canadian study (Orton *et al.* 2006). However, no changes in female to male ratios were seen in a Swedish study in MS patients born between 1931 and 1985 (Bostrom *et al.* 2013). The study revealed that the mean female to male sex ratio in Swedish patients with MS was 2.57. The female to male sex ratio in Finland in regional surveys from central, western and south regions has varied from 1.6 in Seinäjoki to 2.2 in Central Finland to 2.4 in Vaasa and Uusimaa (Sarasoja *et al.* 2004, Sumelahti *et al.* 2000).

Prevalence

The total estimated prevalence rate of MS in Europe for the past three decades has been 83 per 100 000, with higher rates in northern countries. The prevalence rates were higher for women in all countries assessed in the epidemiological study of MS in Europe (Pugliatti *et al.* 2006). In Scandinavia, a few nationwide MS prevalence studies have been published. The following nationwide overall prevalences have been reported: in Iceland, $100/10^5$ (1990), in Denmark, $173/10^5$ (2005) and in Sweden, $188.9/10^5$ (Ahlgren *et al.* 2011). No nationwide MS surveys have been performed in Norway or Finland. In Norway, the regional population-based surveys have revealed that prevalence varies between 73.0 and $163.6/10^5$ with the highest prevalence in the central part of the country (Nord-Trøndelag Country latitude 64° N (Dahl *et al.* 2004). In the small Northern Norway region of Finnmark (68° N, population 75 975), the prevalence was $51.3/10^5$. This region was most densely populated by the Sami people (Gronlie *et al.* 2000).

In Finland, prevalence has been estimated in the south (Uusimaa) and western (Vaasa) districts since 1960s and recently in 2000–2004 by mean of regional population-based surveys in southern, two western and central regions (Table 4). It is known that Finland belongs to the high-risk areas of MS with a continuously increase in the prevalence rates (Rinne *et al.* 1966, Wikström 1975, Kinnunen

et al. 1983, Sumelahti *et al.* 2001, Sarasoja *et al.* 2004). Improved radiological diagnostics with MRI, which became available in Finland from the early 1990s, may explain both the increase of prevalence and incidence of MS (Sarasoja *et al.* 2004). There no data available which would account for the increasing prevalence rates in Finland seen during the last two decades. For example, the MS prevalence is unevenly distributed with a cluster in western Seinäjoki region (Sumelahti *et al.* 2001). The historical to present-day prevalence rates in Finland are demonstrated in Table 4.

Table 4. Prevalence rates (per 10⁵) of defined MS in Finland, previously to recent study.

Region of Finland	Study population size	% of country population	Total defined MS cases (N)	Latitude	Year	Crude prevalence rate	95CI%
Uusimaa	1 227 932	25.1	1 052	60° N	1993	93	87–99
Seinäjoki	197 042	3.9	322	62° N	1993	188	168–211
Central Finland	263 886	5.1	227	62° N	2000	105	93–18
Vaasa	179 079	3.5	156	63° N	1993	107	91–125

Sumelahti *et al.* 2001, Sarasoja *et al.* 2004

Incidence

The incidence of MS is low in childhood and increases after the age of 18, reaching its peak between 20 and 40 years with women being affected approximately 2–5 years earlier than men. The incidence declines at ages above 50 years. (Kamm *et al.* 2014).

Incidence rates seem to fluctuate significantly although there seems to be an increasing trend with time. Some epidemiological studies of patients with MS from different geographical regions of the world have reported an increase in both prevalence and incidence. The estimated European mean annual MS incidence rate is 4.3 cases per 10⁵/year. In this European study for the time-period considered, peaks of incidence rates were registered in Seinäjoki, Finland (11.6/10⁵/year), south-eastern Scotland (9.3/10⁵/year), eastern Norway (8.7/10⁵/year) and northern Sardinia, Italy (6.8/10⁵/year) (Pugliatti *et al.* 2006).

In Finnish regions, the total incidence varies from 9.2/10⁵/year in central Finland to 5.1/10⁵/year in the region of Uusimaa (Sarasoja *et al.* 2004). The incidence in Seinäjoki (11.6/10⁵/year) was more than two times higher than in the neighboring Vaasa region (5.2/10⁵/year) in the time-period 1979–1993, suggesting

that there may be the presence of predisposing genes in the Seinäjoki region (Sumelahti *et al.* 2000).

A profound increase in female incidence of MS in the past three decades has been observed in different populations all around the world i.e. from Iran, South East Wales, Canada, Denmark Crete and Finland (Orton *et al.* 2006, Hirst *et al.* 2009, Maghzi *et al.* 2010, Kotzamani *et al.* 2012, Koch-Henriksen & Sorensen 2011, Kalincik *et al.* 2013, Holmberg *et al.* 2013, Sumelahti *et al.* 2014). The reasons why the incidence has increased only in women is unclear although changes in the female lifestyle occurring in the past three decades have been proposed as one possible explanation. The study from Crete revealed that female subjects residing in urban centers or having relocated at a young age from the countryside to towns were disproportionately affected. This phenomenon might be associated with the changes that accompanied urbanization: cigarette smoking, use of pasteurized cow milk and contraception (Kotzamani *et al.* 2012). In Denmark, the incidence rates have been monitored since 1950 through the population-based nationwide Danish MS Registry. The incidence of onset in men has remained constant from 1950 to 2000 apart from the normal year-to-year stochastic variation, whereas the female incidence of onset has almost doubled since 1970 (Koch-Henriksen & Sorensen 2011).

2.3.3 Survival in multiple sclerosis

Survival analyses are considered time-to-event data. In MS studies, the focus of attention is on the length of time between two events such as time at onset and death or time at diagnosis and death. Long-term survival in MS had been studied mainly in 1960s-1990s, when it represented essentially the natural course of MS, since disease-modifying therapies for RRMS were only developed and released onto the market after 1993. The 25-year survival rates from clinical onset (first symptoms) to all deaths in verified MS cases (by Poser diagnostic criteria) were 78 % in Finland (during the period 1964–1993 (Sumelahti *et al.* 2002) and 62 % in Denmark (Bronnum-Hansen *et al.* 1994) (all verified MS cases by Allison and Millar diagnostic criteria from 1954) (Allison & Millar 1954). In Norway, 20 years' survival probability was 75 % on MS clinically defined by the McAlpine diagnostic criteria from 1961 (McAlpine 1961). There are methodological differences among these studies, and MS disease was verified by different diagnostic criteria. Some studies have claimed that they have detected improved survival (Bronnum-Hansen *et al.* 1994) but others have evaluated stable or

worsening long-term survival. Young age at clinical onset (first symptoms), initial remitting clinical course, and the presence of sensory symptoms at onset have all been significantly associated with longer survival. (Midgard *et al.* 1995).

The 21 years' survival rates from clinical onset in RRMS-patients receiving early IFN β -1b treatment were 80 %, which was significantly better than obtained with placebo as well as better than results in previous studies (Goodin *et al.* 2012).

The results of several studies on mean survival after onset in the past three decades have varied from 28 years in men and 33 years in women in Denmark (Bronnum-Hansen *et al.* 1994) to 45 years in patients with MS in Finland (Sumelahti *et al.* 2002). The median survival time from onset was about 10 years shorter for MS patients than for the age-matched general population. In the Finnish cohort, the 25-year survival rate after diagnosis for all causes of death was 62 % and when followed from clinical onset to all causes of death, the survival rate was 78 % (Sumelahti 2002). In the Danish cohort, if one uses the 25 years after onset criterion, then the survival probability was 57 % for men (80 % for the general male population) and 67 % for women (88 % for the general female population) (Bronnum-Hansen *et al.* 2004b).

2.4 Risk factors of multiple sclerosis

Epidemiological data indicate that both environmental and genetic factors play a key role in the development of MS. As stated above, latitude has a strong influence on MS prevalence. Other major environmental risk factors for MS are infections (Epstein–Barr virus, EBV), low vitamin D levels and lifestyle risk factors.

2.4.1 Genetic factors

The lifetime risk of MS increases if there is a familial history of the disease and about 20 % of MS patients report that they have a relative with the same disease. However, families with more than three affected relatives in more than one generation are extremely rare. The lifetime risk for MS is 0.2 % in the general European population, 2–4 % in siblings of MS patients (sibling recurrence risk 10–20) and 30 % in monozygotic twins of MS patients (Kuusisto *et al.* 2008, Sarkijarvi *et al.* 2006, Willer *et al.* 2003). Today, although over 100 different genetic variants associated with MS have been identified, the risk impact of these

variants is extremely low (Sawcer *et al.* 2014). These risk variants consistently implicate genes associated with immunological processes, overwhelmingly lie in regulatory rather than coding regions and are frequently associated with other autoimmune diseases. The genetic risk variants have been divided into two major types of genes: Human Leukocyte Antigen (HLA) and non-HLA genes.

The HLA region plays a role in nearly all immune-related disorders, including MS (Jersild *et al.* 1972, Olerup & Hillert 1991). The strongest association in MS has been observed with the *HLA-DRB1*1501* allele. Each copy of this allele increases the risk of MS by approximately 3-fold. The *HLA-DRB1*1501* allele frequency is between 3 and 20 % in the European population, with the population frequency increasing with the population risk of MS from southern to northern Europe (Dean *et al.* 2008). The increased risk for MS in association with *HLA-DRB1*03:01*, *HLA-DRB1*13:03*, and *HLA-DPBI*03:0138* alleles has also been confirmed. In contrast, *HLA-A*02:01* has been found to be protective. Together, the HLA alleles explain at most 20 % of the sibling recurrence risk for MS (Sawcer *et al.* 2014).

Genome-wide association studies have confidently identified over 100 different genetic variants outside of the HLA region (non-HLA genes) associated with susceptibility to MS. The majority of these variants have been found to be associated with different immunological genes and genes in immunological pathways. Each of these risk alleles increases the risk of MS rather marginally, from only 1.08 to 1.22-fold, but they may have a more important role in gene-environment interactions (International Multiple Sclerosis Genetics Consortium 2011).

2.4.2 Hygiene hypothesis and EBV infection

The hygiene hypothesis proposes that low exposure to childhood infections because of a clean environment predisposes individuals to pro-inflammatory immune responses to antigens and this is the phenomenon which increases the MS risk. Infection mononucleosis is due to the Epstein–Barr virus (EBV) and it is considered a marker of poor childhood hygiene, where children are infected asymptotically with EBV in the first years of life. In contrast, infection mononucleosis is common in Western countries, where individuals usually escape infection in early childhood and acquire EBV during adolescence or young adulthood. Individuals with a history of infection mononucleosis manifesting in

adolescents and young adults have a 2.3-fold higher relative risk of MS than those without EBV infection (Ascherio 2013, Thacker *et al.* 2006).

2.4.3 Vitamin D

Vitamin D insufficiency has been shown to be associated with increased susceptibility to MS. Case-control studies suggest that vitamin D is the most likely candidate to explain the inverse relationship between ultraviolet (UV) radiation and MS susceptibility (Munger *et al.* 2004).

There are two forms of vitamin D: vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol), which are both available in dietary form but only vitamin D3 is synthesized in the skin by UVB radiation in the sunlight. Vitamin D and its metabolites are transported in the plasma, bound to the vitamin D binding protein. Vitamin D is transformed in the liver into 25-hydroxyvitamin D (25(OH) D). Under stimulation by parathyroid hormone, this metabolite is transformed in the renal proximal tubule to form 1,25-dihydroxyvitamin D (1,25(OH)2D), which is the active metabolite and binds to vitamin D receptors in its target tissues. Vitamin D receptors are not only present in bone, kidneys (i.e. classical target tissues) but also in gonads, breast, pancreas, cardiovascular system, brain (microglia) and circulating immunity cells, i.e. macrophages, monocytes and activated lymphocyte T and B cells. This connection between vitamin D and the basic immunity cells is of particular interest given the potential immunological role of this vitamin in autoimmunity in general and especially in multiple sclerosis (Pierrot-Deseilligny & Souberbielle 2010).

Vitamin D deficiency has been linked to bone diseases such as rickets and osteoporosis and furthermore to some autoimmune diseases including type 1 diabetes, rheumatoid arthritis and system lupus erythematosus. Moreover D vitamin deficiency is believed to play a role in some cancers, in particular colon and breast cancer as well as in diseases of the cardiovascular system and infection.

There is some controversy about the optimal serum levels of vitamin D and no absolute consensus exists about the recommended minimum level of 25(OH)D, i.e. some authors recommend a minimum level of 50 nmol/l (Lips 2001), whereas others state that the serum level should be at least 80 or 100 nmol/l (Bischoff-Ferrari 2007, Holick 2004, Kimball *et al.* 2014, Niino *et al.* 2008).

Sunshine remains the main natural source of vitamin D, providing 80–90 % of the requirement in the absence of vitamin-rich food sources. Exposing a part of the body (for example, the face, trunk and arms) to the sun in summer can provide 10 000 IU of vitamin D in less than half an hour. But this supply disappears within a few weeks and cannot readily be guaranteed throughout the year, except in tropical countries. At latitudes beyond the 55–60th parallel, periods without a solar source of vitamin D may be significant, as long as 6–8 months per year (Diffey 2010, Holick 2004). An epidemiological study from United Kingdom detected a significant correlation between MS prevalence and end-of-summer UV index (Handel *et al.* 2010).

Recent epidemiological studies in temperate countries (mainly beyond the 40th parallels) on the adult population (15–18 years, involving both genders and mostly Caucasian people) have shown that serum levels of 25(OH)D are low, irrespective of what assays are being used (Pierrot-Deseilligny & Souberbielle 2010).

The higher circulating levels of vitamin D have been associated with a lower risk of multiple sclerosis. The protective effect on multiple sclerosis risk was particularly clear for 25(OH)D levels measured before the age of 20 years. (Munger *et al.* 2006). According to this evidence, adequate vitamin D nutrition contributes to the prevention of MS. Moreover the tradition of consuming a high-fat fish diet in a location with low solar UVR might explain why no latitudinal gradient is observed in Northern Norway (Kampman & Brustad 2008).

In Sweden, in a large cohort of women (n = 164 000) who were prospectively examined, the serum 25(OH) D levels. 25(OH) D levels \geq 75 nmol/l were associated with a 60 % decrease in MS risk compared with levels < 75 nmol/l (Salzer *et al.* 2012). Correction to an optimal level (100–150 nmol/l) is achievable with supplementation of 1000–4000 IU/day, but there no experimental evidence to support such a strategy (Ascherio 2013). The beneficial effect of UVB itself in experimental autoimmune encephalomyelitis (EAE) could refer to an independent protective role of UVB in EAE as well as in MS (Becklund *et al.* 2010). However this finding will need additional confirmation.

Vitamin's D supply in Finnish population

The population of Finland is strongly affected by a deficit in solar radiation due to the country's northern geographical location and the short duration and low intensity of sunlight in the winter (UV-index = 3–4). A deficit in dietary vitamin

D was also identified in the Finnish population in the National FINDIET 2007 Survey, which showed the average intakes of vitamin D continued to remain below the recommendations. This tendency existed all over Finland, especially in both males and females in the age group 25–35 years (Pietinen *et al.* 2010). This age at which the vitamin D supply is especially low is closely associated with the age of MS onset.

2.4.4 Life style risk factors

Smoking

Several retrospective and prospective studies have investigated the association between smoking and MS susceptibility. These studies have detected a significant increase in the MS risk among current smokers OR 1.6–1.9 American women from the Nurses' Health study and the Nurses' Health study II revealed a 70 % higher risk of MS in consistently heavy smokers (> 25 pack/years) compared with never-smokers (Hernan *et al.* 2001). In contrast, the evidence, pointing to an association between parental smoking at home and early onset MS in their children, is more controversial. In fact, there is one publication which found no evidence for any possible causal linkage between cigarette smoke and MS onset or clinical course. (Jafari & Hintzen 2011). Changes to smoking behavior may have an impact on MS incidence and MS prevention.

No association has been detected between alcohol or caffeine intakes and the risk of MS (Massa *et al.* 2013).

Childhood obesity

Findings from a population-based case-control study in Sweden and a prospective cohort study in Denmark showed that childhood obesity can be an independent risk factor for the increase in MS incidence (Hedstrom *et al.* 2012, Munger *et al.* 2013). The strongest association of risk for MS has been found in obese patients carrying HLA-DRB1*15, indicative of a genetic and lifestyle interaction (Hedstrom *et al.* 2014). The authors claimed that measures taken against adolescent obesity could well represent a preventive strategy against MS.

2.5 Comorbidity in multiple sclerosis

2.5.1 General aspects

Comorbidity exerts adverse effects on health outcomes, including functional abilities, and mortality. The phenomenon of comorbidity is common in the general population and increases with age. It is also the case that comorbidity occurs in patients with MS at the time of MS diagnosis. It has been found that comorbidity is associated with longer delays in the time between symptom onset and MS diagnosis. Furthermore, comorbidity is associated with increased disability at the time of diagnosis (Marrie *et al.* 2009c).

2.5.2 Physical comorbidity

In the North American Research Committee on Multiple Sclerosis (NARCOMS) Registry, the presence of physical comorbidities was common in MS patients (Marrie *et al.* 2008). This population-based mail-survey included all physical comorbidities. According to the responses from the self-reported diagnosis questionnaires, 77.1 % (n = 6 907) of participants with MS had at least one physical comorbidity, of whom 30.4 % had one comorbidity, 25.6 % had two and 44.1 % three or more comorbidities. The most common individual physical comorbidities were hypercholesterolemia (37 %), hypertension (30 %), arthritis (16 %), irritable bowel syndrome (13 %), and lung disease (13 %).

The age-standardised prevalence of hypertension, dyslipidemia, asthma, psoriasis, eczema and anaemia was significantly higher in MS patients compared to the corresponding values in the general Australian population. Rheumatoid arthritis and anaemia were associated with a greater relapse risk. (Tettey *et al.* 2016).

Vascular comorbidity (heart disease, arterial hypertension, hypercholesterolemia and peripheral vascular disease) and diabetes have been associated with a disability progression in patients with MS (Marrie *et al.* 2010). The nationwide Danish study revealed that patients with MS had about a 30 % higher risk of death from cardiovascular disease than the age-matched general population (Bronnum-Hansen *et al.* 2004a).

2.5.3 Mental comorbidity

According to the NARCOMS study which involved a self-report registry for patients with MS, nearly half of the participants reported a mental comorbidity (most commonly, depression and/or anxiety). Depression was also underdiagnosed and undertreated. Lower socioeconomic status was associated with increased odds of suffering depression (Marrie *et al.* 2009b).

2.5.4 Neurological comorbidity

There is very little data with respect to the prevalence of neurological comorbid diseases in MS. Most previous studies of neurological comorbidity of MS have focused on a narrow spectrum of comorbidities, such as migraine and epilepsy. The majority of the studies have revealed an increased frequency of epilepsy in patients with MS in comparison to the general population (Catenoux *et al.* 2011, Ikeda *et al.* 2010, Kang *et al.* 2010, Knox 2010, Koch *et al.* 2009, Zaccara 2009). In a study originating from Norway, epilepsy was four times more common in MS patients than in the general population (Lund *et al.* 2014). Epilepsy in RRMS patients seems to be the consequence of a particularly severe and rapidly evolving cortical pathology (Calabrese *et al.* 2012).

Several studies have shown that MS patients have an elevated risk for primary headaches such as migraine and tension-type headache, although other studies have not revealed any differences in primary headache prevalence between MS patients and general population (Ikeda *et al.* 2010, Kister *et al.* 2011, La Mantia 2009, Putzki & Katsarava 2010). The prevalence of stroke, movement disorders and peripheral nervous system disorders in MS patients has not been previously systematically evaluated.

2.6 Osteoporosis and fractures in multiple sclerosis

2.6.1 General aspects

Osteoporosis is a metabolic skeletal disorder characterized by low bone mineral density (BMD). The diagnosis of osteoporosis has typically been verified from the World Health Organization (WHO) diagnostic criteria of a T-score of -2.5 or lower as assessed at the lumbar spine, femoral neck, total hip or one-third radius sites with dual-energy x-ray absorptiometry (DXA) (Baim & Leslie 2012).

However, fragility fractures are sufficient to allow a clinical diagnosis of osteoporosis. Osteoporosis is an independent predictor of fragility (low trauma) fractures (Hernlund *et al.* 2013). Hip fractures as well as vertebral fractures are considered as typical osteoporosis-related fragility fractures. Furthermore, fragility fractures occur commonly in the arm, pelvis, ribs and distal forearm. A history of fragility fractures is predictive of further fractures and associated with significant morbidity, mortality, and health care costs all around the world (Kanis *et al.* 2004). The rates of low-trauma fractures increase with older age and decreasing bone density, although individuals without osteoporosis as assessed by DXA could sustain low-trauma fractures (Baim & Leslie 2012).

2.6.2 Osteoporosis and multiple sclerosis

One recent meta-analysis indicated that MS patients have reduced BMD in lumbar spine, femur neck and hip compared with healthy controls. Disease duration > 7 years, total cumulative steroid dose > 15 g and EDSS score > 3 were all risk factors for reduced BMD in MS patients (Huang *et al.* 2014). However, a study from Norway found evidence for low BMD in patients with newly diagnosed MS and CIS, with minor disability (median EDSS score 1.0) (Moen *et al.* 2011). Several studies pointed out that the prevalence of osteoporosis in subjects with MS is higher than in the general population (Marrie *et al.* 2009a, Hearn & Silber 2010). The prevalence of osteoporosis in MS patients in NARCOMS database has been reported to be 15.4 % (Marrie *et al.* 2009a).

Several studies have discussed the possibility that there may be common mediators in the pathogenesis of MS and osteoporosis. Both CD4+ and CD8+ T cells play roles in the pathogenesis of inflammation associated with MS. Cytokines derived from these cells, especially interleukin (IL)-1, tumor necrosis factor-&, IL6, IL11 are known to mediate the pathogenesis of osteoporosis. These cytokines have been shown to promote osteoclastogenesis, causing bone resorption (Manolagas & Jilka 1995). This finding may partly explain the results of the study of Moen *et al.* (2011), which revealed that a high proportion of MS patients have osteoporosis or osteopenia already when they are diagnosed with the disease, even in patients with CIS.

2.6.3 Osteoporosis and vitamin D deficit

Vitamin D plays a primary physiological role in maintaining extracellular calcium ion levels in the human body. Vitamin D influences calcium levels primarily by controlling the absorption of calcium from the intestine, through direct effects on bone and also through its effects on parathyroid hormone (PTH) secretion (Mithal *et al.* 2009). Furthermore, vitamin D deficiency, resulting in decreased bone mineralization, secondary hyperparathyroidism, and increased cortical bone loss, have been linked to the pathogenesis of osteoporosis and hip fractures (Lips 2001).

2.6.4 Osteoporosis and glucocorticoid use

The long-term use of glucocorticoids leads to glucocorticoid induced osteoporosis (GIO). GIO is the most common form of secondary osteoporosis (Mazziotti *et al.* 2006). There is convincing evidence to indicate that a dose of 5 mg per day or more of prednisolone equivalent for 3 months or longer increases the risk for fracture (van Staa *et al.* 2002). The risk of fracture was found to increase rapidly after the start of oral corticosteroid therapy (within 3 to 6 months) and to decrease after termination of therapy. The risk remained independent of underlying disease, age and gender (van Staa *et al.* 2002). With respect to the pathophysiology of GIO, glucocorticoids are known to exert both direct and indirect effects on bones. The primary effects on bone cells are mediated via the osteoblasts and osteocytes. These effects lead to the suppression of bone formation, a central feature in the pathogenesis of GIO. Glucocorticoids also inhibit calcium absorption from the gastrointestinal tract, by antagonizing the effects of vitamin D and by decreasing the expression of specific calcium channels in duodenum. Renal calcium resorption is also inhibited by glucocorticoids which explains why secondary hyperparathyroidism can be present in the context of glucocorticoid use (Canalis *et al.* 2007).

2.7 Risk of fractures in patients with multiple sclerosis

The phenomenon that MS patients suffer more bone fractures than the normal population was recognized in the early 1990s (Stenager & Jensen 1991). In that study, it was noted that the incidence of fractures increased with increasing age, disease duration and disability. In corticosteroid-treated MS patients, relatively

high or low cumulative doses of steroids did not correlate predictably with the occurrence of fractures (Troiano *et al.* 1992). New data have indicated that patients with MS may be at an increased risk of fracture due to the greater risk of falling and decreased bone mineral density when compared with the general population (Bazelier *et al.* 2011, Bazelier *et al.* 2012). Osteoporosis results in bone mineral loss and reduced bone mass leads to increased fracture rates as revealed by Cosman *et al.* (1998). In that study, fractures in the absence of major trauma had occurred in 2 % of controls but many more, 22 %, of MS patients (Cosman *et al.* 1998) Bone loss verified by DXA in the spine was greater in women with MS than in controls (1.6 to 3.5 % per year loss in MS patients versus no change in controls). Furthermore, bone loss in the spine occurred faster in MS patients with low (< 20 ng/mL) 25-hydroxyvitamin D levels.

In a recent study from Kansas University, American patients with MS had a more than two times higher risk for suffering fractures than controls in large retrospective analysis lasting over 20 years (Bhattacharya *et al.* 2014). This risk was independent of age, gender, race with patients sustaining these fractures at a younger age than controls. The limitation of this study was a lack of information concerning the severity and duration of MS and it was not ascertained whether the rate of falls in the MS cohort was different from the non –MS cohort. Several complex factors, may be involved in accounting for the reduced bone mass in MS patients including e.g. low vitamin D levels and use of certain medications such as glucocorticoids and anticonvulsants. Physical inactivity and reduced mechanical load of the bones may also play a role as well as the inflammatory processes of the disease (Gupta *et al.* 2014).

3 Aims of the research

Very few epidemiological studies into MS have been conducted in areas close to the Arctic Circle. The general aim of this work was to study the epidemiology of MS in the Northern Finland population, with a focus on prevalence- and incidence trends, survival, neurological comorbidity and fracture risk.

The specific aims of the study were:

1. To evaluate the incidence and prevalence of MS in Northern Ostrobothnia.
2. To investigate neurological comorbidities in MS and to recognize the associations of neurological comorbid diseases with gender, clinical course, disability level and duration of MS.
3. To study how comorbid neurological diseases may influence on the survival rates of patients with MS.
4. To define the prevalence of low-energy fractures in patients with MS.
5. To assess the differences in the EDSS level and duration of MS at the time of a fracture event.

4 Subjects and methods

4.1 Location of the study population

Northern Ostrobothnia is situated in Northern Finland, near to the Arctic Circle, at 65°1' N latitude and 25°25' E longitude. The population of the region on 31st December 2007 (Study I) was 386 972, accounting for about 7.3 % of the total population of Finland, and the female/male ratio was 0.99. The population of the region on the 31st of December, 2010 (Study II–III) was 398 335, which was equivalent to 7.4 % of the total population of Finland, and the female/male ratio was the same as in Study I. The region is served by Oulu University Hospital (OUH) and a large number of general practitioners. The diagnosis and treatment of MS and other neurological diseases are concentrated in OUH; thus OUH gathers broad information about the comorbidity of other neurological diseases and fractures. The population data were obtained from Statistics Finland (<http://www.tilastokeskus.fi>).

4.2 Ethics

The study protocols of all the studies were approved by the Ethics Committee of the Northern Ostrobothnia hospital district and followed the principles of the Declaration of Helsinki.

4.3 Subjects

The three study cohorts have been collected in the Northern Ostrobothnia district: study cohort 1 (*prevalence cohort*, prevalence day 31st December 2007), study cohort 2 (*incidence cohort*, which covers the time period 1992–2007) were collected for Study 1. Study cohort 3 (*comorbidities cohort*, which covers the time period 2000–2010) was used in Study II and Study III.

Study cohort 1: all living patients with clinically definite MS registered with neurologists at OUH or with the local authority health centers in Northern Ostrobothnia on 31st December 2007 were included into *the prevalence cohort*.

Study cohort 2: All new clinically definite MS cases identified in the 16 years from 1st January 1992 until 31st December 2007 were included into *the incidence*

cohort. Patients with MS already diagnosed in other departments of neurology were excluded from the incidence cohort.

This time interval (1992–2007) was chosen because all definite clinical diagnoses of MS would have been based on the same criteria. The diagnosis of MS in each case was made by a specialist neurologist and verified not only clinically but also with laboratory tests and brain MRI according to the Poser criteria (Poser *et al.* 1983) and early McDonald criteria of 2001 (McDonald *et al.* 2001b). The new revised McDonald criteria of 2005 were taken into use in the Department of Neurology only on January 1st 2008 (Polman *et al.* 2005). All subjects were identified from the hospital records by reference to code 3400A in ICD 9 (1st January 1992–31st December 1995) and code G35 in ICD 10 (1st January 1996–31st December 2007).

Study cohort 3: all new, clinically definite MS cases, $n = 491$ were identified and included to the study cohort 3 (*comorbidities cohort*) during the 21-year time interval from 1st January 1990 through 31st December 2010 among the population of Northern Ostrobothnia. The diagnoses of MS were made by a neurologist who verified the diagnoses according to the Poser criteria (Poser *et al.* 1983), early McDonald criteria of 2001 (McDonald *et al.* 2001a) or the McDonald criteria of 2005 (Berg *et al.* 2010, Polman *et al.* 2005) and details were gathered about sex, age at onset, age at MS-diagnosis, age at death, clinical course type (i.e. RRMS, PPMS and benign) (Poser *et al.* 1983, Weinshenker 1995a). The classification of MS as benign was based on EDSS scores of 0–3 over a duration 10 years from the verified MS diagnosis (Poser *et al.* 1983, Weinshenker 1995a). The severity of the MS (EDSS score) was evaluated in the year of MS diagnosis, at the time 10 years from diagnosis and/or at the time of the last evaluation. In this cohort, 93.3 % of patients suffered from RRMS, and 6.7 % of cases were diagnosed as PPMS. The cases defined as benign in this study represented 17.9 % of all the RRMS cases. The mean age at onset was lower in patients with RRMS compared to patients with PPMS ($p < 0.005$), and the mean age at onset of MS was lower in females than in the males with RRMS ($p = 0.05$).

4.4 Neurological comorbidity

Data concerning neurological comorbidity (Study II) were identified by a systematic review of hospital records according to the following criteria: doctor diagnosed migraine (both without and with aura), clinically verified epilepsy, anamnesis and/or typical clinical status of movement disorders (e.g., Parkinson's

disease, essential tremor and restless legs syndrome), ischemic stroke with diagnostic findings based on brain imaging, peripheral nervous system involvement verified by ENMG, cranial neuralgias with typical anamnesis, and MRI-verified brain tumors. The types of epileptic seizures terms were assigned according to the International League Against Epilepsy (ILAE) Revised Classification of Seizures 2010 (Berg *et al.* 2010).

4.5 Assessment of fractures

All fracture types (Study III) were identified from the patients' hospital records. Patients who had sustained fractures before the age of 15 years or a cranial fracture at any age were excluded from the study. All fracture events were classified as high- or low-energy (fragility) fractures (Flinkkila *et al.* 2011, Morrison *et al.* 2013). A high-energy injury was defined as that occurring from a sports activity, a fall from a height of more than 1 m, a bicycle accident, a motor vehicle collision, or a major intrinsic event (e.g., a stroke, syncope, or epileptic seizure). Fractures that were not associated with these high-energy events were defined as low-energy fractures. A low-trauma fall was defined as a fall from standing height such as slipping, tripping, or stumbling on a flat surface or stairs. Non-fall fractures were also identified.

A diagnosis of osteoporosis was confirmed from the hospital records and was based on the results of DXA or an X-ray based assessment showing an osteoporotic bone structure. A subset of patients received a BMD examination for osteoporosis as part of their clinical treatment and investigations. A clinical osteoporotic fracture has been defined as a fracture of the radius, ulna, humerus, rib, femur, hip, pelvis, or vertebrae. Clinical osteoporotic fractures were limited only to those occurring in these anatomical locations for the purposes of comparison. None of the present cohort had suffered rib or pelvic fractures. Fragility fractures of fingers, toes and ankle were excluded from calculations of clinical osteoporotic fractures. The fracture type as well as the association between the duration of MS and EDSS score with a fracture event were determined.

4.6 Statistical methods

Parametric t-tests were used to analyses baseline differences between the study groups. In all studies P value of less than 0.05 was considered to be statistically

significant. The derivation of P values was made by application of proper statistical tests. All statistical analyses were performed using SPSS for Windows, (IBM Corp. Released 2010–2011. IBM SPSS Statistics for Windows, versions 19.0 and 20.0. Armonk, NY: IBM Corp.).

Statistical methods in Study I Prevalence and incidence of MS

Crude prevalence and incidence were estimated by the direct method, incidence being calculated for one-year time intervals in 1992–2007, both overall and by gender. Rates were given with 95 % confidence intervals. Prevalence was defined as (Armitage & Berry 1995)

$$P = \frac{\text{total number of cases at given time}}{\text{total population at that time}} * 100\ 000 \quad (1)$$

Incidence was defined as (Armitage & Berry 1995)

$$I = \frac{\text{number of new cases in period of time}}{\text{population at risk}} * 100\ 000 \quad (2)$$

Statistical methods in Study II Neurological comorbidities

Associations between neurological comorbidities and MS have been evaluated in study cohort 3. (time interval 2000–2010) according to the duration, gender, disability level and course were systematically analysed for each neurological comorbid nosology and for overall neurological comorbidity by nonparametric tests (Chi-squared or Fisher's exact test). The associations were compared with duration of MS disease subdivided into four groups: less than 5 years, from 5 to 10 years, from 10 to 15 years and more than 15 years from MS diagnosis. In order to analyse the association with disability, patients were divided into two groups. In group one, the EDSS levels were equal to or less than 3, in group two, EDSS levels were greater than 3 at the time of the last evaluation. The survival rate was analysed using Kaplan-Meier curves and the log-rank test. The time points of evaluation were 21 years from both the first symptom and time of MS diagnosis. The use of Kaplan-Meier curves is beneficial because of the time factor, especially when that factor has a major impact on mortality rate. All analyses were performed using a univariate rather than a multivariate approach due to the relatively small subgroup sample sizes. Log rank tests significance was assessed when $p < 0.05$.

Statistical methods in Study III Fractures

The baseline characteristics of MS patients with osteoporosis and fractures were compared using the Chi-square test in study cohort 3 (time interval 2000–2010). A scatter-plot was used to analyse the association of disability and age at the time of a fracture event with the distribution of fractures according to nature of the fracture (low- vs. high-energy). When assessing the association of steroid administration with the likelihood of a fracture, three subgroups were defined based on the administration profile of short courses of steroids during the follow-up: group 1, patients who did not receive steroids; group 2, patients who received fewer than five or five short courses of methylprednisolone; and group 3, patients who received more than five short courses of methylprednisolone. Kaplan-Meier analysis was used to estimate the proportion of MS patients who sustained fractures during the early stage of MS and was performed because of differences in the follow-up duration after MS diagnosis.

5 Results

5.1 Prevalence and incidence of multiple sclerosis in Northern Finland

The province of Northern Ostrobothnia prevalence cohort (Table 5) contained a total of 397 (F/M = 69.5 % / 30.5 %). Patients incidence cohort (Table 6) comprised a total of 374 patients (F/M = 68.4 % / 31.6 %) with a clinically definite diagnosis of MS. The female/male ratio was 2.2. Diagnostic MRI and CSF findings have being reported in Table 7.

Table 5. Characteristics of the prevalence cohort (all MS cases 31.12.2007¹). Study I.

Gender	Count		Age at diagnosis (years)	
	n	%	mean	range
Female	276	69.5	35.1	12–65
Male	121	30.5	36.0	16–62
Total	397	100.0	35.4	12–65

¹ the prevalence date chosen

Table 6. Characteristics of the incident cohort (all new cases 1992–2007). Study I.

Gender	Count						Age			
	PRMS		PPMS		Total		at onset		at diagnosis	
	n	%	n	%	n	%	mean	range	mean	range
Female	240	64.2	16	4.3	256	68.4	30.9	11–58	35.1	12–62
Male	108	28.9	10	2.7	118	31.6	33.5	15–59	37.6	16–62
Total	348	93.0	26	7.0	374	100.0	31.7	11–50	35.9	12–65

Table 7. MRI scan and CSF analysis in study I. Incidence cohort 1992–2007

Gender	Brain MRI ¹ %	Cervical MRI %	TH MRI %	CSF ² -OC ³ %	CSF IgG index %
Female (n = 256)					
available	100.0	52.7	36.7	95.3	95.3
MS related	91.4	32.4	17.2	79.1 ⁴	76.6 ⁵
Male (n = 118)					
available	99.2	56.7	46.6	86.4	93.2
MS related	91.5	36.4	20.3	72.0 ⁴	67.8 ⁵
Total (n = 374)					
available	99.7	53.7	39.8	92.5	94.6
MS related	91.4	33.7	17.2	76.9 ⁴	74.2 ⁵

¹ magnetic resonance image, ² cerebrospinal fluid, ³ oligoclonal fractions, ⁴ MS related means positive,

⁵ MS related means elevated

The overall prevalence of MS in the province of Northern Ostrobothnia was 103/10⁵ (95 % CI, 93–113). The prevalence of MS in women was 144/10⁵ (95 % CI, 128–162) and 62/10⁵ (95 % CI, 52–74) in men.

The mean overall incidence was 6.3/10⁵ (95 % CI, 5.2–7.2), person-years 5844638 (1992–2007). The incidence of MS in women was 8.6/10⁵ (95 % CI, 7–10.2), person-years 2937274.00 and 3.9/10⁵ (95 % CI, 3.1–4.7) in men, person-years 2953414.00. The incidence of MS increased during the 16-year period. This elevated incidence was clearly attributable to the pronounced increase among females, since no corresponding increase occurred in males. The mean age at the onset of MS was significantly lower in the females (31 years) than in the males (33.5 years) $p < 0.023$ (t-test), as also was the age when the clinically definite diagnosis was made (35.1 vs. 37.6 years), $p < 0.036$ (t-test).

5.2 Neurological comorbidities

5.2.1 Neurological comorbidities in general

One or more neurological comorbid disease was present in 17.1 % of MS patients (n = 84/491). The majority of patients were experiencing one (94 %, n = 79) neurological comorbid disease at the time of evaluation. However, 6 % cases suffered from two (n = 5) neurological comorbid diseases.

Table 8. The frequencies of comorbid diseases and their correlation between gender, clinical course, disability impact and duration of MS.

Disease	n	%	Gender ¹	Course ¹	Disability ¹	Duration ¹
Migraine	51	10.4	0.151	0.101	0.669	0.663
Epilepsy	23	4.7	0.102	0.675	0.266	0.181
Stroke	6	1.2	0.672	0.391	0.611	0.023*
Movement ²	4	0.8	0.269	0.075	0.112	1.000
PNSD ³	5	1.0	0.645	0.027*	1.000	0.066
Total	89	18.1	0.903	0.387	0.402	0.121

¹p-value, ² movement disorders, ³ peripheral nervous system disease, * p-value < 0.05

5.2.2 Migraine

Migraine was the most common neurological disease in patients with MS. Doctor-diagnosed migraine was observed in 10.4 % of MS patients (78.4 % female). Migraine was more prevalent in women with benign MS compared to those patients with other types of the disease.

5.2.3 Epilepsy

The frequency of epilepsy was 4.7 %, and it was the second most common comorbid disease encountered in these MS patients. The mean age at the first epileptic seizure was 36.6 years (range 16–62). Epilepsy was diagnosed before MS onset in 20 % of cases. An epileptic seizure was the first symptom of MS disease in one case. EEGs were available from 83 % of the patients with epilepsy. The EEGs were normal in 26.3 % and related to epilepsy in 73.7 % of cases. The most common type of seizure was a focal seizure that evolved into a bilateral tonic-clonic seizure (47.8 %). Generalised tonic-clonic, generalised myoclonic and focal seizures were also diagnosed. Status epilepticus was diagnosed in only one case.

Antiepileptic treatment had been started in 91 % (n = 21) of the patients with epilepsy. Epilepsy was diagnosed, and antiepileptic treatment had been started after the first seizure in 25 % of the patients. At the last evaluation, 61 % of all MS patients with epilepsy were seizure-free, and one patient suffered less than one seizure per year. Three cases (14.3 %) were experiencing more than one seizure per year. One patient had seizures at a rate of more than one per month.

5.2.4 Stroke

The frequency of ischemic stroke was 1.2 % (n = 6, five females) in the cohort. The mean age at stroke occurrence was 43.1 years (range 30–57). The mean age at MS onset in the stroke patients was 39.7 years (range 17–58). Two patients suffered a stroke before MS onset (one 10 years before, in the other the stroke occurred 24 years before MS onset). One patient had both an MRI-verified stroke and the first MS symptoms in the same year. The etiologies of the ischemic strokes remained unknown. However, heart diseases and the risks for cardiac embolism were present in two patients in the follow-up. In the present study, a longer MS disease duration correlated with a higher stroke possibility. Two cases died after the stroke; one of these cases died two years after the stroke, the other died 31 years after the stroke.

5.2.5 Peripheral nervous system disorders

Five MS patients were identified with peripheral nervous system disorders. Two of these cases had PPMS, and three suffered from RRMS. Four patients had polyneuropathy, and one had a bilateral L-5 radiculopathy due to spinal stenosis. ENMG were performed in all of these cases. Peripheral nervous system disease was diagnosed after MS onset in all cases. The mean time between MS onset and peripheral nervous disease onset was 7.8 years (range 2–14). There was a significant correlation between the incidence of peripheral nervous system disease and PPMS. Both of the PPMS patients were males, and one suffered from distal sensorimotor polyneuropathy, while the other had a bilateral ulnar demyelinating neuropathy.

5.2.6 Movement disorders

Four patients with movement disorders were identified in the cohort. Idiopathic Parkinson's disease was diagnosed in one patient eight years after PPMS onset. One patient suffered from essential tremor, and two patients had restless legs syndrome. The prevalence of restless legs syndrome in this cohort was 0.4 %.

5.2.7 Other neurological disorders

Malignant brain tumors were not detected in the cohort.

5.3 Survival in multiple sclerosis

Table 9. Characteristics of the MS cohort 3 (all new cases 1990–2010). Studies II-III.

Cohort	Female	Male	Total
N (%)	340 (69.2)	151 (30.8)	491 (100)
Age at onset mean / range	31.0 / 11–65	33.9 / 15–65	32.0 / 11–65
Age at diagnosis mean / range	35.3 / 12–67	38.2 / 16–70	36.2 / 12–70
Dead N (%)	7 (2.1)	10 (6.6)	17 (3.5)
Age at death mean / range	45.0 / 25–70	57.1 / 45–74	52.1 / 25–74
RRMS ⁴ N (%)	254 (74.7)	122 (80.8)	376 (76.6)
Age at onset mean / range	30.8 / ² 11–65	32.9 / ² 15–59	31.5 / ¹ 11–65
Age at diagnosis mean / range	35.0 / 12–67	37.3 / 17–67	35.8 / 12–67
Dead N (%)	4 (1.6)	7 (5.7)	11 (2.9)
Age at death mean / range	34.0 / ³ 25–49	54.6 / ³ 45–68	47.1 / 25–68
PPMS ⁵ N (%)	19 (5.6)	14 (9.3)	33 (6.7)
Age at onset mean / range	40.8 / 21–58	47.9 / 22–65	43.9 / ¹ 21–65
Age at diagnosis mean / range	44.6 / 21–59	53.2 / 34–70	48.3 / 21–70
Dead N (%)	3 (15.8)	3 (21.4)	6 (18.2)
Age at death mean / range	59.7 / 54–70	63.0 / 57–74	61.3 / 54–74
Benign ⁶ N (%)	67 (19.7)	15 (9.9)	82 (16.7)
Age at onset mean / range	29.5 / 18–49	28.1 / 16–49	29.2 / 16–49
Age at diagnosis mean / range	33.9 / 20–65	31.4 / 16–50	33.5 / 16–65
Dead	0	0	0

¹ p < 0.005 (Total, RRMS vs. PPMS), ² p = 0.05 (Male vs. Female), ³ p = 0.005 (Male vs. Female),

⁴ relapsing-remitting MS, ⁵ primary progressive MS, ⁶ 'benign' cases

Over a 21 year period, a total of 17 (3.5 %) deaths occurred in the study cohort 3 (n = 491). The mean age at death was 52.1 (range 25–74 years). The age at death for females was lower (mean 34.0 years) than that for males (mean 54.6 years) with RRMS (p = 0.005) (Table 9). All cases, which identified as benign MS were still alive at the point of evaluation. The mean time of the delay from onset to diagnosis in total cohort was 4.2 years.

The 21-year survival rate was 90.5 % from the time of MS diagnosis. The 21-year survival rate from the time of MS onset was 95 %, however, this rate declined to 73 % after 40 years from the onset of MS. The 21-year survival rate was significantly lower in MS patients with stroke compared to those patients not suffering a stroke. No other differences in survival were observed between MS patients with or without neurological comorbidities (Figure 1).

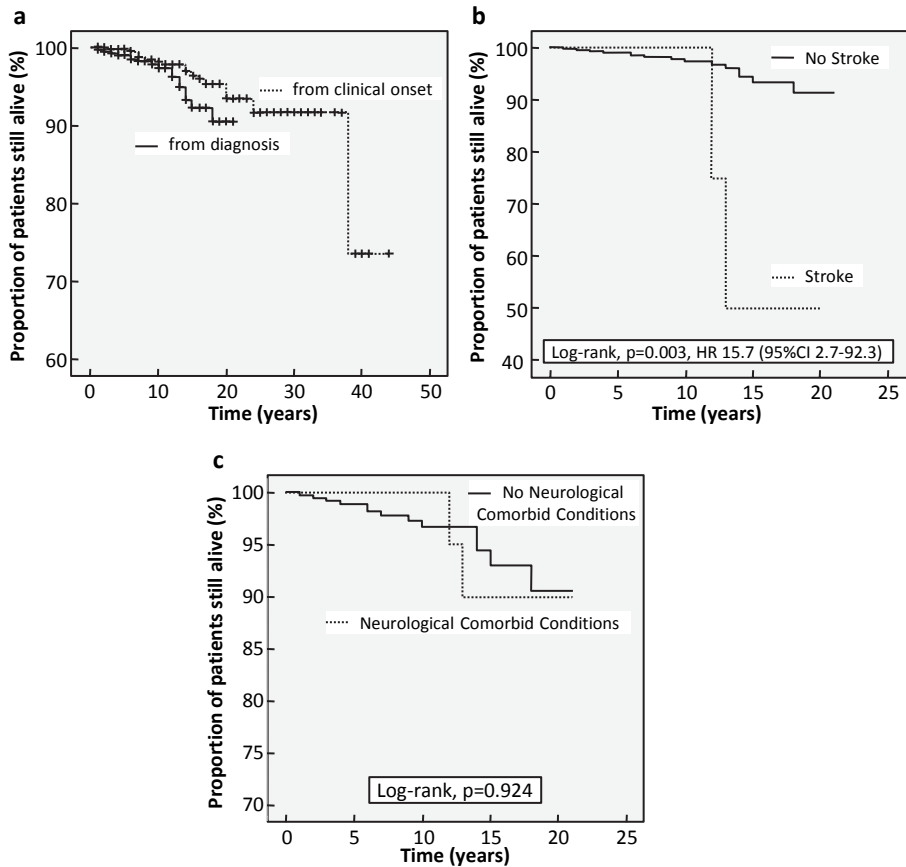


Fig. 1. 21-Year survival rates (a) from time of MS onset and from time of MS-diagnosis in the Finnish Northern Ostrobothnia MS-cohort, (b) with stroke as neurological comorbid disease and (c) with neurological comorbid disease. (Krökki *et al.* 2014, published by permission of Elsevier).

5.4 Fractures and osteoporosis

The fracture types as well as the association between the duration of MS and the EDSS score with a fracture event were determined in *study III*.

The majority of fractures were low-energy fractures and the majority of fractures had occurred within early stage of MS disease in non-disabled to moderately disabled patients (EDSS score 0–4).

In all, 39 MS patients (8.0 %) were identified with at least one fracture event and there were a total of 52 (10.6 %) fracture events in the cohort. The majority of patients (n = 28) with fractures experienced a single fracture event. However, nine patients sustained two fracture events, and two patients suffered three fracture events. The first fracture event occurred within one year after MS diagnosis in 53.8 % (n = 21) of the 39 MS patients having experienced at least one fracture event, with fourteen of those fractures occurring before the MS disease had been diagnosed. The percentage of patients experiencing a first fracture event cumulatively increased to 69.2 % (n = 27) within five years after the verified diagnosis of MS. According to a Kaplan-Meier analysis of the entire MS cohort, the probabilities of sustaining fractures within five, 10, and 20 years after MS diagnosis were 5.7 %, 6.9 % and 14.2 %, respectively.

The most frequent fracture sites were a single vertebra (19.2 %), distal forearm (19.2 %), ankle (25 %), and proximal femur (9.6 %). Thirty-five fracture events (67.3 %) satisfied the criteria of a low-energy fracture, and the overall frequency of low-energy fractures was 7.1 %. The majority of fragility fractures occurred in MS patients who were not disabled or mildly/moderately disabled (EDSS score 0–4) There was no correlation between the location of the fracture and MS severity. When using the same criteria for osteoporotic fractures as previous registry studies, the prevalence of osteoporotic fractures was estimated to be 5.9 % (29/491). Of these fractures, 3.6 % (n = 18) were characterized as low-energy fractures. All of the femur fractures, 60 % of the vertebral fractures, 77 % of the ankle fractures, and 60 % of the distal forearm fractures were classified as low-energy fractures.

Taking into account all of the fracture events, the mean age of patients when fractures occurred was 41 years (range, 17–62 years). The mean patient ages at which high-energy and low-energy fracture events occurred were 37.1 years (95 % CI, 31.3–43.0 years) and 42.5 years (95 % CI, 38.3–46.6 years), respectively. There was no significant difference between the age of patients who sustained fragility fractures and the age of patients who sustained high-energy fractures. Furthermore, when patients were grouped according to their gender or the course of their MS disease course, there were no significant intergroup differences in patient age at the time of their fracture. Similarly when patients were categorized according to the numbers of short courses of methylprednisolone administration, there were no significant intergroup differences in the risk of fragility fractures.

Patients who sustained low-energy fractures were not routinely investigated for osteoporosis. A DXA scan was only performed in 26 % of fragility fracture cases, but 89 % of these patients were suffering from osteoporosis. An osteoporotic bone structure was specified in the X-ray findings of one case.

Table 10. Characteristics of fractures.

Type of fracture ¹	Total fracture patients		Total fracture events		High-energy fracture events		Low-energy fracture events		Fracture events from the cohort % (n = 491)
	N	%	N	%	N	%	N	%	
Femur	3	7.7	5	9.6	0	0.0	5	100.0	1.02
Humerus	4	10.3	4	7.7	3	75.0	1	25.0	0.81
Vertebrae	9	23.1	10	19.2	4	40.0	6	60.0	2.04
Distal forearm	8	20.5	10	19.2	4	40.0	6	60.0	2.04
Ankle	7	17.8	13	25.0	3	23.1	10	76.9	2.65
Shin bone	4	10.3	6	11.5	1	16.7	5	83.3	1.22
Finger, toe	4	10.3	4	7.7	2	50.0	2	50.0	0.81
Total	39	100.0	52	100.0	17	32.7	35	67.3	10.6

¹ Cranial fractures and fracture age under 15 years are excluded.

6 Discussion

6.1 Prevalence and incidence of multiple sclerosis in Northern Finland

6.1.1 Prevalence

This study is the first epidemiological survey conducted near to the Arctic Circle $65^{\circ}1' N$ latitude in Northern Finland. The overall prevalence of MS in Northern Ostrobothnia was determined to be $103/10^5$. The prevalence of MS was over twofold higher in women than in men in the study region. There is no nationwide MS prevalence survey for the whole of Finland, but the prevalence rates have been previously investigated in four regions of Finland: in the southern Uusimaa region, in western regions of Vaasa and Seinäjoki and also in central Finland. The uneven distribution of MS in those regions had been revealed in those previous studies (Kinnunen *et al.* 1983, Rinne *et al.* 1966, Sarasoja *et al.* 2004, Sumelahti *et al.* 2000, Sumelahti *et al.* 2001, Tienari *et al.* 2004). The clear and latitudinal gradient of MS prevalence have been confirmed globally in the recent most comprehensive meta-analysis of MS prevalence in 59 countries in a long time period, from 1923 until 2009 (Simpson *et al.* 2011). The prevalence of MS has been shown to increase with latitude, reaching a peak around 55° , before changing to a significant inverse gradient above 60° . When comparing the present results with previous Finnish epidemiological studies, there is no evidence for the north-to-south latitudinal gradient. In Finland, the highest prevalence of MS has been found in the middle of the Finland in the Seinäjoki region ($188/10^5$) which has a latitude of $62^{\circ} N$ (Sumelahti *et al.* 2001). However, the prevalence of MS seems to be similar in other parts of Finland independently from the latitudinal gradient inside Finland. The same tendency has been found in a recent study from Norway (Berg-Hansen *et al.* 2014). The prevalence of MS in the southern and northern regions of Norway was similar, but a higher MS prevalence was observed in middle Norway. The overall prevalence in the northern region of Tromsø (latitude $69^{\circ}38' N$) was $191/10^5$ and $249/10^5$ in middle Norway (Trondheim latitude $63^{\circ} N$). In Sweden, the prevalence of MS in 2008 was also higher than in Finland being 188.9 per 100 000 and in that country the risk of MS has been found to increase with increasing northern latitude in both men and women (Ahlgren *et al.* 2011).

6.1.2 Incidence

The mean overall incidence of MS in Northern Ostrobothnia was $6.3/10^5$ in this study during the time period 1992–2007. A similar total incidence of MS $6.7/10^5$ has been found also in Pirkanmaa during the time period 1981–2010 (Holmberg *et al.* 2013) and total incidence $5.2/10^5$ in Central Finland region in 1979–1998 (Sarasoja *et al.* 2004). The last-calculated incidence of MS is highest in western Finland, especially in Seinäjoki ($14.7/10^5$) and Vaasa ($11.7/10^5$) (Holmberg *et al.* 2013). The present study estimated an annual fluctuation of MS incidence, however it seemed that there was an evident increase occurring in the incidence from 6.2 to $11.8/10^5$ during the study period 1992–2007. The peak incidence, 14.1 , occurred in 2000 in Northern Finland.

This increase in the MS incidence is due to the increasing incidence among women. Similar findings with the increasing incidence of MS in women throughout a 30-years' follow up have been reported in recent Finnish studies from the University Hospital District of Tampere (including Pirkanmaa, Seinäjoki and Vaasa regions) (Holmberg *et al.* 2013, Sumelahti *et al.* 2014).

A recent study from UK claimed that there was a downward trend in the incidence in both women and men in the two most recent decades, i.e. 1990–2010 (Mackenzie *et al.* 2014) However, an increase in the MS incidence in women has been found in the majority of other epidemiological studies (Alonso & Hernan 2008). In Denmark, the incidence rates for women increased from $4.61/10^5$ to $11.85/10^5$ during the period 1973–2002, whereas only a small increase was observed in men (Koch-Henriksen & Sorensen 2010). The reasons of these differences are still unclear. This phenomenon cannot be explained by genetics and can only partly be explained by better diagnostic certainty due to more sensitive diagnostic criteria and more intensive evaluation. These changes in the female incidence occurring over such a short period of time point to environmental risk factors, such as urbanization of lifestyle (e.g. a relative increase in the numbers of women smokers, oral contraception and use of pasteurized cow milk (Kotzamani *et al.* 2012). Obesity at ages 18–20 ($BMI \geq 30 \text{ kg/m}^2$) has been found to double the risk of MS in women (Munger *et al.* 2009). The important role of a deficit of vitamin D and low life-long levels of UV radiation as well as the role of sex hormones and cytokines have been postulated as the reasons for the gender differences in MS in a few studies (Eikelenboom *et al.* 2009, Kragt *et al.* 2009). The age when a woman has her first child has not been found to influence the risk of MS (Magyari *et al.* 2013).

Lifestyle risk factors, as well as high-latitude geographical location, combined with decreased exposure to solar radiation and vitamin D deficiency may play a role in the disproportionate increase encountered in the MS incidence in females living in Northern Ostrobothnia.

6.2 Neurological comorbidities and their impact on survival

The median survival from birth in the MS population was 75.9 years vs 83.4 years in the matched population (Marrie *et al.* 2015).

Comorbid diseases may exert effects on MS patients' prognosis and survival. The majority of studies into this topic have concentrated on psychiatric and immunological diseases or cancer in MS patients (Marrie *et al.* 2015). Neurological comorbid diseases have been mainly restricted to epilepsy and migraine (Catenoux *et al.* 2011, Kang *et al.* 2010, Koch *et al.* 2009, Zaccara 2009). However, systematical population based studies are rare and in particular, there is virtually nothing known about the association between these other diseases and the survival times of MS patients. The contribution of comorbidities to survival in MS received little attention before the appearance of the present study (Marrie *et al.* 2015). Here, neurological comorbid diseases were examined in MS patients with hospital based settings; the aim was also to evaluate the association between comorbidity status and mortality.

It was found that neurological comorbidity was rather common in MS patients; however, most patients suffered from only one neurological condition in addition to MS. Migraine and epilepsy were the most common comorbid neurological diseases in these MS patients. Several studies have shown an increase in the risk of migraine in patients with MS (Doi *et al.* 2009, Kang *et al.* 2010); however, these findings are controversial (Putzki and Katsarava, 2010). In previous studies, epilepsy has been the most widely studied neurological disease among MS patients and in all reports it has been found to be more prevalent in MS patients when they are compared to non-MS populations (Poser & Brinar 2003, Forsgren *et al.* 2005, Zaccara 2009, Catenoux *et al.* 2011). Here, other neurological comorbid diseases were also identified; there were a few MS patients with peripheral nervous system disorders, restless legs syndrome and one case with idiopathic Parkinson's disease. However, because the lack of population based controls, it is not possible to evaluate the amount of those diseases compared to the non-MS population.

There are rather few reports investigating the prevalence of stroke, cardiovascular diseases, hypertension, diabetes mellitus type II or hyperlipidemia in patients with MS (Roshanisefat *et al.* 2014). Here, the prevalence of stroke in MS patients was similar to the age-matched Finnish population (Meretoja *et al.* 2010). In previous studies, patients with MS have been reported to have a higher risk of suffering ischaemic stroke than those without MS (Allen *et al.* 2008, Christiansen *et al.* 2010). A slightly increased relative risk for cardiovascular diseases (risk ratio (RR) 1.31) in MS patients has been found in Sweden (Roshanisefat *et al.* 2014). These investigators also reported an increased risk for ischemic stroke in RRMS in comparison with the general population (RR 2.57). The strokes were detected closely after MS onset and the researchers speculated that the increased frequency of ischaemic stroke in MS was most probably due to surveillance bias resulting from diagnostic investigations being conducted for MS. However, in the present study, the duration of MS disease, but not the age of the MS patient, significantly increased the risk of stroke occurrence which is at odds with the Swedish study. It was also found that stroke was clearly associated with increased mortality in the patients with MS. All-cause mortality has been found to be 2–2.9-fold higher in the MS patients compared to other populations (Sumelahti *et al.* 2010, Capkun *et al.* 2015, Goodin *et al.* 2015). The causes of mortality of MS patients are variable, but major causes of death have been due to infections, pulmonary and cardiovascular diseases or suicide (Capkun *et al.* 2015, Goodin *et al.* 2012, Goodin *et al.* 2014). An increased mortality from cardiovascular diseases, but not from ischaemic stroke, in MS patients has also been reported (Bronnum-Hansen *et al.* 2004).

One interesting result emerging from our study is that the 21-year survival rate from diagnosis to death by any cause (90.5 %) was higher than in other previous reports, i.e. in the study of Goodin *et al.* (2012) examining 21-year survival rate in interferon- β treated MS patients. In the previous Finnish study conducted by Sumelahti *et al.* (2002) the 25-year survival rate was 62 % from the time of diagnosis to death by any cause. However, in that work (Sumelahti *et al.* 2002) the MS cohort covered the period 1964–1993, before the introduction of immunomodulatory treatments in Finland and therefore, it reflects the survival rate of the natural course of MS. Instead in the present study, most of the patients had received immunomodulatory treatments, which may have influenced their survival. Furthermore, in the present study, the cohort size was somewhat small.

6.3 Low-energy fractures, osteoporosis and multiple sclerosis

In the present cohort, 8 % of MS patients have experienced at least one fracture event and the prevalence of all fractures events was about 10 % (Table 10). Using the same criteria for osteoporotic fractures as applied in previous registry studies, the prevalence of osteoporotic fractures was estimated at about 6 %. This is less than in MS registry based (NARCOMS) data in which more than 25 % of MS patients had low bone mass and 15 % of cases had a history of fracture after age of 13 years (Marrie *et al.* 2009a). However, similar findings than found here have been reported in three other studies in which the prevalence of all fractures in patients with MS has varied between 2 % and 10 % (Bazelier *et al.* 2011, Bazelier *et al.* 2012a, Bazelier *et al.* 2012b). A recent meta-analysis described a significant association between MS and fracture risk (RR = 1.58) (Dong *et al.* 2015). There is no epidemiological data of fractures, fragility fractures or osteoporosis in the general Finnish population. Thus it is not possible to make any comparison between the frequency of fractures in the MS cases and the general population. However, some features were identified which give rise to the speculation that especially fragility fractures may be associated with MS itself.

The symptoms of MS which include muscle weakness, poorer postural balance, stiffness, numbness, tingling, impaired vision, fatigue, dizziness, disability, or spasticity with reduced physical activity and all of these symptoms could be responsible for a fall in an MS patient. However, it was found that the accidents occurred in over half of the MS patients within one year after MS diagnosis and in patients with no or mildly/moderately disability and only occasionally in the advanced phase of the disease. In addition, chronic inflammatory processes, vitamin D deficiency, and glucocorticoid use may increase the risk of osteoporosis and fractures in patients with MS (Hearn & Silber 2010). Low BMD values have been detected even in newly diagnosed MS patients with no or minor physical disability (Moen *et al.* 2011). Low vitamin D levels have been reported in the Finnish population, and low levels of D vitamin are a risk factor for fragility fractures (Heliovaara *et al.* 2009, Huotari & Herzig 2008). Unfortunately, in the present study, D vitamin levels were not measured. Interestingly, low-energy (fragility) fractures appeared also in the femur and vertebral in addition with typical and easily injured ankle and distal forearm. In previous studies, MS patients have also been reported to suffer tibia, femur and hip fractures (Bazelier *et al.* 2011, Bazelier *et al.* 2012). Vertebral fractures have also been associated with MS (RR 1.44) (Dong *et al.* 2015). No correlation was

detected between the extent of glucocorticoid use, gender or age with the likelihood of suffering fragility fractures. These results differ from the previous meta-analysis, where female gender (RR1.80) or glucocorticoid usage (RR 1.33) were associated with an increased fracture risk in MS patients (Dong *et al.* 2015).

Although MS patients are known to be at risk for osteoporosis, only 26 % of our MS patients with low-energy fractures had been investigated for this disease. There are only a few reports which have described the systematic evaluation for osteoporosis or osteopenia in MS patients (Moen *et al.* 2011). In the NARCOMS study, only about every other MS patient reported having undergone bone density testing (Marrie *et al.* 2009a). It is worthwhile remembering that both osteoporosis and fragility fractures can be prevented. These results emphasize that more attention should be paid to the bone health on MS patients in clinical practice.

6.4 Strengths and limitations of the study

This cohort is highly representative of MS during the period 1990–2010 in the area of Northern Ostrobothnia, since almost all the patients with MS were referred to OUH for diagnostics and treatment. In addition, all of the patients remained in follow-up in OUH irrespective of the location where they underwent rehabilitation or received other types of medical treatment. This ensures the most accurate epidemiological as well as neurological comorbidity data. MRI scans were available for diagnosis from the beginning of 1990's and also the diagnostic criteria remained unchanged during the study period. However, some cases with very mild symptoms without referral to diagnostic evaluation or cases with minimal imaging findings may have been missed. In the majority of previous reports, neurological or other comorbidities have been documented by register based methods. In the present study, the clinical patient records were evaluated by the same neurology specialist and thus provided accurate data about all neurological diseases that can be diagnosed and treated in the neurology ward (for example, epilepsy and stroke). The main limitations of the comorbidity studies are the lack of population based controls and that there was no systematic query about migraine or other headaches and restless leg syndrome in the patients. For this reason, the prevalence of migraine may be somewhat underestimated, because migraine is very common and under diagnosed in general population with some migraine patients only suffering from mild headache. RLS may have been under-diagnosed in the present cohort. In addition, the somewhat limited size of the sample influenced the selection of statistical methods.

6.5 Conclusions and future aspects

This is the first report of the epidemiology of MS in the northern part of Finland. The prevalence and incidence are similar than the Finnish average. The postulated south-to-north latitudinal increase in prevalence was not found, which is consistent with the MS epidemiology in Norway. An increasing incidence of MS among women was detected which is a phenomenon also detected in other recent studies. The causes for this increase remain unknown but they may be associated with environmental and/or lifestyle factors.

Neurological comorbidity as well as fragility fracture events was common in this MS cohort. Stroke was associated with increased mortality in the MS patients. Interestingly, fragility fractures were common in this cohort and those fractures occurred already in early stage of the diseases when there was still no major disability. A worrying finding was that the existence of osteoporosis had not been taken into consideration as only a very small number of patients had had their bone density investigated.

Further investigations with population or register based cohorts including all Finnish MS patients are needed to evaluate the role not only of neurological comorbidities but also other comorbid diseases in the prognosis and survival of MS patients. In addition, more investigations should be focused on bone health of MS patients. The comorbid diseases and especially the risk for osteoporosis and fractures in these patients should also be better taken into account in clinical practice.

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Original publications

- I Krökki O, Bloigu R, Reunanen M & Remes AM (2011) Increasing incidence of multiple sclerosis in women in Northern Finland. *Mult Scler* 17 (2):133–8.
- II Krökki O, Bloigu R, Ansakorpi H, Reunanen M & Remes AM (2014) Neurological comorbidity and survival in multiple sclerosis. *Mult Scler Relat Disord* 3(1):72–7.
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