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Katariina Kuutti

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Dedicated to Joonas and our upcoming new family member

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ABSTRACT

Background: Multiple sclerosis (MS) is an inflammatory disease of the central nervous system (CNS). Although great advances in MS treatment have been made, it remains one of the most common causes of neurological disability in young adults globally. The disease course can be highly variable. The emergence of effective disease modifying therapies (DMTs) for relapsing-remitting MS (RRMS) has created a need for blood and imaging biomarkers for disease activity and prognosis for improved treatment decisions.

Aims of the study: To study which brain volume measures correlate with disability and cognition in a clinical cohort of relapsing and secondary progressive Finnish MS patients, and to evaluate whether a single-time-point brain volume measure could predict disability progression. A further aim was to study whether serum neurofilament light chain (sNfL) levels are affected by supplementation of vitamin D and correlate with magnetic resonance imaging (MRI) activity in interferon-beta-1b (IFN)-treated MS patients. Another aim was to compare outcomes of initial treatment with high-efficacy therapies vs starting therapy with medium-efficacy therapy in a propensity-matched cohort of Finnish RRMS patients.

Results: Worse performance in Symbol Digit Modalities Test (SDMT) for cognition and higher Expanded Disability Status Scale (EDSS) for physical disability significantly correlated with smaller deep grey matter and total brain volumes. Even very early brain atrophy, such that atrophy was detected in thalamus only, was a negative prognostic marker for disability. Patients with only thalamic atrophy were at a higher risk for not reaching 2-year No Evidence of Disease Activity (NEDA) and for EDSS increase at 2 and 5 years than patients with no brain atrophy. In a small cohort of clinically stable IFN-treated patients, sNfL levels were similarly low in patients supplemented with high-dose vitamin D or placebo. Subclinical disease activity in MRI was associated with higher sNfL levels. Initiating MS-therapy with high-efficacy DMTs significantly reduced the risk of 5-year disability progression and relapse compared to using moderate-efficacy DMT as first treatment.

Conclusions: Measuring brain volume early in the disease course could aid therapeutic decision making. In clinically stable patients, elevated sNfL levels may assist detection of subclinical inflammatory disease activity. Early high-efficacy therapy in RRMS patients leads to better prognosis than initiating treatment with moderate-efficacy therapies.

KEYWORDS: Multiple Sclerosis, biomarker, prognosis, magnetic resonance imaging, disease modifying therapies

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TIIVISTELMÄ

Tausta: MS-tauti on tulehduksellinen demyelinisoiva, aksonaaliseen vaurioon ja neurodegeneratioon johtava keskushermoston sairaus. Vaikka taudin hoidossa on tapahtunut suurta edistystä, on se edelleen yleisin nuorten aikuisten vammautumiselle altistava neurologinen sairaus. Taudinkulku on vaihtelevaa. Aaltomaisen MS-taudin taudinkulkua muokkaavien lääkkeiden kehityksen myötä mahdollisimman varhainen diagnosointi ja hoidon nopea aloitus ovat muodostuneet tärkeiksi tavoitteiksi. Tautiaktiivisuuden ja taudin etenemisen ennustamiseen tarvittaisiin verestä mitattavia ja kuvantamisen biomarkkereita.

Tavoitteet: Selvittää, minkä aivoalueiden tilavuudella on yhteys fyysisen ja kognitiivisen haitta-asteen lisääntymiseen sekä arvioida, voiko yhdellä ajanhetkellä tehty aivojen tilavuusmittaus ennustaa fyysisen haitta-asteen etenemistä suomalaisilla MS-potilailla 2 ja 5 vuoden kohdalla. Toinen tavoite oli tutkia, miten D-vitamiinilisä verrattuna lumelääkkeeseen vaikuttaa seerumin neurofilamentti (NfL)-tasoihin ja tautiaktiivisuuteen magneettikuvauksessa (MK) beetainterferonilla (IFN) hoidetuilla MS-potilailla. Tavoitteena oli myös tarkastella taudin etenemistä aaltomaista MS-tautia sairastavilla potilailla, joita hoidettiin alusta lähtien hyvin tehokkailla taudinkulkua muokkaavilla lääkkeillä verrattuna kohtalaisen tehokkailla lääkkeillä hoidon aloittaneisiin potilaisiin.

Tulokset: Pienempi harmaan aineen alueiden ja koko aivojen tilavuus korreloivat huonompien kognitiivisen testin, Symbol Digit Modalities Test (SDMT) ja korkeampien fyysistä haittaa mittaavien Expanded Disability Status Scale (EDSS)-pisteiden kanssa. Jopa hyvin varhainen aivoatrofia, jota havaittiin vain talamuksessa, oli yhteydessä huonompaan ennusteeseen. Potilailla, joilla oli vain talamusatrofiaa, oli suurempi todennäköisyys olla saavuttamatta 2 vuoden No Evidence of Disease Activity (NEDA)-tilaa sekä EDSS-pisteiden huononemiseen 2 ja 5 vuoden kohdalla verrattuna niihin, joilla ei ollut aivoatrofiaa. Kliinisesti hoitotasapainon saavuttaneilla, IFN-hoidetuilla MS-potilailla, sNfL-tasot olivat yhtä matalia D-vitamiini- ja lumelääkeryhmissä. Subkliininen tautiaktiivisuus pään MK:ssa oli yhteydessä korkeampiin sNfL-tasoihin. Hoidon aloittaminen hyvin tehokkailla lääkkeillä verrattuna kohtalaisen tehon lääkkeisiin johti parempaan ennusteeseen 5 vuoden seurannassa.

Johtopäätökset: Aivojen tilavuuden mittaus taudin alkuvaiheessa voisi tukea kliinistä päätöksentekoa. Seerumin NfL saattaisi olla hyödyllinen biomarkkeri hiljaisen tautiaktiivisuuden arviointiin. Aikaisin aloitettu hyvin tehokas lääkehoito johtaa parempaan ennusteeseen kuin kohtalaisen tehokkailla lääkkeillä aloittaminen.

AVAINSANAT: MS-tauti, biomarkkeri, ennuste, magneettikuvaus, taudinkulkuun vaikuttava lääkehoito

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Abbreviations

25(OH)D	25-hydroxyvitamin D
AE	Adverse event
APC	Antigen-presenting cell
ARR	Annual relapse rate
BBB	Blood-brain barrier
BET	Brain Extraction Tool
BMI	Body mass index
BOD	Burden of disease
CDP	Confirmed disability progression
CI	Confidence interval
CIS	Clinically isolated syndrome
CMSC	Consortium of Multiple Sclerosis Centers
CMV	Cytomegalovirus
CNS	Central nervous system
CSF	Cerebrospinal fluid
CXCL-13	C-X-C Motif Chemokine Ligand 13
DIR	Double inversion recovery imaging
DIS	Dissemination in space
DIT	Dissemination in time
DMT	Disease modifying therapy
EBV	Ebstein-Barr virus
EDSS	Expanded disability status scale
ESP	European Standard Population
FDR	False discovery rate
FLAIR	Fluid Attenuated Inversion Recovery
FS	Functional system
GWAS	Genome-wide association study
Gd	Gadolinium
GFAP	Glial fibrillary acidic protein
GM	Grey matter
HR	Hazard ratio

IFN	Interferon beta
IgG	Immunoglobulin G
JCV	John Cunningham virus
JCV-Ab	John Cunningham virus antibodies
MAGNIMS	Magnetic Resonance Imaging in MS
MHC	Major histocompatibility complex
MPRAGE	Magnetization-prepared rapid gradient-echo sequence
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
MxA	Myxovirus A
NEDA	No evidence of disease activity
NfL	Neurofilament light chain
OCB	Oligoclonal band
OCT	Optical coherence tomography
OR	Odds ratio
PET	Positron emission tomography
PPMS	Primary progressive multiple sclerosis
PML	Progressive multifocal leukoencephalopathy
RCT	Randomized controlled trial
RR	Risk ratio
RIS	Radiologically isolated syndrome
RRMS	Relapsing-remitting multiple sclerosis
SD	Standard deviation
SIMOA	Single molecule array
SPMS	Secondary progressive multiple sclerosis
T25FW	Timed 25-Foot Walk
SDMT	Symbol Digit Modalitites Test
T	Tesla
WM	White matter

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Hänninen K, Viitala M, Paavilainen T, Karhu JO, Rinne J, Koikkalainen J, Lötjönen J, Soilu-Hänninen M. Thalamic atrophy without whole brain atrophy is associated with absence of 2-year NEDA in multiple sclerosis. Frontiers in Neurology, 2019; 10:459.

II Hänninen K, Viitala M, Paavilainen T, Karhu JO, Rinne J, Koikkalainen J, Lötjönen J, Soilu-Hänninen M. Thalamic atrophy predicts 5-year disability progression in multiple sclerosis. Frontiers in Neurology, 2020; 11:606.

III Hänninen K, Jääskeläinen O, Herukka S-K, Soilu-Hänninen M. Vitamin D supplementation and serum neurofilament light chain in interferon-beta-1b-treated MS patients. Brain and Behaviour, 2020; 10e01772.

IV Hänninen K, Viitala M, Laakso SM, Kuusisto H, Soilu-Hänninen M. Initial treatment strategy and clinical outcomes in Finnish MS patients: a propensity-matched study. Journal of Neurology, Epub 2021-06-25, 2022 Feb; 269(2):913–922.

1 Introduction

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system (CNS) characterized by inflammation, demyelination, and axonal loss. Although significant advances in MS treatment have been accomplished, it remains one of the most common causes of neurological disability in young adults globally. The symptoms are diverse, because they depend on which part of the CNS is affected.

The disease course can be remarkably variable among individual patients. The emergence of effective disease modifying therapies (DMTs) for the most common form of the disease, relapsing-remitting MS (RRMS), has created a need to diagnose and initiate treatment as early as possible (Thompson et al., 2018). Treatment selection has become a real challenge for the MS clinician: It is required to stay up-to-date on all the newest available treatments and to give careful consideration of disease profile, prognosis, treatment response and adverse events. In recent years, the term personalized medicine has become a hot topic in MS treatment (Comabella et al., 2016; Gafson et al., 2017). To aid the complex clinical decision making, potential biomarkers for prediction of disease progression have been studied. These include serum biomarkers, such as neurofilament light chain (NfL) and magnetic resonance imaging (MRI)-derived brain volume measures (Bhan et al., 2018; Housley et al., 2015; Sotirchos et al., 2020).

Better understanding of predictive factors in MS is needed. Although DMTs have shown several beneficial effects on relapses, disability accumulation, and transition to secondary progressive MS (Armoiry et al. 2018; Freedman, 2014; Palace et al., 2015; Tedeholm et al., 2013), they have shown little measurable improvement in long-term disability outcomes. The reasons behind these suboptimal outcomes have been studied and one reason may be that current treatment paradigms miss a window of therapeutic opportunity (Buron et al. 2020; Harding et al., 2019; Kalincik et al., 2017). Prognostic biomarkers could help identify the MS patients in need of more aggressive treatment from early on, and thus improve the future disability outcomes.

2 Review of the Literature

2.1 Etiology of multiple sclerosis

2.1.1 Pathogenesis

MS is a chronic autoimmune disease of the CNS, involving both humoral and cellular immune systems. The mechanisms behind CNS injury are still incompletely understood. The current understanding is, that some environmental antigen or infectious agent could activate a loss of self-tolerance towards CNS antigens in genetically susceptible individuals, leading to a release of autoantigens and activation of autoreactive T cells, which can then migrate across the blood-brain barrier (BBB) (Fujinami & Oldstone, 1985). After infiltrating into the CNS, antigen-presenting cells (APCs) can reactivate autoreactive T cells, further leading to a release of chemokines and cytokines, recruitment of additional T cells, B cells, monocytes and activation of microglia and macrophages resulting in myelin damage (Hemmer & Selter, 2013).

MS has classically been considered a T-cell-mediated disease. Especially Th-17 cells (CD4+ T-cells that secrete interleukin-17) have been thought to be involved in MS pathogenesis (Ontaneda et al., 2012). Th-17 cells are able to open the BBB and cause axonal damage and neuronal death (Kebir et al., 2007). During an acute relapse, there is an increased number of circulating Th-17 cells in MS patients (Durelli et al., 2009). CD8+ T cells are believed to induce axonal pathology by direct injury to major histocompatibility complex I/antigen-expressing cells, such as neurons and oligodendrocytes (Neumann et al., 2002). Increasing evidence shows, that B cells are also important in MS pathogenesis. B cells can differentiate into plasma cells and secrete autoantibodies that contribute to the CNS inflammation. They can also act as APCs to activate CNS-specific pathogenic T cells. Pathologic studies have shown antibody build-up and activated complement in MS lesions. Cerebrospinal fluid (CSF) oligoclonal bands, which represent antibodies produced within the CNS, are a characteristic feature of MS (O'Connor et al., 2005). Since the role of B cells has been discovered, new monoclonal antibody treatments targeting B cells have been introduced and are found to be very effective in MS (Hauser et al., 2008).

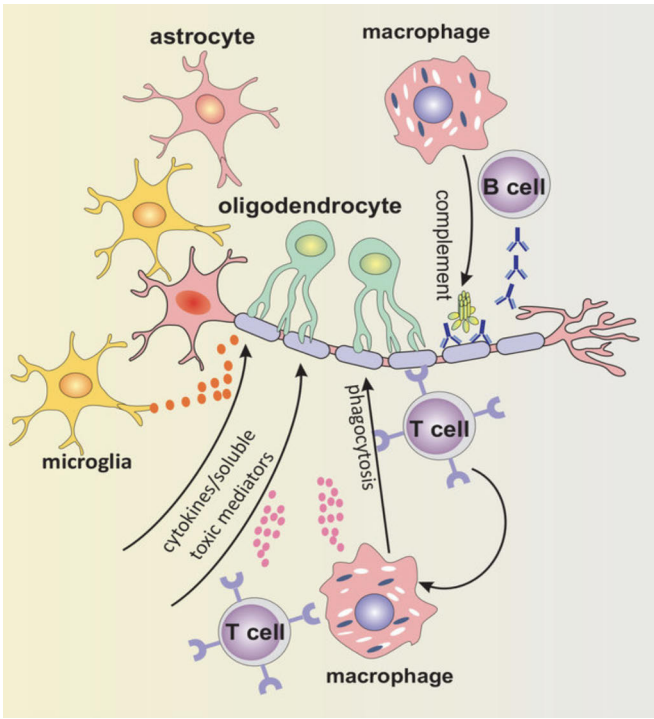

Figure 1. Different immune cells' participation in the pathogenesis of MS. T cells recognize myelin epitopes and activate macrophages to damage myelin by phagocytosis. Cytokines and toxic mediators released by T cells, macrophages, and microglia cause myelin damage. Autoantibodies facilitate phagocytosis mediated by macrophages through binding to myelin and activating complement. Reprinted with permission from Elieh-Ali-Komi et al. Clinic Rev Allerg Immunol (2017) 52:436–445 DOI 10.1007/s12016-016-8595-y.

As a result of the inflammation process described above, oligodendrocyte damage and demyelination occur. MS pathology is characterized by focal inflammatory infiltrates, demyelination, microglia activation, gliosis, proliferation of astrocytes and axonal damage associated with oxidative stress and mitochondrial injury (Lassmann, 2014; Simons et al., 2014). Focal demyelinated lesions can be partially or completely repaired by remyelination. The presence of demyelinating activity in MS has been recognized for decades, but since then, axonal loss also has been found to be present even in the earliest stages of MS (Singh et al. 2013). MS has earlier been considered a demyelinating disease of the white matter, but in recent years, demyelination of the cortical and deep grey matter has been recognized and even suggested to exceed white matter demyelination. Cortical atrophy has been associated with grey matter atrophy and disease progression (Kutzelnigg et al., 2005).

It has been stated, that different kinds of pathologies dominate during different stages of MS. Focal demyelinated plaques are present in all stages of the disease,

however, active plaques are primarily formed in the acute or relapsing stage of MS. In progressive MS, focal white matter plaques are either inactive or show slow expansion on their edges. In contrast, cortical demyelination and diffuse white matter injury are most prominent in patients with progressive MS, whereas they are rare or absent in the relapsing stage. These different pathological processes have been suggested to develop, at least to some extent, independently from each other (Kutzelnigg et al. 2005).

2.1.2 Risk factors

2.1.2.1 The role of vitamin D in the risk of multiple sclerosis

Several environmental and lifestyle risk factors for MS have been recognized. Migration studies have shown that the greater the distance from the equator, the higher the MS prevalence (Simpson et al., 2011). The latitude effect can be explained by the role of vitamin D and ultraviolet radiation in MS susceptibility. Vitamin D is a precursor of a potent steroid hormone with several biological effects including immunomodulation and neuroprotection (Peelen et al., 2011). Increased vitamin D levels, especially before the age of 20, are associated with a lower risk of MS in later life (Munger et al., 2006). Month of birth and thus sunlight exposure during pregnancy also appears to have an influence, because the risk of MS has been found to be significantly greater for people born in the spring than for those born in the autumn (Willer et al., 2005). UV radiation also protects against MS, probably both through its effects on vitamin D and through independent beneficial effects (Lucas et al., 2015).

2.1.2.2 Other environmental and lifestyle risk factors

Several viral infections have been suspected to have an association with MS. Only Epstein-Barr virus (EBV) has been consistently linked to MS risk. Compared to the population without EBV infection, individuals with undiagnosed EBV infection in childhood have been shown to be at an approximately 10-fold greater MS risk, and those with clinical EBV infection at least at a 20-fold greater risk (Ascherio & Munger, 2007). A recent large study with 10 million young adults in the United States military showed a 32-fold increased risk of MS after infection with EBV, but not with other viruses, including the similarly transmitted cytomegalovirus (Bjornevik et al., 2022). It is not clear how EBV infection contributes to MS. Several mechanisms have been discussed; EBV could have a specific effect through molecular mimicry, or general immune effects on B cells or on other immune regulatory elements, or it could be a secondary phenomenon (Baecher-Allan et al., 2018).

Cigarette smoking is another well-known risk factor for being diagnosed with MS and disease activity (Wingerchuk, 2012). The risk of MS increases with the intensity and duration of smoking.

The risk for MS has shown to decrease when moving from a high-risk to a low-risk country before adolescence, whereas the risk increases in those who move from a low-risk to a high-risk country (Ahlgren et al., 2012; Berg-Hansen et al., 2015). These findings suggest that the exposure to an environmental factor in childhood and adolescence can influence disease risk.

Obesity in childhood and adolescence has been linked to a higher MS risk in several studies around the world (Hedström et al., 2012; Munger et al., 2009; Wesnes et al., 2015). The role of obesity in MS could also be related to vitamin D, because the prevalence of vitamin D deficiency has been shown to be higher in obese individuals (Pereira-Santos et al, 2015).

2.1.2.3 Genetic risk factors

MS predominantly affects northern Europeans and there is a familial recurrence rate of approximately 15%. For monozygotic twins the recurrence rate is around 35%. The age-adjusted risk is higher for siblings (3%), children (2%), and parents (2%) than for second- or third-degree relatives (Compston & Coles, 2002).

Genome-wide association studies (GWAS) have identified over 200 genes linked to MS (Bashinskaya et al., 2015). More than 50 gene loci have been associated with MS, with the HLADRB1*1501 allele being the most important with an odds ratio (OR) of 3 (Hollenbach & Oksenberg, 2015). Subsequent GWAS have shown that the interleukin-2 receptor alpha gene (IL2RA) and interleukin-7 receptor alpha gene (IL7R) are also risk factors of MS (Hafler et al., 2017). The class I variant HLA-A*02 is associated with protection from disease (Sawcer et al., 2014).

2.2 Epidemiology of multiple sclerosis

2.2.1 Epidemiology globally

According to the latest updated Atlas of MS, with a prevalence of 50– to 300 per 100,000 people, approximately 2.8 million are estimated to have MS globally [The Multiple Sclerosis International Federation (MSIF), 2020]. The previous estimate of the number of MS patients from 2013 was 2.3 million (Browne et al 2015). Several factors are likely to be contributing to the increase, including: better counting methods, improved diagnosis, people with MS living longer and global population growth (Kingwell et al. 2019; Schwenkenbecher et al. 2019). However, it cannot be ruled out that there may also be some increase in the risk of developing MS.

The global distribution of MS increases with increasing distance from the equator, with some exceptions (Browne et al., 2015). This latitude gradient was observed already in the 1970s (Kurtzke, 1975). In Europe, MS prevalence and incidence are higher in the Nordic countries and northern regions of the British Isles, which supports the role of latitude (Kingwell et al., 2013). However, the latitude gradient of MS incidence has been reported to attenuate over time and even its presence has been questioned recently in the northern hemisphere (Alonso & Hernán, 2008; Koch-Henriksen & Sørensen, 2010).

2.2.2 Epidemiology in Finland

Finland is a high-risk MS region based on regional studies (Holmberg et al., 2013; Pirttisalo et al., 2019; Åivo et al., 2017). A national MS register was launched in 2014 and is currently used in 17/21 Finnish hospital districts, covering over 90% of the estimated Finnish MS population (Laakso et al., 2019). The prevalence of MS in Finland has steadily increased in parallel with the global increase. In a study from 2018 using data from the MS register, there were 8722 MS patients in the Finnish MS register (71.5% females). By combining MS register data with data of the hospitals that had not joined the register, the nationwide prevalence was between 10 000 and 11 000 patients, corresponding to a crude prevalence of approximately 180-200/100,000 (Laakso et al., 2019). The latest estimate of the number of MS patients in Finland is 12 000 (Sipilä et al., 2021).

Like the global distribution, the geographical distribution of MS in Finland is not even. There is no latitude gradient of MS in Finland, but the distribution is concentrated in the western and southwestern parts of the country. The prevalence has shown to be highest in the districts of South Ostrobothnia, Turku, Vaasa, and Åland and lowest in the eastern districts of Finland. In Vaasa, South Ostrobothnia, and Pirkanmaa, MS prevalence increased by 45% from 2000 to 2010, being 192/100,000 in the whole western area (Krökki et al., 2011; Murtonen & Sumelahti, 2019), whereas in North Ostrobothnia, the estimated prevalence was 103/100,000 at the end of 2007 (Krökki et al., 2011). In 2012, the prevalence of Southwest Finland hospital district was reported to be 213/100,000 (Åivo et al., 2017). In a recent epidemiological study of MS in Finland from 2016, the prevalence was 280/100,000 in Southwest Finland and 168/100,000 in Northern Karelia. The annual age-standardized incidence [European Standard Population (ESP), 2013] was 12.1/100 000 person-years in Southwest Finland and 8.6/100,000 person-years in North Karelia in the age group of 10– to 69 years (Pirttisalo et al., 2019).

Due to increased life expectancy of MS patients, the mean age of patients has increased significantly to 51 years, compared to 38.2 years in a previous study between the years 1979 and 1993 (Laakso et al., 2019; Sumelahti et al. 2003). In

recent studies conducted in the 2000s, the prevalence has been highest in the age group of 40- to 49 years (Pirttisalo et al., 2020).

The female-to-male sex ratio has increased because of increased incidence of MS in women globally (Orton et al., 2006). An increased female-to-male ratio has also been found in Finnish MS studies. In the Finnish MS register study from 2018, the female-to-male ratio was 2.5, compared to ratios ranging from 1.6 to 2.4 in different regions of Finland in previous studies (Laakso et al., 2019; Sumelahti et al., 2003). The factors causing the increasing number of women with MS have been speculated. Given the short time period over which this has occurred, genetic change can be excluded, so the factors must be environmental, perhaps the changes in lifestyle factors of women (Orton et al., 2006).

2.3 Clinical multiple sclerosis

2.3.1 Clinical features of multiple sclerosis

Multiple sclerosis, as the name suggests, is a disease with multiple clinical presentations. The signs and symptoms of MS vary widely depending on the affected area of the CNS. Most patients develop multiple MS lesions in different locations, along with multiple relapses, which can each have different symptoms.

Current MS classifications into different phenotypes include clinically isolated syndrome (CIS), radiologically isolated syndrome (RIS), relapsing-remitting MS (RRMS), primary-progressive MS (PPMS), and secondary-progressive MS (SPMS) (Lublin, 2014; Sand, 2015). In a majority of MS patients (80%–85%), the disease starts with an acute phase, followed by a relapsing-remitting course. RRMS is defined by acute relapses from which patients typically recover partially or completely, with clinically stable periods in between. A relapse is defined as signs of an acute inflammatory demyelinating event in the CNS, which last for at least 24 hours, with the exclusion of fever or infection (Polman et al., 2011). The definition of CIS is having clinical evidence of a single relapse but MRI findings are not fully meeting RRMS criteria. As MRI use has increased in different conditions such as headache and trauma, incidental MS-type lesions are found more often in patients with no clinical MS symptoms. To describe these situations, the term radiologically isolated syndrome (RIS) was introduced in 2009, and was later added to the revised MS phenotype classification (Okuda & Mowry, 2009). A third of RIS patients will develop clinical symptoms of MS in 5 years of follow-up (Okuda et al., 2014).

Most RRMS patients eventually convert to SPMS after approximately 10–15 years in untreated patients. SPMS in characterized by a gradual worsening of neurological disability. The transition from RRMS to SPMS is often subtle and thus difficult to define exactly. In PPMS (10%–15% of MS population), the progressive

decline in neurological functions starts from disease onset with no acute relapse episodes (Lassmann et al., 2012).

The symptoms in MS are not specific; they can occur in several other neurological or medical conditions. However, certain symptoms occur frequently in MS patients. The most common ones include fatigue, abnormal sensations, walking difficulties, muscle spasms and stiffness, bowel or bladder problems, memory or other cognitive difficulties, emotional or mood problems, pain and other unpleasant sensations, vision problems, dizziness, and swallowing or speech problems (Lezzoni, 2010).

2.3.2 Diagnostic criteria

The diagnosis of MS is based on neurological symptoms and signs combined with evidence of dissemination of CNS lesions in space (DIS) and dissemination in time (DIT), and exclusion of differential diagnoses (Brownlee, Hardy, et al., 2017). An early and accurate diagnosis is critical because of the available effective treatments that have shown best results on disease progression when started in the early stage of the disease (Chalmer et al., 2018).

The first diagnostic criteria from 1965 were purely clinical (Schumacher et al., 1965). In the updated Poser criteria from 1983, dissemination in time and space was still based on only clinical findings, but clinical criteria were supplemented with paraclinical evidence using CSF, neuroimaging, and evoked potentials (Poser et al., 1983).

The McDonald criteria were first introduced in 2001 (McDonald et al., 2001) and have since been updated in 2005, 2010, and 2017. In the McDonald 2010 criteria, MRI was used to provide evidence for DIT and DIS. Revisions of MRI criteria proposed by the European Magnetic Resonance Imaging in Multiple Sclerosis (MAGNIMS) network were incorporated in the 2017 version of the McDonald criteria, which are the latest diagnostic criteria and currently in clinical use in Finland (Filippi et al., 2016; Thompson, Banwell, et al., 2018). The 2017 revisions of the McDonald criteria (shown in Table 1) reinforced the importance of brain and spinal cord MRI examinations, in addition to the clinical presentation and CSF analysis under certain circumstances (Wattjes et al., 2021). The important changes to the earlier criteria were the following: CSF Oligoclonal bands (OCBs) may substitute for a second clinical event or MRI finding for DIT, both symptomatic and asymptomatic MRI lesions can be considered in the determination of DIS or DIT (the only exception being lesions in the optic nerve in a patient with optic neuritis), and cortical and juxtacortical lesions are given the same weighting to fulfil the MRI criteria for DIS (Carroll, 2018).

Table 1. The 2017 McDonald criteria for diagnosis of multiple sclerosis in patients with an attack at onset, modified from Thompson et al., 2018.

	Number of lesions with objective clinical evidence	**Additional data needed for a diagnosis of MS**
≥2 clinical attacks	≥2	None*
≥2 clinical attacks	1 (as well as clear-cut historical evidence of a previous attack involving a lesion in a distinct anatomical location**)	None*
≥2 clinical attacks	1	Dissemination in space demonstrated by an additional clinical attack or by MRI
1 clinical attack	≥2	Dissemination in time demonstrated by an additional clinical attack or by MRI OR demonstration of CSF-specific OCBs***
1 clinical attack	1	Dissemination in space demonstrated by an additional clinical attack implicating a different CNS site or by MRI AND dissemination in time demonstrated by an additional clinical attack or by MRI OR demonstration of CSF-specific OCBs***

*No additional tests required to demonstrate DIT and DIS. However, brain MRI should be obtained in all patients suspected of having MS. Spinal cord MRI or CSF examination should be considered in patients with insufficient clinical and MRI evidence, a presentation other than a typical CIS, or with atypical features. If imaging or other tests are negative, caution needs to be taken before diagnosing MS, and alternative diagnoses should be considered. **Clinical diagnosis based on objective clinical findings for 2 attacks is most secure. Reasonable historical evidence for one past attack can include events characteristic for a previous inflammatory demyelinating attack, but at least one attack must be supported by objective findings. ***The presence of OCBs does not demonstrate DIT but can substitute for the requirement for demonstration of this measure.

2.3.3 Follow-up

After diagnosis, which most commonly is RRMS at disease onset, most patients start a DMT that is selected based on multiple factors, including the activity of the disease, potential risks, possible comorbidities and current medications, family planning, and the patients' own preferences. The follow-up of MS patients on DMTs in Finland takes place in neurological clinics. The effectiveness of the treatment is evaluated at least once a year, and follow-up consists of clinical evaluation including Expanded Disability Status Scale (EDSS) assessment; information on relapses; and MRI usually 6– to 12 months after treatment onset and thereafter every 6– to 12 months, depending on the DMT of choice (Current Care Guidelines 2020,

www.kaypahoito.fi). The data of each patient visit are collected in the national Finnish MS register (www.neurorekisteri.fi).

2.3.4 MRI in diagnosis and follow-up

The latest update on the international consensus recommendations on the use of MRI in MS patients has been published in 2021 (Wattjes et al., 2021). The recommendations for standardized brain MRI protocol include imaging with at least 1.5 Tesla (T), 3 T if available, with the following core sequences: T2-weighted 3D-fluid-attenuated inversion recovery, axial T2-weighted, and T1-weighted with gadolinium (Gd). Identical slice positioning, pulse sequences, magnetic field strengths, and spatial resolution are highly recommended for each imaging event (Wattjes et al., 2021).

In MS diagnostics, MRI is used to provide evidence for DIT and DIS. For RRMS diagnosis, MRI evidence of DIS requires at least one T2 lesion in at least 2 of 4 sites: juxtacortical, periventricular, and infratentorial regions; and the spinal cord. In addition to juxtacortical lesions, cortical lesions can be used to fulfil MRI criteria for DIS. DIT requires either Gd-enhancing and non-enhancing lesions on the same MRI scan or a new lesion on a follow-up scan (Thompson, Banwell, et al., 2018).

A new brain MRI is recommended every 6– to 12 months in CIS and subclinical RIS with risk factors for conversion to MS and paraclinical features of MS. In MS follow-up, a baseline brain MRI before starting or switching DMT is recommended. A new baseline MRI is recommended at 3– to 6 months after treatment onset, or at longer intervals in patients who are receiving slow-acting DMTs. After the new baseline MRI, a yearly control MRI is suggested when the patient is on DMT; longer intervals can be considered after the first few years of treatment in clinically stable patients, particularly if safety monitoring is not required.

The paramagnetic contrast agent gadolinium can only cross the BBB in sites of inflammation or damage. The presence of Gd-enhancing lesions on MRI in MS indicates active inflammation and lesion burden, and the size and number of Gd-enhancing lesions predict both onset and severity of relapses (Brück et al., 1997; Katz et al., 1993). In the 2015 MAGNIMS guidelines, the use of Gd-based contrast agents for the assessment of disease activity, particularly for DMT effectiveness monitoring purposes, was recommended. Given the evidence regarding Gd deposition in the brain, the European Medicines Agency later suspended the use of linear Gd-enhanced CNS MRI and recommended that gadolinium should be used only if essential, and at the lowest possible dose (Wattjes et al., 2021).

Spinal cord imaging at symptom onset is recommended in patients with clinical findings suggestive of spinal cord involvement to exclude alternative cord pathology (such as compression, tumor, vasculitis, or neuromyelitis optica) and in those with

non-spinal CIS that do not fulfil the brain MRI criteria for dissemination in space. In MS follow-up, spinal cord MRI is not routinely recommended, but it should be considered in patients with a spinal cord phenotype (ie, no or few brain lesions), in patients with disability worsening that cannot be explained by brain MRI, and in patients with symptoms of a spinal cord relapse (Wattjes et al., 2021).

2.4 Prognosis

2.4.1 Clinical outcome measures

The prognosis of MS is highly variable, ranging from benign to more severe cases. The concept of benign MS has been challenged because the definitions vary and do not take into account non-physical disability (Ellenberger et al., 2020; Reynders et al., 2017). The establishment of clinical factors detectable early in the disease that can aid prediction of the long-term outcome of an individual patient is highly desirable. Historically, MS has led to irreversible limitation in ambulation and eventually patients becoming wheelchair-bound, however, several factors, including the evolution of DMTs and earlier and more accurate diagnosis, have improved the prognosis of MS during the last decades (Sorensen et al., 2020). Data from large cohort registries have shown that life expectancy in the MS population is reduced by 7– to 14 years compared with the healthy general population (Brønnum-Hansen et al., 2004; Ragonese et al., 2008; Sumelahti et al., 2010). At least half of patients die from causes directly related to MS. Nevertheless, survival of patients with MS has significantly improved compared with a generation ago (Scalfari et al., 2010).

One of the most used primary clinical outcome measures in clinical trials is the EDSS (Kurtzke, 1983). EDSS is a clinician-administered assessment scale evaluating the functional systems of the CNS. EDSS steps 1.0 to 4.5 refer to people with MS who are able to walk without any aid and is based on measures of impairment in eight functional systems (FS):

- pyramidal – muscle weakness or difficulty moving limbs
- cerebellar – ataxia, loss of balance, coordination or tremor
- brainstem – problems with speech, swallowing and nystagmus
- sensory – numbness or loss of sensations
- bowel and bladder function
- visual function
- cerebral functions – problems with thinking and memory
- other

A functional system represents a network of neurons in the brain with responsibility for particular tasks. Each FS is scored on a scale of 0 (no disability) to 5 or 6 (more

severe disability). EDSS steps 5.0 to 9.5 are defined by the impairment to walking. The EDSS scale is sometimes criticised for its reliance on walking as the main measure of disability and its weaknesses in reliability and sensitivity to change. However, EDSS is the most widely used and best-known instrument to assess disease progression in MS (Meyer-Moock et al., 2014).

Figure 2. The Extended Disability Status Scale (EDSS) (Kurtzke, 1983) Image source: https://myms.org/ms_progression.htm.

Another commonly used primary outcome measure is relapse rate, often in the form of annual relapse rate (ARR) (van Munster & Uitdehaag, 2017). No Evidence of Disease Activity, NEDA, has emerged as a new outcome measure and treatment target as the DMTs have led to better treatment expectations, optimally potent remission. NEDA-3 is determined by no clinical relapse, no confirmed EDSS progression during the follow-up, no new or enlarging T2 lesions and no new Gd-enhancing lesions in MRI. NEDA-4 is determined by the same factors as NEDA-3, but with annualized whole brain volume loss ≤0.4% as a fourth variable (Lu et al., 2018).

Clinical outcome measures in frequent use also include cognitive measures, of which Symbol Digit Modalities Test (SDMT) is one of the most widely used for the evaluation of cognitive processing efficacy and speed in MS (Grothe et al., 2020). In the SDMT test, the patient substitutes a number, either orally or written, for randomized presentations of geometric figures. After completing the first 10 items with guidance, the patient is timed to determine how many responses can be made in 90 seconds. In the standard administration of the SDMT, the written response task is conducted first, followed by an oral response task, using the same stimuli (Benedict et al., 2017).

Some clinical variables have been found to predict future disease activity and disability progression and can be used to determine optimal treatment for each patient. Initial symptoms can help with this prediction: Optic neuritis or initial involvement of only sensory systems as the first symptom have been shown to lead to better long-term outcomes than other initial symptoms, such as bowel or bladder

involvement (Amato et al., 1999; Confavreux et al., 2003; Langer-Gould et al., 2006). A polysymptomatic onset has been reported as an unfavorable prognostic factor (Amato et al., 1999; Bsteh et al., 2016). Among the clinical factors found to be predictors of an unfavorable prognosis are older age at onset (Bergamaschi et al., 2001; Trojano et al., 1995) and male gender (Bergamaschi et al., 2001; Kantarci et al., 1998). When the time between the first and second relapse is over 5 years vs under 2 years, reaching an EDSS score of 4 has been shown to be significantly slower (16.1 years vs 6.6 years) (Confavreux et al., 2003). Frequent relapses during the first 2 years after diagnosis have been shown to predict shorter time to EDSS progression and to SPMS conversion, but after 2 years such a connection was no longer seen (Scalfari et al., 2010). Poor recovery from relapses during the first 5 years after MS onset leads to developing progressive MS significantly faster than those who recover well from relapses (progressive MS by 8.3 years vs 30.2 years after MS onset) (Novotna et al., 2015). Patients with a more aggressive disease course have shown worsening of cognitive function on SDMT at a significantly higher rate when compared to patients with a more benign disease course (Crielaard et al., 2019). Clinical and cognitive measures combined have been shown to predict outcomes better than each one isolated (Damasceno et al., 2020).

2.4.2 Radiologic outcome measures

MRI is widely used for diagnostic, prognostic, and monitoring purposes in MS. In RRMS, MRI measures of disease activity, based on new T2 and Gd-enhancing lesions and lesion volumes, provide a good surrogacy of treatment effect on relapse rate and disability progression (Filippi et al., 2014; Sormani et al., 2010; Sormani & Bruzzi, 2013). Asymptomatic infratentorial (Minneboo et al., 2004; Tintore et al., 2010), spinal cord (Brownlee, Altmann, et al., 2017), and Gd-enhancing lesions (Swanton et al., 2010) are associated with the development of physical disability over the first 5– to 7 years after a CIS. MRI-based evaluation of inflammatory activity in MS is established as the main efficacy outcome in phase II clinical trials (Miller et al., 1991). However, the value of these MRI measures in progressive MS remains elusive. The conventional MRI measures are sensitive only to inflammatory demyelination and are not able to assess the extent of irreversible tissue loss and the diversity of clinical outcomes in MS. This mismatch has been termed the clinico-radiological paradox in MS (Barkhof, 1999).

The focus of MRI studies has been redirected in the past years on diffuse grey matter (GM) damage. The quantification of brain atrophy by MRI has become an increasingly important part of evaluating neurodegeneration in MS. Brain atrophy occurs in all clinical stages of untreated MS patients at a rate of 0.5%–1.35%/year, compared to 0.1%–0.3%/year in healthy individuals (de Stefano et al., 2014). Brain

atrophy and lesion load have been shown to predict long term disability in MS (Popescu et al., 2013). Thalamus and other deep GM nuclei are among the first GM structures to be affected in MS. Multiple studies have shown associations between GM atrophy and measures of disease progression (Fisher et al., 2008; Jacobsen et al., 2014), as well as cognitive impairment (Filippi et al., 2013). Thalamic atrophy occurs from the earliest stages of the disease course (Bergsland et al., 2012), and it has been associated with the transition from CIS to definite MS (Zivadinov et al., 2013). Thalamic atrophy has been shown to correlate with accumulation of disability in patients with MS (Rocca et al., 2017). Quantification of whole brain atrophy, grey matter atrophy, and spinal cord atrophy have shown promise as outcome measures in the recent years, but still need to be validated (Filippi et al., 2014). Currently, brain and spinal cord volume measures have no role in the MS diagnostic criteria (Thompson, Banwell, et al., 2018) or disease course classification (Lublin et al., 2014), but there is emerging evidence that these measures are valuable for early evaluation of treatment responses and prediction of disease prognosis. A need for improvements in methodology that could facilitate the implementation of these measures in clinical practice has been recognized (Sastre-Garriga et al., 2020).

In the past decade, pathological and MRI studies have shown that MS lesions are not only white matter lesions, but are often located in the grey matter, especially in the cerebral cortex. The histopathological characteristics of these cortical lesions differ significantly from white matter lesions, suggesting a location-dependent expression of the immunopathological process of MS. Double inversion recovery imaging (DIR) is an MRI technique that has enabled researchers to image cortical lesions in vivo. Selectively imaging grey matter and lesions with DIR has shown that it is possible to detect cortical lesions at the earliest clinical stages of MS, and cortical lesion burden positively correlates with the severity of cognitive and physical impairments. These grey matter lesions have also been shown to be independent predictors of disease evolution (Calabrese et al., 2010).

Peripapillary nerve layer thickness can be measured non-invasively and inexpensively by optical coherence tomography (OCT) and has been shown to be a marker of axonal degeneration in MS (Bsteh et al., 2019). Positron emission tomography (PET) imaging of microglial activation has recently shown promise in the prediction of disability progression (Airas et al., 2017; Sucksdorff et al., 2020). Clinical applicability of PET imaging is restricted, but also MRI-based methods based on detection of iron in the slowly expanding rim lesions to detect microglial activation are being developed (Gillen et al., 2021).

2.4.3 Serum and CSF biomarkers

2.4.3.1 Neurofilament light chain

Substantial effort has been made to identify biomarkers for MS that can improve diagnosis, aid prediction of disease progression, and improve clinical outcomes. The main aims regarding MS biomarker research are the following: identifying disease-specific biomarkers that can predict individuals at risk for developing MS, determining biomarkers that can help identify individuals at high risk of developing severe attacks or progressive disease, and defining individuals who may be responsive to specific treatments, thereby allowing personalized treatment plans (Housley et al., 2015).

Neurofilament light chain (NfL) proteins are part of the neuronal cytoskeleton and upon axonal damage, NfL is released into the CSF, and subsequently, peripheral blood. Although initially discovered in the CSF, the recent development of ultrasensitive digital immunoassay technologies has enabled reliable detection of NfL also in serum or plasma, obviating the need for invasive lumbar punctures, which is especially desirable in longitudinal assessment (Novakova et al., 2017; Thebault et al., 2020). The structure of NfL is shown in Figure 3.

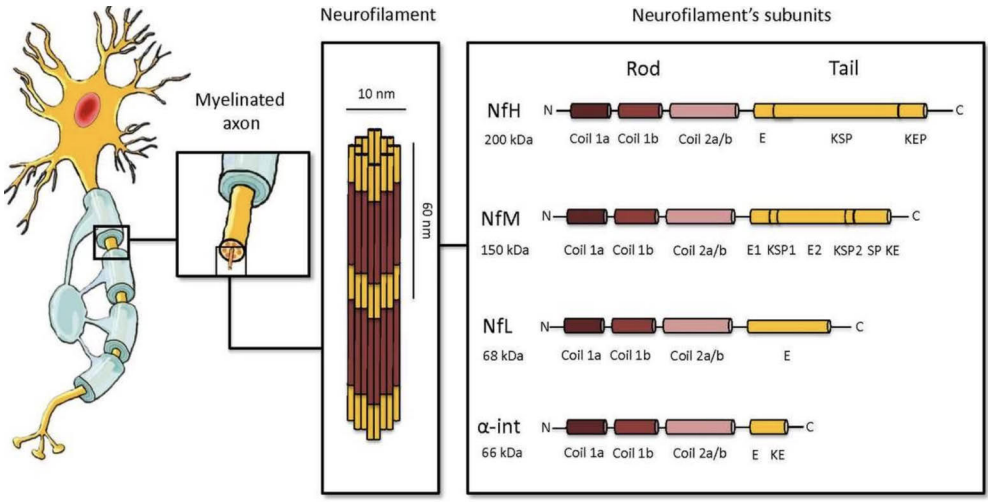

Figure 3. Neurofilament proteins add to the diameter of the axon and therefore influence its function. They all have a central, highly conserved alpha helical rod domain in the middle, neighboured by 2 variable regions: the head domain on the end terminus and the tail domain at the C terminus. Reprinted with permission from Gaetani et al. J Neurol Neurosurg Psychiatry. 2019 Aug;90(8):870–881.

NfL is elevated in various neurological diseases associated with neuronal damage, including neurodegenerative diseases (Preische et al., 2019), stroke (Gattringer et al., 2017) and MS (Bittner et al., 2020; Siller et al., 2019). In MS, elevated NfL levels in the CSF have been associated to relapses (Malmeström et al., 2004), MRI activity (Arrambide et al., 2016), disability progression (Salzer et al., 2010), and conversion from CIS to definite MS and from RRMS to SPMS (Martínez et al., 2015). Brain atrophy and sNfL together predict the time to EDSS of 6 over 8 years (Summary of Panel Recommendations Consortium of MS, 2021). Taken together, these findings suggest that NfL levels could be a prognostic biomarker for detection of an aggressive disease course and risk for secondary progression.

There are limitations to the potential clinical use of NfL. Although NfL correlates to disease severity and progression, it generally increases and decreases in correlation with clinical relapses, rather than predicting relapses. However, even though NfL levels in the CSF fluctuate with relapses, the fluctuations are slower than other markers and the levels remain much higher even during remission (Burman et al., 2014). This suggests that NfL alone, or in combination with other markers of CNS damage, may be the best marker of neuronal damage. Another difficulty with NfL is that it is not unique to MS, but can indicate neuronal degeneration from a wide range of causes. Therefore it offers limited diagnostic value in MS but can be useful in prognosis. A recent summary of panel recommendations from the Consortium of Multiple Sclerosis Centers (CMSC) on integrating the measurement of NfL into MS research and clinical practice has been published. The panel recommends evaluation of NfL to be used in conjunction with other measures of MS severity and prognosis, including MRI, other imaging biomarkers, and findings of neurologic examination. If a patient shows clinical worsening and/or MRI changes while on therapy, elevations in sNfL levels may signal the need to perform further examination or consider a change in therapy. For a patient who appears to be clinically stable but has elevations in sNfL, this may warrant closer monitoring and/or escalation of therapy (Summary of Panel Recommendations Consortium of MS, 2021).

2.4.3.2 Vitamin D

The effects of vitamin D in MS are related to its role in the immune system. In vitro and in animal models of MS, $1,25(OH)_2D$ is mostly attributed in anti-inflammatory properties in activated lymphocytes, myeloid cells, and glia (Smolders et al., 2008, 2011). In neurons, homeostatic functions have been observed. These observations have led to suggestions of vitamin D acting as a promotor of a homeostatic state both in immune and CNS-resident cells (Smolders et al., 2019).

Vitamin D levels have been associated with MS disease activity and progression in several studies, making it a potential biomarker in MS. MS patients have been found to have lower vitamin D levels during relapse than remission (Correale et al., 2009; Soilu-Hänninen et al., 2005, 2008). MRI activity, as seen as new T2 and Gd-enhancing lesions, has been found to have an inverse correlation with levels of 25(OH)D (Ferre' et al., 2018; Løken-Amsrud et al., 2012). In a large prospective study, average serum 25(OH)D levels in the first 12 months following a CIS strongly predicted MS activity and progression during the following 4 years. By the end of the 5-year follow-up, patients with serum concentrations of 25(OH)D equal to 50 nmol/L had a 4 times lower change in T2 lesion volume, a 2-fold lower rate of brain atrophy, and lower disability than those below 50nmol/L (Ascherio et al., 2014).

Vitamin D supplementation in patients with low 25(OH)D levels has been anticipated as a potential treatment strategy in MS. The results of the first large randomized controlled trials (RCTs) have been published in the recent years. None of the RCTs on vitamin D supplementation in MS have met their primary clinical endpoint of NEDA-3, however, these studies suggested modest effects on secondary endpoints. In three RCTs, vitamin D reduced the number of new or enlarging lesions and new T2 lesions, volume of hypointense T1 lesions and number of new T1 lesions, ARR, and disability progression (Camu et al., 2019; Hupperts et al., 2019; Soilu-Hänninen et al., 2012).

Studies on the effect of vitamin D supplementation on NfL levels have shown inconsistent results. The findings of two studies investigating the effect of vitamin D supplementation among patients participating in RCTs with high-dose vitamin D or placebo did not support an effect of vitamin D on serum NfL (Holmøy et al., 2019; Smolders et al., 2020). Among Swedish MS patients, an inverse association between serum 25(OH)D and CSF-NfL levels has been shown (Sandberg et al., 2016).

2.4.3.3 Other serum and CSF biomarkers

Biomarkers in diagnostic use

Oligoclonal bands and immunoglobulin G (IgG) ratio are currently in use in MS diagnostics. Oligoclonal bands serve as a marker of synthesis of immunoglobulins in the central nervous system. Up to 90% of MS patients have OCBs in the CSF, leading to the inclusion of OCBs as the first biomarker in the 1983 MS diagnostic criteria (Davenport & Keren, 1988; Poser et al., 1983). OCBs were removed from the 2010 McDonald criteria for RRMS although they are still a criterion for the diagnosis of PPMS (Housley et al., 2015). In the 2017 revisions of the McDonald criteria, OCBs in the CSF can substitute clinical or MRI evidence of DIT

(Thompson, et al., 2018). In recent years, new interest in OCBs has emerged as the presence of OCBs in the CSF has been shown to predict conversion from CIS to MS (Kuhle et al., 2015). The presence of oligoclonal bands has also been associated with worse disability outcomes in MS (Joseph et al., 2009).

Biomarkers used in treatment follow-up

John Cunningham virus (JCV) antibodies are in clinical use to assess the risk of progressive multifocal leukoencephalopathy (PML) in MS patients on natalizumab treatment. JCV titers are measured prior to initiating and during natalizumab treatment. Natalizumab is one of the most effective MS treatments available, but PML has emerged as a rare but serious adverse event. The risk of PML can be evaluated based on JC viral antibodies, because it is extremely rare that PML would occur in a patient with seronegative JCV antibodies (Antoniol & Stankoff, 2015). Antibodies against natalizumab have been found in 4.5% to-14.1% of natalizumab-treated MS patients. If antibodies persist, they are associated with an adverse effect on treatment response (Vennegoor et al., 2013).

Potential future biomarkers

Glial fibrillary acidic protein, GFAP, is one of the potential biomarkers in MS. GFAP is a component of intermediate filaments in astrocytes and is released in astrocyte damage (Housley et al., 2015). MS patients have been found to have elevated GFAP levels in the CSF compared to healthy controls (Malmeström et al., 2004; Norgren et al., 2004; Rosengren et al., 1995). Increased levels of GFAP correlate with diminished ambulation and more severe disability in MS (Malmeström et al., 2004; Petzold et al., 2002). As well as from the CSF, GFAP can be measured from serum samples. A recent Finnish study showed that serum GFAP is elevated also in patients with benign MS, indicating astrocytic damage and challenging the concept of true benign nature of the disease (Niiranen et al., 2021).

YKL-40 (chitinase-3-like 1) is a glial activation marker that is also expressed on activated macrophages, airway epithelia, smooth muscle cells, and chondrocytes. Increased levels of serum YKL-40 have been discovered in inflammatory conditions, including rheumatoid arthritis (Canto et al., 2015). In MS, elevated CSF YKL-40 has been associated to the risk of conversion from CIS to MS (Comabella et al., 2010). High levels of both YKL-40 and GFAP have been associated with earlier progression to EDSS of 3. Additionally, high levels of YKL-40 are associated to earlier progression to EDSS of 6 (Martínez et al., 2015).

CXCL-13, a B cell chemoattractant, is required for the development of B cell follicles and secondary lymphoid structures (Housley et al., 2015). CXCL13

expression in the CSF has been shown to be elevated in all MS subtypes and CIS (Stilund et al., 2015). During active disease, both CSF and serum CXCL-13 are increased (Festa et al., 2009). Higher CSF CXCL-13 levels have been associated to conversion from CIS to MS, and increased relapse rate, EDSS score, and lesion burden (Khademi et al., 2011; Stilund et al., 2015). However, highest levels of CXCL-13 have been found in viral encephalitic infections, suggesting that it is not specific for MS (Khademi et al., 2011). CXCL-13 is also used in the diagnostics of neuroborreliosis (Waiß et al., 2017). Other potential biomarkers studied in MS include CD163, a monocyte/macrophage-specific membrane marker; miRNA and mRNA; anti-microbial antibodies against measles, rubella and varicella zoster; KIR4.1; potassium channel antibodies; serum osteopontin; myelin-reactive T cells; microbiome-associated lipopeptides; and genetic biomarkers (Housley et al., 2015).

Table 2. Currently known prognostic factors in MS.

Good prognosis	Poor prognosis
Onset at a younger age	Onset at an older age[a]
Female gender	Male gender[b]
Monosymptomatic onset	Polysymptomatic onset[c]
A single sensory symptom or optical neuritis at onset	Motor symptoms/ -brainstem symptoms/ bladder symptoms at onset[d]
Full recovery from relapse	Incomplete or no recovery from relapse[e]
Long time before second relapse	High relapse rate during the first 2 years after onset[f]
Low MRI activity/ low lesion burden	High lesion burden[g]
No lesions in cerebellum or brainstem	Lesions in cerebellum or brainstem[h]
No brain atrophy	Brain atrophy[i]
No oligoclonal bands in CSF	Oligoclonal bands in CSF[j]
Low levels of NfL	High levels of NfL[k]

[a] Bergamaschi et al., 2001, [b] Bergamaschi et al., 2001, [c]Amato et al., 1999, [d] Confavreux et al., 2003, [e] Novotna et al., 2015, [f] Scalfari et al., 2010, [g]Popescu et al., 2013, [h]Minneboo et al., 2004, [i]Popescu et al., 2013, [j]Joseph et al., 2009, [k]Salzer et al., 2010.

2.5 Treatment

2.5.1 Disease modifying treatment

Over the last 20 years, MS treatment has gone through a revolution, particularly in recent years with the advent of the more potent DMTs (Doshi & Chataway, 2017). Relapsing MS is characterized by immune cells migrating into the CNS. Relapsing MS treatments act on the following common pathways: They decrease the number

and/or function of regulatory cells, increase the number and/or function of effector cells, and prevent trafficking of cells to the CNS (Baecher-Allan et al., 2018).

The first DMTs for MS, interferons (IFN), became available in Finland in the 1990s. IFN-beta 1b was approved for MS treatment in 1995 but reimbursement by the Social Insurance Institution was not granted until 1998. Glatiramer acetate became available in 2004, natalizumab in 2006, fingolimod in 2011, alemtuzumab and teriflunomide in 2013, pegylated IFN-beta and dimethyl fumarate in 2014, ocrelizumab and cladribine in 2018, and siponimod in 2020. Mitoxantone was approved for the treatment of worsening RRMS in 2000. In 2022 ponesimod and ofatumumab became reimbursed for the treatment of relapsing MS in Finland. Diroximel fumarate was approved for RRMS treatment in Finland in 2021 but is not yet reimbursed. Ozanimod was approved by the European Medicines Agency for the treatment of RRMS in 2021, but it is not yet available in Finland. Rituximab is not officially approved for MS treatment in Finland, but is in some cases used off-label.

The DMTs for MS in Finland according to the Current Care Guidelines are shown in Table 3. The latest update of the Current Care Guidelines for MS is from 2020, and additional DMTs have already became available since then, which emphasizes the rapid progress in MS treatment in recent years.

Table 3. Disease modifying treatments available for different subtypes of MS in Finland. Modified from Multiple Sclerosis Current Care Guidelines, 2020.

MS Type	DMT
Active RRMS	Interferon beta Dimethyl fumarate Glatiramer acetate Ocrelizumab Teriflunomide
Highly active RRMS	Alemtuzumab Fingolimod Cladribine Mitoxantrone Natalizumab Ocrelizumab
Active PPMS	Ocrelizumab
Active SPMS	Siponimod

DMTs for active MS

The DMTs are categorized based on their effectiveness in those used in active RRMS and those used in highly active RRMS. The DMTs used for active MS in Finland

include interferon beta, teriflunomide, glatiramer acetate, dimethyl fumarate, and ocrelizumab. The self-injectable interferon beta 1a and 1b are well-established and have comparable long-term efficacy and safety data. They have been shown to reduce relapses and T2 lesions on MRI and to postpone RRMS diagnosis in CIS patients (Ebers, 1998; Jacobs et al., 1996; Simon et al., 1998). Pegylated interferon, which became available later and has an advantage of a longer interval between injections, has been shown to reduce relapses and T2 lesions on MRI (Calabresi et al., 2014).

Another self-injectable DMT, glatiramer acetate, has shown similar efficacy results when compared to interferon beta (la Mantia et al., 2015). Studies on long-term outcomes of interferons and glatiramer acetate have not shown consistent findings on their effect on disability. In some studies, there was no detectable effect on disability (Shirani et al., 2012), and some suggest that they only slow down the progress of disability (Palace et al., 2015).

Dimethyl fumarate is an oral treatment that has been shown to reduce relapse rate, new Gd-enhancing and T2 lesions on MRI, and to slow down EDSS progression (Fox et al., 2012; Gold et al., 2012; Kappos et al., 2008; Xu et al., 2014). Diroximel fumarate is a newer oral treatment for active MS, similar to dimethyl fumarate, but with less gastrointestinal adverse events (Jonasson & Sejbaek, 2020).

Another oral treatment, teriflunomide, has been shown to decrease relapse rate as well as T2 and Gd-enhancing lesions on MRI compared to placebo (Confavreux et al., 2014; O'Connor et al., 2011; Vermersch et al., 2014). Safety data has shown that it is also safe in long-term use (O'Connor et al., 2016).

Ocrelizumab is a humanized monoclonal antibody that selectively depletes CD20+ B cells. It is administered as an intravenous infusion every 6 months. In a study comparing ocrelizumab and interferon beta, ocrelizumab reduced relapse rate and T2 and Gd-enhancing lesions on MRI more effectively, and the percentage of patients with confirmed disability progression at 12 weeks was markedly lower than with interferon beta (Hauser et al., 2017).

DMTs for highly active MS

The treatments for highly active MS according to the Current Care Guidelines of MS in Finland are fingolimod, alemtuzumab, natalizumab, cladribine, ocrelizumab, and mitoxantrone. Ocrelizumab is used both in active and highly active MS. In addition, rituximab is used off-label. Ofatumumab and ponesimod are not yet included in the latest Current Care Guidelines, but they have become available in 2022 and can be considered as treatments for highly active MS, because ofatamumab has a similar efficacy profile to ocrelizumab, and ponesimod to fingolimod.

Ofatumumab is a second-generation anti-CD20 fully human monoclonal antibody, which has been shown in phase II and III clinical trials to be effective in reducing the ARR and clinical disability worsening, and in suppressing MRI disease activity. The safety profile of ofatamumab has been proven to be favourable, with good tolerability and low immunogenic risk (Gajofatto & Orlandi, 2022).

Alemtuzumab, a humanized monoclonal antibody that targets CD52 on monocytes and lymphocytes, has been compared to interferon beta in 3 randomized controlled trials with 2- to 3 years of follow-up. Alemutuzumab significantly reduced relapse rate, T2 and Gd-enhancing lesions on MRI, and disability worsening (Cohen et al., 2012; Coles et al., 2008, 2012). It is administered as an intravenous infusion for 5 consecutive days, which is repeated as a 3-day infusion after a year.

Natalizumab is an α_4 integrin antagonist that has been shown to reduce relapse rate, new T2 and Gd-enhancing lesions on MRI, and slow down EDSS progression in RRMS (Miller et al., 2003; Polman et al., 2006). Natalizumab is given as an intravenous infusion every 4 weeks. The most important safety concern of natalizumab is the risk of PML, which is 27/1000 in JCV-positive patients and 0.07/1000 in JCV-negative patients (Ho et al., 2017).

Fingolimod, a sphingosine 1-phosphate (S1P) receptor modulator, is an oral treatment that has shown an effect on relapse rate and MRI activity and it has slowed down disability worsening when compared to placebo in randomized controlled trials (Kappos et al., 2006, 2010). After fingolimod, additional S1P receptor modulators, ozanimod and ponesimod, have been approved for relapsing MS treatment. Ozanimod has been shown to be well tolerated and to reduce relapse rate more efficiently than interferon beta in RRMS patients (Comi et al, 2019). Ponesimod has shown to reduce relapse rate, fatique, MRI activity and brain volume loss compared to teriflunomide in the treatment of relapsing MS (Kappos et al., 2021).

Cladribine is an oral treatment that has an immunomodulative effect through selectively targeting lymphocyte subtypes. It has been shown to reduce relapse rate and MRI activity and to slow down EDSS worsening in RRMS (Giovannoni et al., 2010). In CIS patients, cladribine led to 50% less conversion to clinically definite MS in 96-week follow-up (Leist et al., 2014).

Mitoxantrone is an antineoplastic agent that intercalates with DNA and exerts a potent immunomodulating effect that reduces T cell numbers, abrogates helper activity, suppresses humoral immunity, and enhances suppressor function (Edan et al., 1997). It is an old drug that was used in cancer treatment before the more modern antineoplastic drugs were developed. The effect of mitoxantrone in RRMS and SPMS has been studied in three randomized controlled trials, which have shown that it may reduce relapses and slow the rate of disability progression in comparison to placebo (Edan et al., 1997; Hartung et al., 2002; Millefiorini et al., 1997;). Its use in

MS began in the early 2000s before natalizumab became available. Currently, it is seldom used due to the availability of better DMTs and its potential risk of causing malignancies and severe adverse events. (Achiron, et al. 2005; Ragonese et al., 2017). It has a cumulative cardiotoxic effect, but cardiotoxicity can arise even after a single dose (Paul et al., 2007).

Rituximab, a monoclonal antibody, is not officially an MS treatment in Finland, but it is used off-label in highly active MS. One randomized placebo-controlled trial on rituximab in RRMS patients showed that it reduces MRI activity and relapse rate (Hauser et al., 2008).

DMTs in progressive MS

The mechanisms of progressive MS are both immune dependent and immune independent. An innate immune response established in the brain involves macrophages, microglia, lymphoid follicles, and B cells. There may also be chronic activation of peripheral T cells and innate cells. In immune-independent forms, oxidative stress, mitochondrial injury, and ion imbalance occur. Current DMTs do not effectively target these processes (Baecher-Allan et al., 2018). Most DMTs for MS are indicated for RRMS and SPMS, in which relapses still occur. These therapies have not shown efficacy in slowing disability progression in SPMS, until recently. Among the latest of the current DMTs, siponimod, is an oral treatment that selectively modulates S1P-receptors. Siponimod is the first DMT that has shown a reduction of the risk of disability progression in SPMS and it has a safety profile typical for S1P-receptor modulators. In a large RCT, siponimod significantly reduced 3-month confirmed disability progression (CDP) compared to placebo, and the safety profile was similar to other drugs in the class (Kappos et al., 2018). Ocrelizumab has shown reduction of disability progression and MRI activity not only in RRMS, but also in PPMS (Montalban, et al., 2017).

Treatment of relapses

The challenge with relapse treatment is defining whether an episode is a true relapse or an exacerbation or fluctuation of symptoms (Doshi & Chataway, 2017). If the relapse is of at least moderate functional severity, high-dose methylprednisolone therapy should be considered and any concomitant infection should be treated before corticosteroid therapy initiation. Oral vs intravenous administration have been shown to be equally effective, provided the dose is high enough (le Page et al., 2015). Corticosteroid treatment tends to shorten the duration of the relapse, but there appears to be no long-term benefits (Gal et al., 2015). Plasma exchange is occasionally used for severe or rapidly progressive relapses, as an adjunctive therapy

or alone. Therapy interventions, such as physiotherapy, should also be used early to enhance recovery from the relapse (Doshi & Chataway, 2017).

2.5.2 Treatment strategies

Due to the vast selection of available DMTs, one of the key questions in MS treatment is which treatment to choose for a patient. There are two common treatment approaches used in MS clinics. In the escalation approach, lower-efficacy therapies with a known and relatively safe risk profile are selected for initial treatment. If disease activity persists or recurs during sufficiently long and regular treatment on the initial DMT, treatment is escalated to a more potent option. The DMTs classified as moderate efficacy include beta interferons, teriflunomide, glatiramer acetate and dimethyl fumarate. Fingolimod has been classified as either moderate or high efficacy, depending on the source (Scolding et al., 2015; Wiendl et al., 2021).

Alternatively, in the early intensive approach, a high-efficacy DMT is initiated already at the time of diagnosis. The highly effective DMTs include alemtuzumab, cladribine, natalizumab, ocrelizumab, fingolimod, ofatumumab, ozanimod, and ponesimod (Wiendl et al., 2021). This early intensive treatment algorithm is currently suggested to be reserved only for the patients considered at highest risk of accumulating disability, usually those who have a high level of clinical or radiologic MS activity. Recent studies on optimal treatment strategies suggest, that the current treatment algorithms may miss an opportunity for achieving the highest effectiveness of DMTs (Buron et al., 2020; Harding et al., 2019; Kalincik et al., 2017; Rush et al., 2015). DMTs have been most effective when aggressive treatments have been applied in the earliest stages of the disease course (Coles et al., 2006; Edan et al., 2011).

3 Aims

Better understanding of predictive factors in MS is needed. Although DMTs have shown several well-established beneficial effects, they have shown little measurable improvement on long-term disability outcomes. The reasons behind these suboptimal outcomes have been studied, and one reason may be that current treatment paradigms miss a window of therapeutic opportunity. Prognostic biomarkers could help identify the MS patients in need of more aggressive treatment from early on, and thus improve the future disability outcomes. The specific aims of this study were the following:

1. To investigate which brain volume measures correlate with disability and cognition in a clinical cohort of relapsing and secondary progressive Finnish MS patients.

2. To evaluate whether brain volume measurement at a single time point could predict No Evidence of Disease Activity (NEDA) at 2 years and disability progression at 5 years in relapsing MS patients.

3. To address whether serum neurofilament light chain (NfL) concentrations correlate with MRI activity during the first year of interferon therapy in interferon-beta-1b-treated Finnish MS patients and whether the NfL levels are affected by supplementation of vitamin D.

4. To compare outcomes of initiating treatment with high-efficacy infusion therapies vs starting therapy with medium-efficacy therapy in a propensity-matched cohort of Finnish RRMS patients.

This work consists of three separate studies (1-3). The Original articles I and II are based on study 1, the Original article III is based on study 2, and the Original article IV is based on study 3.

4 Materials and Methods

4.1 Patients

Table 4. Summary and baseline characteristics of all the patients included in the studies.

	Number of patients and MS subtype	Age (y, mean)	Females/males	Baseline EDSS (median)
Study 1, Original article I and II	24 RRMS	36.3	20/4	1.2
	36 SPMS	52.8	21/15	4.5
Study 2, Original article III	32 RRMS	38.4	20/12	2.0
Study 3, Original article IV	154 RRMS– initial high-efficacy treatment	30.4	109/45	2.0
	1771 RRMS– initial medium-efficacy treatment	32.3	1293/478	1.0

4.1.1 Study 1, Original articles I–II

Original article I. Twenty-four patients with newly diagnosed RRMS and 36 SPMS patients were included. Inclusion criteria for the RRMS patients were fulfilling McDonald 2010 criteria, EDSS 0– to 3.5 and having interferon-beta or glatiramer acetate as the first DMT, initiated within 12 months. Inclusion criteria for the SPMS patients were: SPMS diagnosis defined by the treating neurologist and EDSS 4– to 6.5. The patients were recruited from the outpatient clinic of Turku University Hospital. Exclusion criteria included the following: malignancy, pregnancy or planning a pregnancy, contraindications to MRI, and failure to obtain informed consent. The first patient enrolled in March 2014 and the last patient in January 2015.

Original article II. The patients were the same as in Original article I (Hänninen et al., 2019), with the same inclusion and exclusion criteria; 24 patients with newly diagnosed RRMS and 36 SPMS patients were included. In the analyses of Original article II, 5-year follow-up data for the patients in question was available, whereas in Original article I, follow-up data was only available for up to 2 years.

4.1.2 Study 2, Original article III

A total of 66 RRMS patients were included and randomized to either 20,000 IU (500 µg) of cholecalciferol (Swiss-Caps), or identical placebo capsules, for 52 weeks. Of these 66 patients, 32 had available serum samples and were included in the analyses of this study. The study design and clinical and MRI results of the Finnish vitamin D study (cholecalciferol as an add-on treatment to subcutaneously administered interferon b-1b for the treatment of MS) have been previously published (Soilu-Hänninen et al., 2012). The aim of the present study was to address whether sNfL concentrations are affected by supplementation of vitamin D and correlate with disability and MRI markers of disease burden and activity in interferon-beta-1b (IFNb-1b)-treated Finnish MS patiens.

Inclusion criteria were the following: age 18– to 55 years; RRMS according to the McDonald criteria (Polman et al., 2005), with IFNB-1b use for at least 1 month; no neutralizing antibodies to IFNB, as measured by the indirect myxovirus A (MxA) test; EDSS score ≤5.0; using appropriate contraceptive methods (women of childbearing potential); and signed written informed consent.

Exclusion criteria were the following: serum 25(OH)D >85 nmol/l; serum calcium >2.6 mmol/l; pregnancy or unwillingness to use contraception; drug or alcohol abuse; use of other immunomodulatory therapy than IFNB-1b; primary hyperparathyroidism; known allergy to cholecalciferol or peanuts; therapy with calcitonin, vitamin D, or vitamin D3 analogues; digitalis; any condition predisposing to hypercalcaemia (such as any type of cancer); sarcoidosis; renal insufficiency or nephrolithiasis; significant hypertension (blood pressure <180/110 mm Hg); hyperthyroidism or hypothyroidism in the year before study onset; a history of kidney stones in the previous 5 years; cardiac insufficiency; unstable ischaemic heart disease or significant cardiac dysrhythmia; depression; and inability to perform serial MRI scans. Patients were recruited from the outpatient clinics of Helsinki, Tampere, Turku, Oulu, and Kuopio University Hospitals and the Central Hospitals of Central Finland and Ostrobotnia.

4.1.3 Study 3, Original article IV

The inclusion and exclusion criteria and patient enrollment are presented as a flow-chart in Figure 4. Patients receiving azathioprine, fingolimod, mitoxantrone, or cladribine as first-line treatment were excluded due to the following reasons: azatiophrine and mitoxantrone are not used as first-line DMTs for MS in Finland; fingolimod is reimbursed in Finland as first DMT only for patients with highly active disease, but was not categorized as high-efficacy treatment in this study on the basis of comparative data (Kalincik, Brown, et al., 2017); and cladribine use did not begin in Finland until 2018. However, additional analyses with fingolimod included in the high-efficacy DMT group were performed. The eligible treatment–naïve patients were categorized into two groups: initiating high-efficacy infusion therapy (heDMT) or moderate-efficacy therapies (meDMT). Alemtuzumab, ocrelizumab, rituximab, and natalizumab were categorized as high-efficacy and interferon beta, glatiramer acetate, dimethyl fumarate and teriflunomide as moderate-efficacy DMTs.

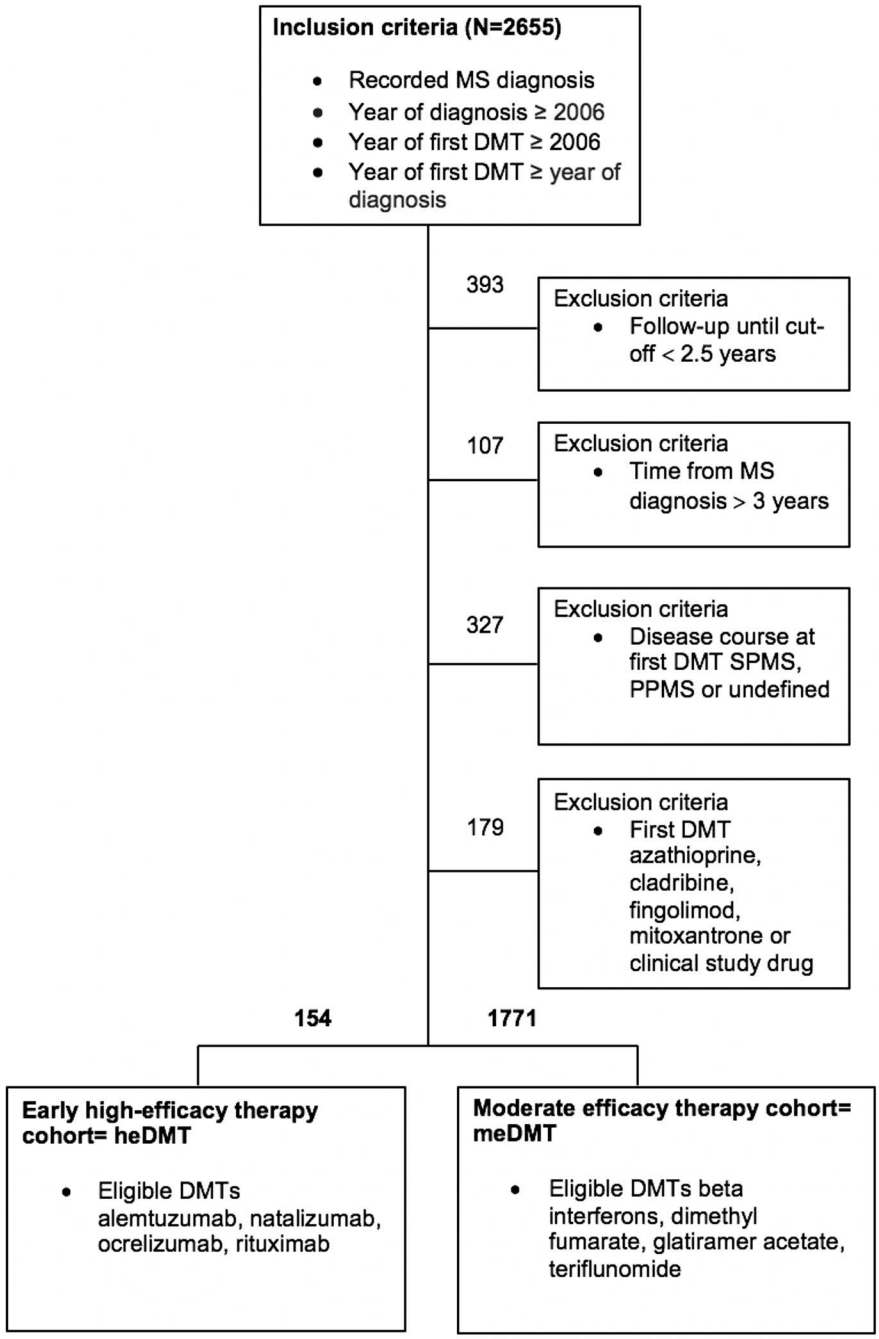

Figure 4. Flowchart of participants. Of the 1925 eligible patients enrolled, 154 (8.0%) had initiated a high-efficacy DMT as first-line treatment (heDMT), and 1771 (92.0%) had begun treatment with a moderate-efficacy DMT (meDMT). Modified from Original article IV, Figure 1.

4.2 Methods

Table 5. Summary of the prognostic and outcome variables of each study.

	Prognostic variable studied	Outcome variable
Study 1, Original article I	Regional atrophy in the brain MRI (several predefined loci)	EDSS- and SDMT-change, NEDA-3, NEDA-4
Study 1, Original article II	MS-subgroups based on thalamic and whole brain parenchymal atrophy: Group 1 = No atrophy (thalamic or parenchymal) Group 2 = Thalamic atrophy only Group 3 = Thalamic and parenchymal atrophy	Difference between study groups 1-3 in EDSS, relapses, and MRI findings
Study 2, Original article III	Serum NfL-level	EDSS and 25-Foot Walk test, MRI (activity, burden of disease)
	Serum vitamin-D levels	NfL
Study 3, Original article IV	Initial high- or moderate-intensity treatment	Time to 6-month EDSS progression (primary), time to first relapse (secondary), relapse rate

4.2.1 Study 1

4.2.1.1 Clinical evaluation

Original article I. Structured StellarQ MS registry (SQ-MS, www.stellarq.com) was used for collection of the following background data: age, sex, neurological status findings, date of MS diagnosis, first symptoms, socioeconomic status, data on immunomodulatory drug treatment, EDSS at baseline and at 2 years, serum concentration of 25-OH(D), and data on relapses from the date of first symptoms until the end of the study. SDMT was performed at baseline and at 24 months by the study nurse. EDSS was performed by a neurologist with 21 years of experience in EDSS evaluation. The SDMT and EDSS evaluations were performed within 2 weeks of the MRI acquisition. No evidence of disease activity status, NEDA-3, was determined by no relapses, no new or enhancing lesions on MRI, and no disability progression from the study baseline 6 months to 2 years later. NEDA-4 was determined by NEDA-3 and annualized whole brain volume loss ≤0.4%.

Standard scores (z-scores) were defined by comparing individual brain volumes with corresponding volumes from the Open Access Series of Imaging Studies

(OASIS) cohort of 295 healthy controls acquired with Siemens 3T scanners (Marcus et al., 2010). A cut-off value of −1.96 was applied for the z-scores to separate pathologically atrophic from normal brain volumes for thalamus and whole brain parenchymal (BP) volume (accepting a 2.5% error probability). Patients were then divided into groups based on the z-scores.

Original article II. The same patients and background data as in Original article I were used in Original article II. The 5-year analysis included information on MRI findings at the study baseline, annual EDSS, relapses, immunomodulatory drug usage, and possible changes in treatment during the follow-up. EDSS increase was defined by a 1-point increase in EDSS in patients with baseline EDSS <5.5 (1.5-point increase for EDSS = 0), or an increase of 0.5 for patients with EDSS 5.5 or greater.

4.2.1.2 MRI evaluation

Original article I. MRIs were obtained from the newly diagnosed RRMS patients 6 months after initiating DMT and 2 years later, and from the SPMS patients at study baseline and 2 years later. All MRIs were analysed by the same neuro-radiologist with extensive experience in MS MRI analyses. Two female patients did not undergo the 2-year MRI analysis, one because of a total atrioventricular block necessitating a pacemaker before the 2-year MRI, and the other because of refusal. The patients had to be clinically stable with no corticosteroid administration within 30 days prior to the MRI. All the MRIs were acquired at the same radiological facility with the same scanner, and the same acquisition protocol settings. The MRIs were obtained using a Siemens Skyra 3.0 Tesla scanner. The details of the MRI acquisition protocols are published in Original article I (Hänninen et al., 2019). The effect of pseudo-atrophy was minimized by timing the baseline MRI 6 months after the onset of the DMT in the RRMS patients. Pseudo-atrophy is the phenomenon of paradoxical acceleration of brain volume loss in MS patients following the initiation of most DMTs (Zivadinov et al., 2008). Baseline grey- and white–matter volumes, normalized for head size, were determined by SIENAX and volume change between baseline and 2 years by SIENA on the 3D T1 MRI images prior to gadolinium administration by an experienced neuro radiologist. Manually generated lesion masks and the lesion-filling tool (part of FSL) were used to minimize the impact of hypo intense T1 lesions on the volume measurements. In the SIENAX analyses, the same BET (Brain Extraction Tool) parameters (f = 0.1; g = 0; option “B”) were used for all images as previously described (Popescu et al., 2012). Quality control was performed to exclude imaging artefacts and BET parameters were further individually refined when necessary after manual correction of the segmentations.

Regional brain volumes were determined at baseline and 2 years by a fully automated multi-atlas segmentation tool cNeuro (Combinostics Ltd, Tampere, Finland), from the 3D T1 MRI images that were obtained prior to gadolinium administration. The volumes used in the statistical analyses were normalized for head size, sex and age. The cNeuro method is a fully automated, CE-marked tool for brain atrophy measurement, which is in clinical use at the Turku University Hospital. The method is illustrated in Figure 5. The segmentation method described in previous studies (Koikkalainen et al., 2016; Wang et al., 2012), was used to compute the volumes of white-matter lesions from 3D FLAIR images.

Original article II. The same MRIs and brain volume measures as in Original article I were used in the 5-year follow-up study and are described in the Original article II manuscript (Hänninen et al., 2020).

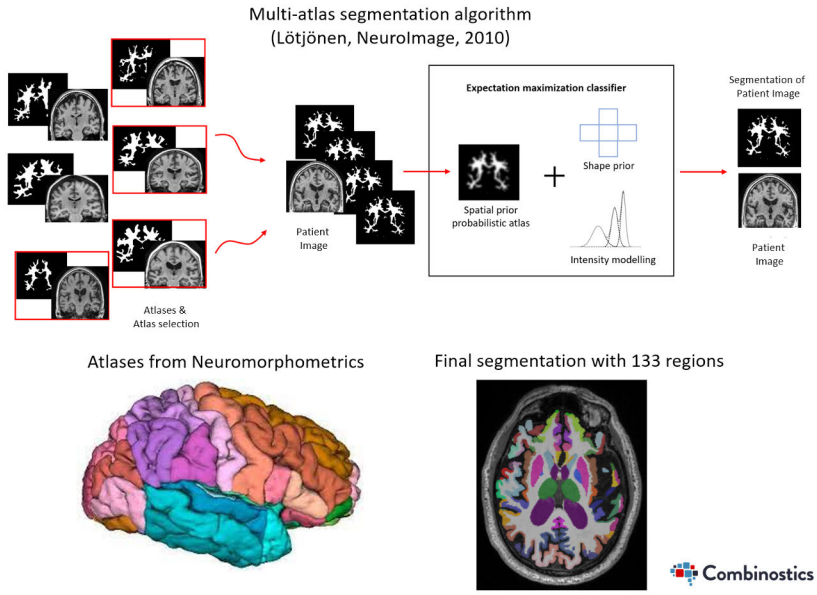

Figure 5. The cNeuro method. Reprinted with permission from Lötjönen et al., *NeuroImage* 2010;49(3):2352–2365. doi: 10.1016/j.neuroimage.2009.10.026.

4.2.2 Study 2

4.2.2.1 Laboratory analyses

Serum samples for the 25(OH)D analysis were gathered at the study baseline, at 6 and 12 months, and were freshly frozen and stored at −70°C until analyses. Serum

25(OH)D was measured using the DiaSorin Radioimmunoassay Kit. The sensitivity of the assay is 4.0 nmol/L, and intra-assay coefficient of variations were <10%. Serum NfL concentrations were measured in duplicate according to the manufacturer's instructions using the SIMOA (single molecule array) NfL Advantage Kit (Catalog number 103186; Quanterix). The SIMOA Kit and DiaSorin Kit details are described in Original article III (Hänninen, Jääskeläinen, et al., 2020).

4.2.2.2 MRI analyses

Brain MRIs were obtained at the study baseline and at 52 weeks using a 1.5 Tesla scanner and dual echo T2/PD and postcontrast T1-weighted sequences covering the whole brain in transverse imaging plane with 3 mm slice thickness. The images were analysed centrally at the Neuroimaging Research Unit, Vita-Salute University, Milan, Italy. Analyses included the number of new/enlarging T2/PD lesions and new Gd-enhancing lesions, quantification of the total number of Gd-enhancing lesions, and T2 lesion volume (burden of disease, BOD) (mm3), and T1-enhancing lesion volume (mm3).

4.2.3 Study 3

The study was a population-based, propensity-matched register study of Finnish RRMS patients from four Finnish hospital districts: Southwest Finland (SwF), Helsinki and Uusimaa (HUS), Pirkanmaa and Kanta-Häme, jointly covering a population of 2.8 million inhabitants. Data collection was conducted from 1 January 2006 to 31 December 2020. The clinical and demographic data were collected using the Finnish MS register.

The eligible treatment–naïve RRMS patients were categorized into 2 groups: initiating high-efficacy infusion therapy (heDMT) or moderate-efficacy therapies (meDMT). Alemtuzumab, ocrelizumab, rituximab and natalizumab were categorized as high-efficacy, and interferon beta, glatiramer acetate, dimethyl fumarate and teriflunomide as moderate-efficacy DMTs. All DMTs were administered according to published protocols. Rituximab was administered as one dose of 1.0 g intravenously, followed by 0.5 g every 6 months.

4.3 Statistics

4.3.1 Study 1

Original article I. Fisher's exact test was used for categorical parameters and Wilcoxon rank-sum test for continuous variables in the group comparisons. The

Pearson correlation coefficients were used in the correlation analyses and the significances of correlations were obtained from the t distribution. Benjamini-Hochberg procedure (1995) was used as correction for multiple comparisons for controlling the False Discovery Rate. Differences in NEDA-3, NEDA-4, SDMT, and EDSS were evaluated using Fisher's exact test and validation of p-values was conducted using the Monte Carlo simulated chi-squared test. Additional analysis for the 2 × 2 contingency tables was conducted using predictive values, logistic regression derived odds ratios, and confidence intervals. NEDA-3 positive predictive value was determined by the ratio of patients with thalamus atrophy and not reaching NEDA-3 among all the patients with thalamus atrophy (true positives). NEDA-3 negative predictive value was determined by the ratio of patients with no thalamic atrophy and reaching NEDA-3 among all patients with no thalamic atrophy (true negatives). EDSS positive and negative predictive value was determined similarly. The number of patients reaching NEDA-4 was too small for a meaningful predictive value analysis. R was used for all the analyses and p-values <0.05 were considered significant.

Original article II. Fisher's exact test was used for calculating the baseline group comparison p-values for categorical variables. Conditional probabilities were obtained from hypergeometrical distribution. Group comparison p-values for continuous variables were calculated using non-parametric Wilcoxon rank-sum test, testing the location shift difference between two samples. The p-values of the baseline characteristics and logistic regression analysis were adjusted using the Benjamini-Hochberg method, in which false discovery rate is controlled by penalizing smaller p-values more than higher p-values. The prevalence, sensitivity, specificity, and positive and negative predictive value derivations were calculated for EDSS change as described previously (Akobeng, 2007).

Reaching EDSS increase in 5 years was modelled with the whole study population, where all baseline MRI covariates and 5-year EDSS could be assessed. The dependent variable reaching EDSS increase in 5 years is represented as a binary (0/1) variable. All MRI variables included in the baseline characteristics were considered to explain EDSS increase, except for total white matter volume, and cerebellum white matter volume, due to the fact that grey matter atrophy has shown to have a greater clinical relevance in MS (Fisher et al., 2008). Age at baseline was added to adjust baseline characteristics of the modeled population. A logistic regression model was fitted using a generalized linear model with logit link function to explain EDSS increase. The four most important covariates were identified based on univariate analysis. Underlying multicollinearity was checked, and use of selected variables was confirmed using variance inflation factors. The best model was selected using manual forward selection, where a simple model is the starting point

and terms were added based on deviance analysis until the model shows no significant improvement. In addition, automatic forward and backward Akaike information criteria-based stepwise algorithms support the model choice. A few influential outliers were detected by diagnostic checks, but based on studentised residuals and Cook's distance, they were not influential enough to justify refitting the model without those observations.

4.3.2 Study 2

Statistical analyses were conducted with SPSS (SPSS Inc., version 20.0) and GraphPad Prism (GraphPad Software). Descriptive statistics were provided as mean and standard deviations for normally distributed data and median (95% confidence interval) for skewed variables. Due to the non-normal distribution of the sNfL levels, natural logarithm transformation was used to create normally distributed data. Unpaired t test was used to compare differences in the logarithmic sNfL levels between the treatment arms at baseline and 52 weeks, and between patients with and without Gd-enhancing lesions. Correlation of log-transformed sNfL levels to other variables was tested with Pearson's correlation. The p-values <0.05 were considered significant.

4.3.3 Study 3

The primary outcome was time to 6-month confirmed disability progression (CDP) and the secondary outcome was time to first relapse. As an additional outcome, odds for disability progression at 3 and 5 years in the whole study cohort and matched cohorts was studied. Safety was assessed as an exploratory outcome.

Disability was evaluated with EDSS and disability progression was defined by the following criteria: ≥1.5-point increase from baseline EDSS of 0; ≥ 1-point increase for baseline 1 to 5.5; and ≥ 0.5 increase for baseline ≥ 6. The 6-month CDP was defined by the same EDSS criteria and a 6-month confirmation period. As baseline EDSS, the closest date to DMT onset (12 months prior to or 6 months after) was used, prioritizing EDSS assessments within 6 months prior. EDSS assessments within 1 month after relapse were excluded at baseline and follow-up, unless verified by a 6-month confirmation period.

Group comparisons for continuous variables were conducted using the Wilcoxon rank-sum test or Student's t test, depending on the normality of the groups. Group comparisons for categorical variables were done using Fisher's exact test. For controlling and checking the False Discovery Rate, Benjamini–Hochberg procedure was used as a correction for multiple comparisons. All significant raw p values remained under 0.05 after adjustment. Separate complete case matching was

performed for all outcome analyses. Propensity scores were matched by Nearest Neighbor matching with a 1:1 ratio and a calliper of 0.1 standard deviations (SDs) controlling adequate pair similarities. Matching variables for outcome analyses were sex, age, baseline EDSS, time since MS onset, and ARR 1 year prior DMT onset. Additionally, time difference between baseline EDSS and DMT onset was added to the matching for the logistic regression analyses.

Hazard ratios (HR) and corresponding confidence intervals were analysed using a semiparametric Cox proportional hazard regression model. Significance testing for rate differences between the patient groups was based on Wald test. Probabilities of 6-month CDP and first relapse at specific timepoints were analysed using cumulated events analysis based on 1-Kaplan–Meier estimates and curves. Log-rank test was used to assess differences among overall event probabilities. In the time to 6-month CDP analysis, time origin for patients with baseline EDSS before DMT onset was fixed to the DMT initiation date. Group balances were checked with standardized differences before and after matching. In the 6-month CDP analysis, standardized mean difference for sex falling over 0.2 was considered acceptable based on non-significant omnibus test using chi-squared test. Diagnostics for the Cox regression model included testing the proportional-hazards assumptions and visual residual checks. Univariate and multivariate analyses for unmatched data were conducted to detect the matching effect and raw data bias.

4.4 Ethical considerations

4.4.1 Study 1

The study was approved by the Ethics Committee of Turku University Hospital and University of Turku on 21.1.2014. A written informed consent was obtained from all patients participating in the study. The study was conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments

4.4.2 Study 2

The study was approved by the Ethics Committee of Turku University and Turku University Hospital and the National Agency of Medicine, Helsinki, Finland. All participants gave written informed consent.

4.4.3 Study 3

According to Finnish law, ethics committee approval was not required because the study was based on administrative register data and did not involve any contact with patients. The study was approved by the Helsinki, Tampere, and Turku University Hospitals and Kanta-Häme Central Hospital Research Services. The EU Data Protection Directive rules were followed in the data processing practices (permission numbers T16/2017 for SwF, R19613S for Pirkanmaa, HUS/163/2019 for Helsinki, and KHSHP/1571/13.00.01/2018 for Kanta-Häme).

5 Results

Table 6. Summary of key findings of the studies.

	Clinical prognostic variable studied	Key result	Conclusion
Study 1, Original article I	Global and regional brain atrophy	Worse performance in SDMT for cognition and higher EDSS for physical disability significantly correlated with smaller deep grey matter and total brain volumes. Even very early brain atrophy, such that atrophy was detected in thalamus only, was a negative prognostic marker for disability.	Brain volume measurement at one time-point could help predict disability progression in MS and complement clinical and routine MRI evaluation in therapeutic decision-making.
Study 1, Original article II	Thalamic atrophy	Patients with only thalamic atrophy were at a higher risk for not reaching 2-year No Evidence of Disease activity (NEDA) and for EDSS increase at 2 and 5 years than patients with no brain atrophy.	Brain volume measurement at one time-point could help predict disability progression in MS and complement clinical and routine MRI evaluation in therapeutic decision-making.
Study 2	Serum NFL	sNfL levels were similarly low in patients supplemented with high-dose vitamin D or placebo. Subclinical disease activity in MRI was associated with higher sNfL levels.	In clinically stable patients, elevated sNfL levels may assist in detection of subclinical inflammatory disease activity.
Study 3	Initial high efficacy infusion therapy	Patients who initiated first treatment with high-efficacy infusion therapy, had a 40% lower risk for developing physical disability and 30% lower risk for a relapse during the follow-up compared to patients initially treated with moderate-efficacy therapies.	Initiating MS therapy with high-efficacy DMTs results in better clinical outcome than with moderate-efficacy therapies. Randomized clinical trials are needed to directly compare these different treatment strategies in relapsing MS.

5.1 Study 1

5.1.1 Clinical and MRI characteristics of the RRMS and SPMS patients

At baseline, the SPMS patients were older, had longer disease duration, lower SDMT scores, and higher EDSS points compared to RRMS patients. There were no significant differences between the RRMS and SPMS patients in their comorbidities, alcohol consumption, smoking habits, education status, serum vitamin D levels or BMI. All patients in the RRMS group started either glatiramer acetate (GA) or interferon-beta (IFNB) 6 months prior to the baseline MRI. In the SPMS group, 30% of the patients were using DMTs. As a local treatment practice, all patients were supplemented with vitamin D3, with a mean dose of 65.9 μg (range 50–150) in the RRMS patients and 55.2 μg (range 10–100) in the SPMS patients. One patient in the SPMS group had ulcerative colitis and took azathioprine medication. Other comorbidities included arterial hypertension, depression, glaucoma, epilepsy, irritable bowel syndrome, hypercholesterolemia, hypothyroidism, mitral valve prolapse, and frozen shoulder, with no clustering of any comorbidities in either patient group. The baseline characteristics are presented in Table 7.

Table 7. Baseline demographic and clinical characteristics of the patients included in the study. Modified from Original article I, Table 1.

Variable	RRMS	SPMS	p-value
N	24	36	
Age, years (mean, SD)	36.3 (7.53)	52.8 (7.28)	**<0.001**
Females/males	20/4	21/15	0.069
Disease duration, years (mean, range)	0.8 (0.7-1.6)	19.7 (5.4-35.7)	**<0.001**
EDSS (median, range)	1.2 (0.0-4.0)	4.5 (2.0-6.5)	**<0.001**
Education, years (mean, SD)	12.5 (2.30)	12.5 (2.91)	0.825
SDMT (mean, SD)	49.5 (11.82)	36.3 (10.72)	**<0.001**
BMI kg/m2 (mean, range)	26.7 (18.4-41.0)	25.3 (16.1-36.3)	0.417
Smoking, yes/no	11/13	14/22	0.662
25(OH)D, nmol/l (mean, range)	122.8 (59.7-271.6)	108.4 (25.7-268.7)	0.248
Number of relapses (mean, range)	0.7 (0.0-3.0)	0.1 (0.0-2.0)	**0.005**
Percentage of patients with DMTs	100	31	**<0.001**

In the MRI analyses, significant differences between RRMS and SPMS patients were found in the white matter lesion volume, total brain parenchymal volume (BP), total grey and white matter, cerebellar white matter, and hippocampus, thalamus and putamen volumes at study baseline and at 2 years. The most significant differences between RRMS and SPMS patients were found in thalamic volume ($p < 0.001$) and cerebellar white matter volume ($p < 0.001$) at both time points. The total and regional brain volumes at baseline and at 2 years are presented in Table 8.

Table 8. Brain MRI findings in the RRMS and SPMS patients at study baseline and at 2 years. Modified from Original article I, Table 2.

	RRMS		SPMS		p-value	
Variable	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
N	24	23	36	35		
Gd+ lesions (N,%)	4/24 (17)	1/23 (4)	2/36 (6)	4/35 (11)	0.248	0.677
Total BP vol. (ml, SD)	1509.5 (109.26)	1482.3 (110.87)	1397.9 (107.11)	1404.7 (107.49)	**0.001**	**0.012**
Total WM vol. (ml, SD)	688.6 (51.40)	678.0 (45.09)	648.1 (49.05)	652.9 (53.10)	**0.011**	0.084
Total GM vol. (ml, SD)	820.7 (67.03)	804.1 (74.16)	749.9 (66.24)	751.7 (67.66)	**<0.001**	**0.007**
WM lesion vol. (ml, SD)	11.0 (8.35)	10.2 (8.43)	20.2 (12.26)	20.4 (13.29)	**<0.001**	**<0.001**
Putamen vol. (ml, SD)	4.4 (0.58)	4.4 (0.61)	4.0 (0.63)	4.0 (0.67)	**0.020**	0.065
Hippocampus vol. (ml, SD)	3.9 (0.48)	3.9 (0.55)	3.5 (0.44)	3.4 (0.48)	**0.008**	**0.006**
Thalamus vol. (ml, SD)	7.2 (0.80)	7.3 (0.82)	6.2 (0.95)	6.2 (0.97)	**<0.001**	**<0.001**
Nucleus caudatus vol. (ml, SD)	2.9 (0.41)	2.8 (0.45)	2.5 (0.40)	2.5 (0.43)	**<0.001**	**0.014**
Globus pallidus vol. (ml, SD)	1.3 (0.15)	1.2 (0.16)	1.1 (0.14)	1.1 (0.14)	**0.003**	**0.020**
Cerebellum WM vol. (ml, SD)	15.7 (2.36)	15.8 (2.25)	11.9 (2.97)	11.6 (2.62)	**<0.001**	**<0.001**
Cerebellum GM vol. (ml, SD)	55.1 (5.18)	57.1 (5.92)	54.7 (7.21)	54.1 (7.34)	0.769	0.127

5.1.2 Correlation of brain volume measures with cognition and disability

Better performance in SDMT test showed a significant correlation with larger cerebellum white matter, thalamus, hippocampus, total grey matter, and total brain volumes at both baseline and 2 years. Higher EDSS significantly correlated with smaller cerebellum white matter, putamen, thalamus, hippocampus, total grey matter, and total brain volumes at baseline and at 2 years. White matter lesion volume positively correlated with EDSS ($p = 0.045$ at baseline, $p = 0.015$ at follow-up) and negatively with SDMT ($p < 0.001$ at baseline and at 2 years).

5.1.3 Grouping of the patients based on atrophy measures and subgroup comparisons

After the comparisons between the RRMS and SPMS groups, the patients were divided into new subgroups based on thalamic and whole brain parenchymal (BP) atrophy using z-scores. At the study baseline, 7 SPMS and 12 RRMS patients had no brain atrophy, 8 SPMS and 10 RRMS patients had isolated thalamic atrophy, and 2 RRMS and 20 SPMS patients had both thalamic and BP atrophy. Only 1 SPMS patient had BP atrophy without thalamic atrophy.

The patient group with neither thalamic nor BP atrophy was named group 1 ($n = 19$). The group with thalamic atrophy but no BP atrophy was named group 2 ($n = 18$). The group of patients with both BP and thalamic atrophy was named group 3 ($n = 22$). The grouping of the patients according to their thalamic and BP atrophy is illustrated in Figure 6. The one SPMS patient who had BP atrophy without thalamic atrophy was very close to the cut-off value of the patients in group 3 (thalamus z-score −1.88, whole BP −3.34), and 1 patient fell in between groups 1 and 2 and could not be categorized in either group. In group 2, EDSS change could not be determined for 1 patient due to missing two-year EDSS data and NEDA could not be determined for 2 patients because of missing 2-year MRI.

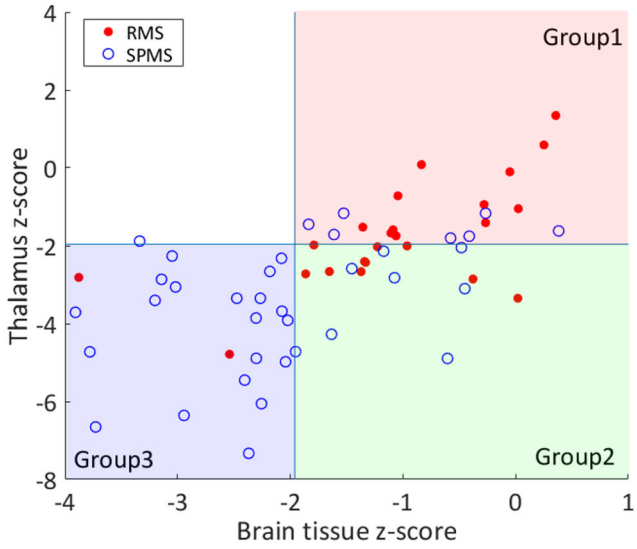

Figure 6. Grouping of the patients based on thalamic and BP atrophy. Relapsing MS (RMS) patients are marked with red circles and SPMS patients with blue rings. Most of the RMS patients fell into group 1 (both normal BP and thalamus). Group 2 patients had thalamic atrophy without BP atrophy. Most of the SPMS patients had both thalamic and BP atrophy (group 3). As shown, only 1 patient had BP atrophy without thalamic atrophy (upper left quadrant). Figure source: Original article I, Figure 1.

After grouping of the patients according to atrophy measures, between-group differences in SDMT and EDSS at baseline and at 2 years and the achievement of NEDA-3 and NEDA-4 during the 2-year follow-up were assessed. There was no significant difference in the EDSS, SDMT, or relapses between groups 1 and 2 at baseline. In group 3, most patients (20/22) were SPMS patients and had lower SDMT, higher EDSS, and fewer relapses compared to patients in group 1 and 2 and were therefore not included in the between-group outcomes analyses.

At 2 years, a significant difference was detected between groups 1 and 2 in EDSS change. In group 1, EDSS was same or better at the 2-year follow-up in 16/19 patients, but only in 5/17 patients in group 2 ($p = 0.002$). NEDA-3 was reached in 11/19 patients in group 1, but only in 2/17 patients in group 2 ($p = 0.012$). NEDA-4 was reached in 7/19 patients in group 1, and only in 1/16 in group 2 ($p = 0.047$). There was no significant difference in SDMT scores or relapses between the groups at baseline or at follow-up. SDMT scores had deteriorated during the follow-up in 6/19 patients in group 1, and in 10/16 patients in group 2 ($p = 0.179$). In group 1, 13/19 patients and in group 2, 13/18 patients had no relapses during the follow-up. A total of 6/19 patients in group 1 and 5/18 patients in group 2 had at least 1 relapse during the follow-up ($p = 1.000$). The achievement of NEDA-3, NEDA-4, and presence of relapses are presented in Table 9.

Table 9. Achievement of NEDA-3 and NEDA-4 and relapses during the 2-year follow-up in group 1 and group 2. Modified from Original article I, Table 4.

	NEDA-3		NEDA-4		Relapses	
Group	No (n)	Yes (n)	No (n)	Yes (n)	>0 (n)	0 (n)
Group 1	8	11	12	7	6	13
Group 2	14	2	15	1	5	13
p-value	**0.012**		**0.047**		1.000	

Group 1= Patients with no thalamic and no whole BP atrophy
Group 2= Patients with thalamic atrophy but no whole BP atrophy
Prevalence of NEDA3-No: 63 %. Sensitivity: 64 %. Specificity: 85 %. Positive predictive value: 88 %. Negative predictive value: 58 %. Odds ratio (OR) [95% CI]: 9.6 [1.69, 54.79]

In the 5-year follow-up analyses of Study 1, published in Original article II (Hänninen et al., 2020), the same grouping of patients based on thalamus and whole BP z-scores was used: the patient group that had no thalamic and no BP atrophy was group 1 (n = 19). The group with thalamic atrophy without whole BP atrophy was group 2 (n = 18). The group with both thalamic and whole brain atrophy was group 3 (n = 22). There was no significant difference in the demographic and clinical characteristics between groups 1 and 2, but in group 3, the patients were older and had a more advanced disease at baseline compared to group 1. The baseline demographic, clinical and MRI characteristics of the groups are shown in Table 10.

Table 10. Baseline demographic, clinical, and MRI characteristics of the patients with and without thalamus atrophy. Modified from Original article II, Table 1.

Variable	Group 1	Group 2	p-value	Group 3	p-value
N	19	18		22	
Age, years (mean, SD)	42.8 (10.04)	40.5 (10.39)	0.860	53.2 (7.97)	**0.002**
RRMS/SPMS	14/5	10/8	0.903	2/20	**0.001**
Female/male	17/2	13/5	0.696	10/12	**0.006**
Disease duration, years (mean, SD)	4.9 (6.07)	9.9 (11.54)	0.903	20.5 (10.63)	**<0.001**
EDSS (median, range)	2.0 (0.0–6.5)	1.8 (0.0–6.5)	0.970	4.0 (2.0–6.0)	**0.040**
Education, years (mean, SD)	13.0 (2.62)	11.7 (2.35)	0.403	12.7 (2.97)	0.719
SDMT (mean, SD)	48.5 (10.87)	45.1 (12.56)	0.818	33.1 (10.05)	**<0.001**
BMI kg/m2 (mean, SD)	27.3 (5.57)	24.9 (3.86)	0.696	25.5 (4.72)	0.602
Smoking, yes/no	7/12	10/8	0.720	7/15	0.822
25(OH)D, nmol/L (mean, SD)	118.9 (53.58)	111.5 (48.63)	0.903	112.8 (60.3)	0.616
Number of relapses (mean, SD)	0.4 (0.69)	0.5 (0.92)	0.970	0.1 (0.47)	0.120
Total BP vol. (ml, SD)	1505.3 (93.33)	1476.7(111.44)	0.903	1354.0 (101.58)	**<0.001**
Total WM vol. (ml, SD)	694.2 (43.38)	672.8 (48.78)	0.709	628.5 (47.30)	**<0.001**
Total GM vol. (ml, SD)	810.8 (59.14)	803.9 (69.55)	0.970	725.7 (65.83)	**<0.001**
WM lesion vol. (ml, SD)	11.4 (5.53)	14.7 (6.75)	0.322	26.6 (16.99)	**<0.001**
Putamen vol.	3.9 (0.40)	3.8 (0.35)	0.822	3.4 (0.53)	**<0.001**
Hippocampus vol. (ml, SD)	3.4 (0.24)	3.4 (0.33)	0.818	3.0 (0.32)	**<0.001**
Thalamus vol. (ml, SD)	6.6 (0.43)	5.7 (0.41)	**<0.001**	5.3 (0.78)	**<0.001**
Nucleus caudatus vol. (ml, SD)	2.8 (0.24)	2.6 (0.32)	0.194	2.3 (0.32)	**<0.001**
Globus pallidus vol. (ml, SD)	1.3 (0.14)	1.2 (0.12)	0.322	1.1 (0.12)	**<0.001**
Cerebellum GM vol. (ml, SD)	47.9 (4.89)	46.2 (4.59)	0.903	8.9 (1.02)	**<0.001**
Cerebellum WM vol. (ml, SD).	11.1 (1.24)	10.5 (0.97)	0.322	47.4 (6.45)	0.883
Gd + lesions (N, %)	0/19 (0.0)	5/18 (27.8)	0.194	1/22 (4.5)	1.000

Group 1: no brain atrophy at baseline; group 2: isolated thalamic atrophy at baseline; group 3: whole brain and thalamic atrophy at baseline.
p-values calculated against group 1.

Reaching EDSS increase in 5 years was modelled with the whole study population where 5-year EDSS could be assessed. The final model and its estimates are presented in Table 11. Baseline thalamus volume was significant ($p = 0.031$). Every unit decrease in baseline thalamus volume increased the odds to reach disability progression over 5 years to 2.4-fold.

Table 11. Logistic regression for prediction of disability in 5 years by baseline thalamic volume. Modified from Original article II, Table 2.

Variable	Estimate	Std. error	p-value	OR (95% CI)
Intercept		6.46	3.034	0.033
Age	−0.04	0.027	0.196	0.95 (0.91, 1.02)
Thalamus vol.	–0.86	0.398	**0.031**	0.42 (0.18, 0.88)

The prevalence, sensitivity, specificity, and positive and negative predictive values for EDSS change at 5 years in groups 1– to 3 are shown in Table 12. At 5 years, EDSS was the same or better in 12/18 patients in group 1, while in group 2, EDSS was the same or better only in 5/18 patients. In group 3, EDSS was the same or better in 7/22 patients.

Table 12. The predictive value of baseline brain atrophy measures to EDSS change at 5 years. Modified from Original article II, Table 3.

	Group 1 vs. group 2	Group 1 vs. group 3	Group 1 vs. group 2+3
Prevalence	53%	52%	59%
Sensitivity	68%	71%	82%
Specificity	71%	63%	50%
Positive predictive value	72%	68%	70%
Negative predictive value	67%	67%	67%
Odds ratio (OR, 95% CI)	5.2 (1.25, 21.57)	4.3 (1.14, 16.18)	4.7 (1.42, 15.35)

All the comparisons were made using group 1 (patients with no brain atrophy at baseline) as the reference group. Group 1: EDSS same/better (n) = 12, EDSS worse (n) = 6, one patient excluded because of missing control value. Group 2: EDSS same/better (n) = 5, EDSS worse (n) = 13. Group 3: EDSS same/better (n) = 7, EDSS worse (n) = 15.

5.2 Study 2

5.2.1 Patient characteristics

Serum samples were available from 32 of the 66 patients in the Finnish vitamin D study, (17 vitamin D/15 placebo) at baseline, N = 20 patients (12 vitamin D and 8 placebo) at 6 months, and of N = 25 patients (13 vitamin D/12 placebo) at both the baseline and 52 weeks. MRI data and 25(OH)D results from the study baseline and the week 52 visit were available for all these patients. The vitamin D and placebo groups were balanced for the baseline characteristics and did not differ from the total Finnish vitamin D study population. Cohort characteristics of the 32 patients are shown in Table 13.

Table 13. Patient characteristics. Modified from Original article III.

	Vitamin D group (N=17)	Placebo group (N=15)	p-value
Age (y), mean (SD)	38.3 (8.17)	38.5 (7.32)	0.91
EDSS score, median (95% CI)	2.0 (1.5–3.0)	1.5 (1.0–3.0)	0.16
Body mass index, mean (SD)	26.2 (5.15)	24.72 (4.96)	0.42
Immunomodulatory treatment*, N	17	15	0.60
Smoking, N	8	4	0.99
25-hydroxyvitamin D (nmol/L), mean (SD)	52.2 (16.86)	58.5 (20.71)	0.37
25 FW, mean (SD)	4.87 (1.27)	4.90 (1.75)	0.67
NfL (pg/ml), median (95%CI)	12.44 (9.9–16.8)	11.86 (8.5–14.8)	0.47
T2 BOD, Mean (SD), Median (95% CI)	8,452 (10,698) 3,465 (2224–12016)	8,702 (8.743) 6,421 (2187–11272)	0.71
No. of Gd-enhancing lesions, mean (SD)	0.29 (0.69)	0.20 (0.56)	0.89
T1 enhancing lesion volume ($mm^{2)}$, mean (SD)	30.47 (71.3)	30.20 (89.12)	0.93
Disease duration (y), mean			
From onset of symptoms	7.79 (6.94)	7.31 (5.92)	0.75
From diagnosis	6.08 (6.22)	4.96 (4.48)	0.79
Annual relapse rate, median (95% CI)	0.49 (0.31–0.66)	0.52 (0.33–0.7)	0.67
Interferon therapy (y), mean	2.78	2.78	0.67

Note: All patients received interferon β-1b therapy. Annual relapse rate was from 2 years before the study onset. Abbreviations: BOD, burden of disease, mm3; NfL, neurofilament light in serum, 25 FW, timed 25-Foot Walk test in s, EDSS, Kurtzke's Expanded Disability Status Scale.

5.2.2 Correlation of serum NfL with vitamin D, MRI activity and disability

Age, disease duration, BMI, or smoking status did not significantly correlate with serum NfL. Disability was measured by EDSS and the Timed 25-Foot Walk test as described earlier (Soilu-Hänninen et al., 2012). In the original sample, there was no significant difference in the clinical endpoints at week 52, but there was a trend for less increase in the BOD on MRI and significantly less Gd-enhancing lesions in the vitamin D group than in the placebo group at week 52.

As previously reported (Soilu-Hänninen et al., 2012), in the patient group randomized into vitamin D supplementation, the 25(OH) D levels doubled from the baseline to the 52-week follow-up. The number of patients with vitamin D level >100 nmol/ L were 8/13 (61.5%) in the vitamin D group and 0/12 in the placebo group. Median (95% CI) sNfL was similar: 11.66 (6.5–52.8) pg/ml, in the 8 patients reaching serum 25(OH)D levels above 100 nmol/L at 12 months, in comparison with all patients in whom 25(OH)-D was below 100 nmol/L at 12 months, median (95%) sNflL 11.96 (10.39–16.88) pg/ml, p-value for difference .09.

The correlations of NfL with vitamin D by categories of 25(OH)D values 0<25, 25<50, 50<75, 75<100 and ≥100 were analysed. All the 79 sampling occasions at the study baseline, 6-, and 12 months were included (17 vitamin D, 15 placebo at baseline, 12 vitamin D and 8 placebo at 6 months, and 13 vitamin D, 12 placebo at 52 weeks). At baseline, there was a nonsignificant trend for a positive correlation of sNfL and serum 25(OH)D (r =.30, p = .09), but at month 12 no correlation could be seen (r = −.001, p = .99). No significant difference was found in the serum NfL levels between the vitamin D and placebo groups at the study baseline or at 52 weeks, as shown in Figure 7.

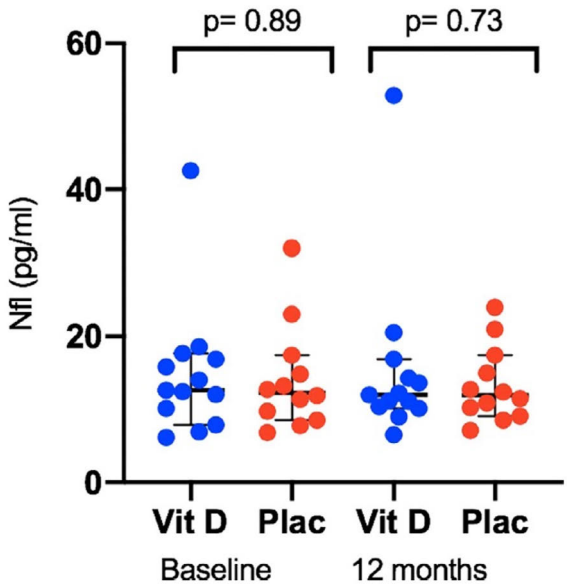

Figure 7. Serum neurofilament light chain (NfL) levels (pg/ml) stratified by treatment and timepoint. Plac= placebo group; Vit D=vitamin D treatment group. Bars present median and 95% confidence interval. Significance was tested with unpaired t test of logtransformed sNfL values. Figure source: Original article III, Figure 1.

None of the 25 patients experienced relapses during the 52 weeks of the study. No correlation between the EDSS and sNfL was found at study baseline (r = .181, p = .32) or at week 52 (r = −.047, p = .86). Levels of sNfL did not correlate with the 25-foot walk test results at the study baseline (r = −.090 p = .61) or at 12 months (r = −.05, p = .8).

A total of 5/32 patients at the study baseline and 4/25 patients at 52 weeks had Gd+ lesions on the brain MRI. At baseline, levels of sNfL were higher in patients with Gd+ lesions than in patients without Gd+ lesions (p < .014), presented in Figure 8, but there was no difference at week 52 (not shown). There were only 2 patients with Gd+-lesions at week 52 in the vitamin D arm of the whole Finnish vitamin D study population (N = 66 patients), and both were included in the current study. The Gd-enhancing lesion volumes at baseline among the 5 patients with enhancing lesions ranged from 102 to 332 m3 and significantly correlated with sNfL (Pearson .36, p = .037). At week 52, the enhancing lesions were smaller, ranging from 48 to 52 mm3, and did not significantly correlate with the sNfL concentration (not shown). No correlation was seen between MRI BOD and sNfL either at baseline (r = −.12, p = .48) or at 12 months (r = .08, p = .67).

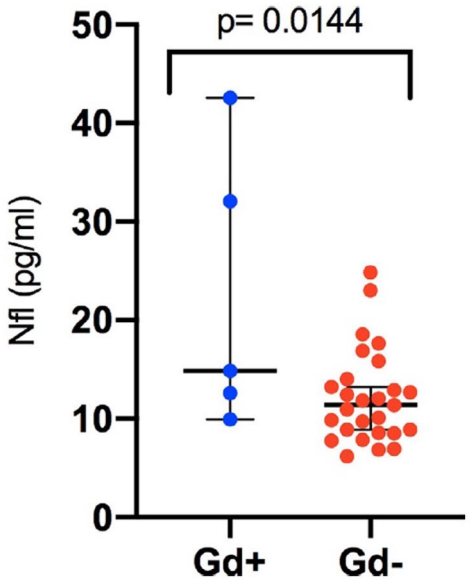

Figure 8. Serum neurofilament light chain (Nfl) levels (pg/ml) in the patients with Gd+ levels and without Gd+ lesions on brain MRI at the study baseline. Bars present median and 95% confidence interval. Significance was tested with unpaired t test of log transformed sNfL values. Figure source: Original article III, Figure 2.

5.3 Study 3

5.3.1 Patient characteristics and disability and relapse outcomes in the unmatched cohort

The clinical and demographic characteristics of all the study patients are presented in Table 14. The mean ARR prior to treatment was higher in the heDMT group vs meDMT (1.6 vs 1.1, $p < 0.001$), as well as baseline EDSS (median 2.0 vs. 1.0, $p < 0.001$). A conditional regression analysis with raw data indicated that the odds for disability progression at 3 years in the heDMT group (n=100) was significantly lower than in the meDMT group (n=308, OR 0.51 95% CI 0.31–0.76, $p = 0.002$) in a univariate model. In a multivariate model, the OR was 0.53 (95% CI 0.32–0.84, $p = 0.010$). At 5 years, the odds for disability progression in the heDMT patients (n = 72) compared to 233 meDMT patients was 0.46 (95% CI 0.29–0.79, $p < 0.001$) in a univariate model, and 0.58 (95% CI 0.34–0.95, $p = 0.034$) in a multivariate model. In the heDMT group, ARR reduction from baseline was 69% at 3 years and 75% at 5 years, and in the meDMT group 45% at 3 years and 64% at 5 years, respectively. The patients in the heDMT group had more Gd + lesions ($p < 0.001$) and T2 lesions ($p < 0.001$) on brain MRI at study onset, however, MRI data was not available in all the patients. Therefore, propensity matching based on MRI characteristics was not feasible.

Table 14. Demographic and clinical characteristics of all the study patients. Modified from Original article IV, Table 1.

Variable	heDMT (N=154)	meDMT (N=1771)	p-value
Sex- female, n (%)	109 (70.8)	1293 (73.0)	0.571
Age (y) at symptom onset, mean (SD)	30.4 (8.99)	32.3 (9.31)	**0.013**
Age (y) at MS diagnosis, mean (SD)	32.0 (9.38)	35.1 (9.69)	**<0.001**
Age (y) at DMT onset, mean (SD)	32.3 (9.40)	35.4 (9.69)	**<0.001**
Time since symptom onset (y), median (Q1,Q3)	0.5 (0.3, 1.3)	1.3 (0.6, 3.4)	**<0.001**
Time since MS diagnosis (y), mean (SD)	0.2 (0.27)	0.3 (0.45)	**<0.001**
ARR 1 year prior DMT onset, mean (SD)	1.6 (0.95)	1.1 (0.81)	**<0.001**
ARR 3 years after DMT onset, mean (SD)	0.5 (0.33)	0.6 (0.45)	0.116
ARR 5 years after DMT onset, mean (SD)	0.4 (0.31)	0.4 (0.34)	0.377
Time on first DMT (y), mean (SD)	2.9 (1.89)	2.9 (2.17)	0.928
Time on any DMT (y), mean (SD)	4.4 (1.44)	4.9 (1.43)	**<0.001**
Baseline EDSS, median (Q1,Q3)	2.0 (1.5, 3.0)	1.0 (0.0, 2.0)	**<0.001**
Follow-up EDSS at 3 years, median (Q1,Q3)	2.0 (1.0, 3.0)	1.5 (1.0, 2.5)	**<0.001**
Follow-up EDSS at 5 years, median (Q1,Q3)	2.0 (1.0, 3.5)	2.0 (1.0, 3.0)	0.154
Any AEs during first-line therapy, n (%)	13 (8.4)	252 (14.2)	0.050
>0 Gd + lesions on MRI at DMT onset, n (%) a	85 (55.2)	354 (20.0)	**<0.001**
>9 T2 lesions at DMT onset, n (%) b	50 (32.5)	143 (8.1)	**<0.001**
First-line therapy, n (%)			
Alemtuzumab	14 (9.1)	-	
Natalizumab	124 (80.5)	-	
Ocrelizumab	7 (4.5)	-	
Rituximab	9 (5.8)	-	
Dimethyl fumarate	-	182 (10.3)	
Glatiramer acetate	-	229 (12.9)	
Interferon beta	-	1284 (72.5)	
Teriflunomide	-	76 (4.3)	

Abbreviations: heDMT high-efficacy disease-modifying therap; meDMT moderate-efficacy disease-modifying therapy; ARR annual relapse rate; AE adverse event.
a Data missing in 29% of heDMT and 53% of meDMT patients.
b Data missing in 58% of heDMT and 82% of meDMT patients.

5.3.2 Patient characteristics and disability and relapse outcomes in the propensity-matched cohorts

5.3.2.1 Time to 6-month confirmed disability progression

A total of 66 heDMT patients had frequent EDSS data enabling time to 6-month CDP analysis [a mean of 5.5 (SD 1.97) EDSS evaluations during the follow-up].

They were propensity-matched to 66 meDMT patients with a mean of 5.1 (SD 2.22) EDSS evaluations. The characteristics of the propensity-matched groups are published as tables in Original article IV (Hänninen et al., 2021). The median (Q1, Q3) follow-up time to an event or censoring was 4.7 (3.1, 5.8) years in the heDMT group and 4.0 (2.3, 5.7) years in the meDMT group. Patients were censored at death, data cut-off, or at 6-year follow-up mark.

The probability of 6-month CDP at 3 years after initiating DMT was 15.2% (95% CI 6.1–23.4) in the heDMT group and 35.0% (95% CI 22.4–45.6) in the meDMT group and at 5 years, 28.4% (95% CI 15.7–39.3) vs 47.0% (95% CI 33.1–58.1), respectively. The absolute risk reduction for CDP was 19.8% at 3 years and 18.6% at 5 years. The event probabilities between the groups differed significantly ($p = 0.013$). The heDMT group had a 40% lower rate of 6-month CDP compared to the meDMT group (HR 0.60, 95% CI 0.39–0.91, $p = 0.015$). The results are presented as a cumulated events (1-KM) curve displayed in Figure 9. When fingolimod was included in the heDMT-group, the rate of 6-month CDP compared to meDMT was no longer significant (n = 73; HR 0.74 95% CI 0.49–1.11, p = 0.143.

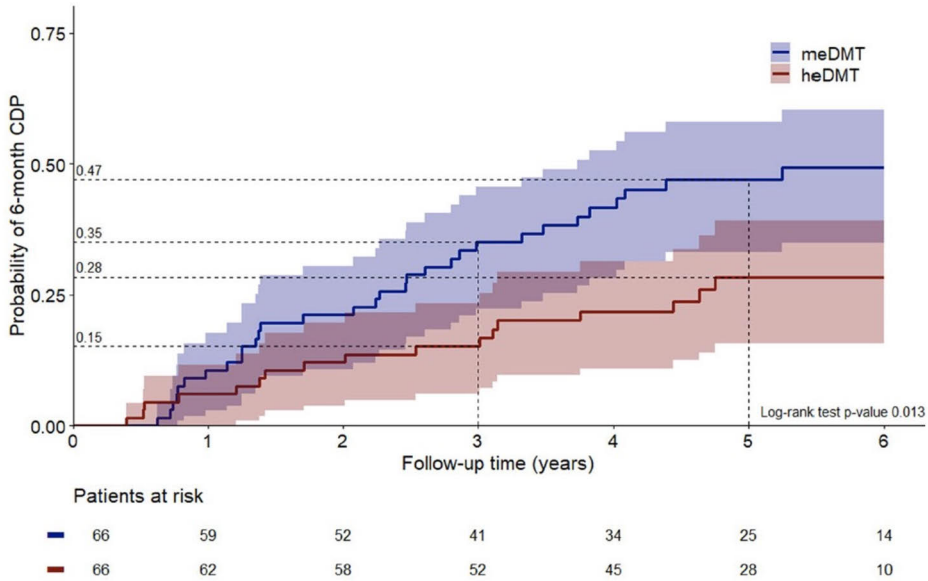

Figure 9. Probability of 6-month CDP in the propensity-matched heDMT vs meDMT groups. Figure source: Original article IV, Figure 2.

5.3.2.2 Time to first relapse

A total of 105 heDMT patients were propensity matched with 105 meDMT patients. The characteristics of the propensity-matched groups are published as tables in

Original article IV (Hänninen et al., 2021). The probability of the first relapse at 3 years was 27.6% (95% CI 18.5–35.7) in the heDMT group and 43.9% (95% CI 33.5–52.6) in the meDMT group. Probability of the first relapse at 5 years was 34.6% (95% CI 24.1–43.6) in the heDMT group and 47.2% (95% CI 36.6–56.1) in the meDMT group. The absolute risk reduction for relapse was 16.3% at 3 years and 12.6% at 5 years. The mean (SD) number of relapses during the follow-up was 0.7 (1.54) in the heDMT group and 1.4 (2.5) in the meDMT group. The event probabilities between the groups differed significantly (p = 0.019). The heDMT group had a 30% lower rate of the first relapse compared to meDMT (HR 0.70, 95% CI 0.52–0.94, p = 0.020). The results are presented as a cumulated events (1-KM) curve displayed in Figure 10. When fingolimod was included in the heDMT group, the results remained similar (n = 115; HR 0.64 95% CI 0.52–0.94, p = 0.020, p = 0.003).

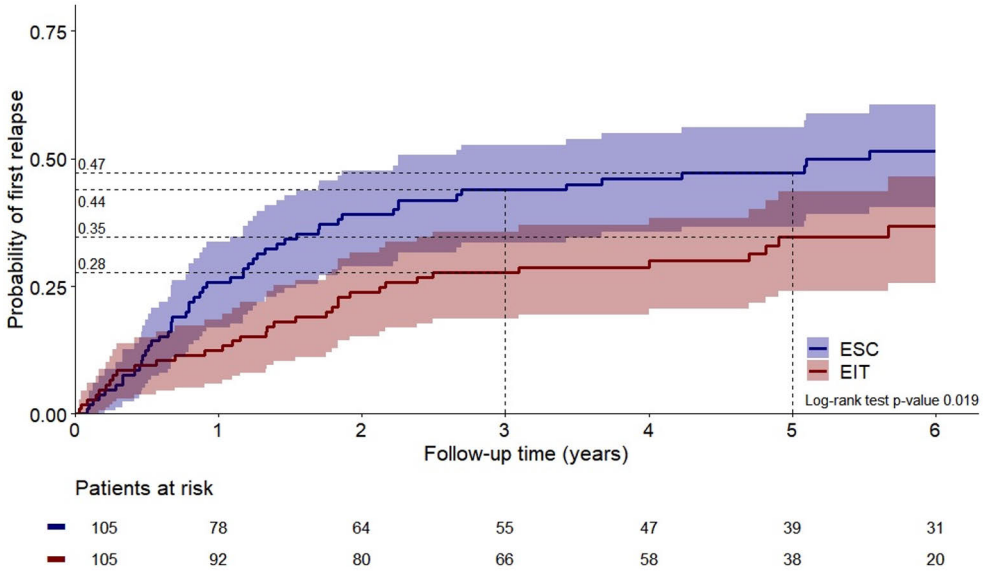

Figure 10. Probability of the first relapse in the propensity matched heDMT vs meDMT groups. Figure source: Original article IV, Figure 3.

5.3.2.3 Disability progression at 3 and 5 years

A conditional regression analysis was performed to assess the difference in disability progression in the propensity matched cohorts of meDMT and heDMT at 3 and 5 years after onset of first DMT. The results were in line with the raw data results; at 3 years, the OR (95% CI) for disability progression in a univariate model comparing heDMT (n = 76) and meDMT (n = 76) was 0.43 (95% CI 0.24–0.75, p = 0.004), and

at 5 years, the OR was 0.54 (95% CI 0.30–0.94, p = 0.032), (n = 57 for both groups). When fingolimod was included in the heDMT group, the results remained significant for the 3-year disability progression (n = 76; OR 0.47, 95% CI 0.26–0.80, p = 0.006), but not for 5-year disability progression (n = 56; OR 0.60, 95% CI 0.34–1.05, p = 0.076).

5.3.3 Treatment escalations

A total of 219 out of 1771 (12.4%) patients in the whole meDMT group escalated into natalizumab, alemtuzumab, ocrelizumab, or rituximab at a median of 2.4 years after first DMT initiation. The treatment escalations of the whole group are presented in Figure 11. In most of the patients going through treatment escalation (80.8%), the reason for escalation was lack of efficacy. A relapse within 1 year before the switch was observed in 74.2% of the patients switching due to a lack of treatment efficacy. In the matched subcohort of 105 meDMT patients in the time to first relapse analysis, 24.8% of the patients escalated at a median of 2.5 years, and in the matched subcohort of 66 patients in the meDMT group in the time to 6-month CDP analysis, 36.3% escalated at a median of 2.5 years. A total of 20 patients had a relapse within a year before the escalation in both of the propensity-matched meDMT groups (76.9% and 83.3%, respectively).

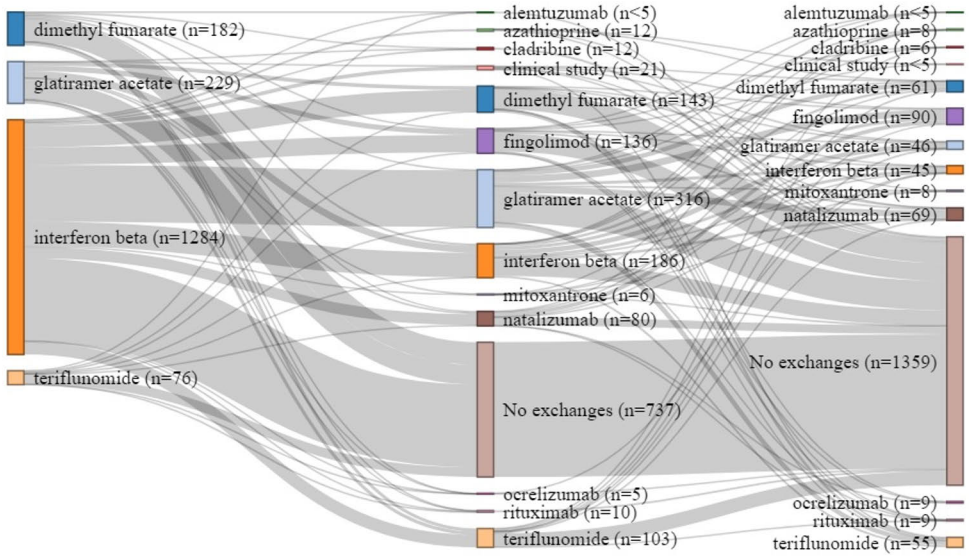

Figure 11. Treatment escalations in the meDMT group of the whole study population.

5.3.4 Safety

There was no significant difference in adverse events between the groups. In the meDMT group, adverse events were recorded for 252 (14.2%) patients, and in the heDMT group, for 13 patients (8.4%). There was one case of PML in the meDMT group after escalation from interferon to natalizumab; the patient was not included in the propensity-matched group comparisons. There were 7 deaths among the 1771 patients in the meDMT group during the 6-year follow-up, and no deaths among the 154 patients in the heDMT group. The mean age at death was 48.4 years (range 29–58 years) and the causes of death were stroke, subarachnoid hemorrhage, lung cancer, breast cancer, amyotrophic lateral sclerosis (patient not included in the matched comparisons), advanced MS, aspiration pneumonia and 1 unknown.

6 Discussion

The prognosis of MS is highly variable and the therapeutic landscape is rapidly evolving. Choosing optimal therapy for each treatment-naïve patient early in the disease course has become very complex. Currently, the clinical presentation and brain MRI findings are the major disease-activity-related measures driving the DMT choice. The most common treatment algorithm of newly diagnosed RRMS is starting therapy with a low-risk, moderate-efficacy DMT and escalating treatment in the presence of continued disease activity. Initial treatment with high-efficacy DMTs is an approach often reserved for a minority of patients with high disease activity. Novel methods to assist early therapeutic decision making and prognostication are needed. In this thesis, the utility of automated brain volume measurement and sNfL as early prognostic and disease activity markers, as well as prognostic implications of the first treatment choice among Finnish MS patients, were studied.

6.1 Interpretation of the study results

Brain volume measurement as a prognostic tool

We found that higher EDSS and worse cognitive performance on SDMT significantly correlated with smaller deep grey matter and total brain volumes. Additionally, we discovered that thalamic atrophy is measurable before whole brain atrophy is detectable. Patients who had only thalamic atrophy had a greater risk of not reaching 2-year NEDA-3 and for EDSS increase at 2 and 5 years than patients with no brain atrophy. Also the patients that had already developed whole brain atrophy were at an increased risk for 5-year disability progression compared to those with no brain atrophy, but these patients had a more advanced disease at the study baseline and were older, whereas the patients with only thalamic atrophy were clinically not distinguishable from the patients with no brain atrophy at baseline. The odds ratio for disability progression was higher in the patients with isolated thalamic atrophy than in the patients with whole brain parenchymal atrophy, supporting the view that thalamic atrophy might be a more sensitive tool than whole brain atrophy in predicting disability progression in MS. This is in line with earlier studies

demonstrating that subcortical atrophy may be present before whole brain atrophy in MS (Bergsland et al., 2012; Datta et al., 2015; Raji et al., 2018) and with previous studies that have suggested the unique role of thalamus in predicting future disability (Eshaghi et al., 2018; Gaetano et al., 2018; Raji et al., 2018).

A total of 36% of the patients with no thalamic or whole brain atrophy at baseline and reaching NEDA-3 at 2 years still developed brain atrophy exceeding 0.4% annual atrophy rate in the 2-year follow up (>0.8% brain atrophy at 2 years). This result is in line with the study by Uher et al. (2017), suggesting that reaching NEDA-4 is a hard goal to achieve, at least on platform MS therapies.

Serum NfL

We found that during the first year of IFN therapy, sNfL levels were higher in patients with Gd-enhancing lesions on brain MRI than in patients without MRI activity, and sNfL levels correlated with enhancing lesion volume. Vitamin D had no effect on the sNfL concentrations. Similar results were found in 2 previous RCTs finding no effect of vitamin D supplementation on sNfL or in clinical study endpoints (Holmøy et al., 2019; Smolders et al., 2020). In our study and the study by Smolders et al., MRIs were included allowing assessment of correlation of sNfL levels with MRI activity.

All the patients in our study were previously treatment-naïve RRMS patients who had used IFNb-1b for at least 1 month and with no neutralizing antibodies detected. Previously, treatment with IFN has been shown to lead to a drop in sNfL and higher sNfL levels have been found in patients with Gd-enhancing lesions (Varhaug et al., 2018). The sNfL levels in our patients without enhancing lesions were low and in the same range as reported by Holmøy et al. (2019) from the Norwegian vitamin D study, and somewhat lower than in the study by Smolders et al. (2020). Similar low on-treatment sNfL levels were recently reported in a large series of Swedish RRMS patients, suggesting a role for sNfL as a drug-response marker (Delcoigne et al., 2020).

We did not find a correlation of sNfL and disability, but our patients were relatively mildly disabled with a median disease duration of approximately 3 years and no SPMS patients were included. In a previous Finnish study including older MS patients with longer disease duration and patients with progressive MS, a correlation of sNfL with disability was found (Högel et al., 2020). The MRI T2 lesion volume can be considered a cumulative measure of total lesion formation in MS (Chitnis et al., 2018). We did not find any correlation of sNfL and the MRI burden of disease in our patients. This may have been related to our small sample size, but may also be explained by the serum samples being collected approximately 3 years after the initiation of the IFN therapy. In a 10-year follow-up study of MS patients

followed from the disease onset, the strongest correlation between sNfL and long-term lesion volume accumulation and brain atrophy was observed among sNfL samples collected during the first year after MS onset (Chitnis et al., 2018).

Treatment strategy

We found that early high-efficacy infusion therapy resulted in a lower probability of 5-year confirmed disability progression and first relapse than initiating first treatment with moderate-efficacy therapies. The probabilities of disability progression in the heDMT and meDMT groups at 5 years were 28% and 47% and the probabilities of relapse 35% and 47%, respectively. The relative risk for disability progression was 40% lower and for relapse 30% lower in the heDMT group in comparison to the meDMT group at any time point during the 5 years of follow up. Previous studies have shown similar results (Buron et al., 2020; Harding et al., 2019). A recent register study covering over 2000 RRMS patients from Italy also showed similar results: The mean change in EDSS was significantly higher in patients treated with the escalation approach compared to patients on early intensive treatment in 6−11 years of follow-up (Iaffaldano, Lucisano, Caputo, et al., 2021).

High-efficacy therapy that had begun early in the disease course, within 2 years of onset, compared with a later onset has been associated with less disability in up to 10 years of follow-up (He et al., 2020). In our study, the mean time from MS onset to treatment was 1.7 years in the meDMT group. In the propensity-matched meDMT groups, 25% to- 36% of the patients escalated into infusion therapies at a median of 2.5 years after DMT onset, meaning that in the majority of the patients escalating therapy, the start of high-efficacy treatment was delayed beyond 4 years. Subgroup analyses of patients escalating before or after 2 years did not significantly change our results. The numbers of patients escalating within the first year after DMT start and without relapse activity was too small for statistical analyses. It is thence possible, that using minimal or no evidence of disease activity as a treatment goal and escalating treatment within the first year after disease onset, could have led to similarly good outcomes in the meDMT group as in the heDMT group.

A recent large MS-register-based study suggested that DMTs should be commenced within 1.2 years after MS onset to delay long-term disability accrual (Iaffaldano, Lucisano, Butzkueven, et al., 2021). In our study, the median intervals from MS onset to DMT were less than a year in both groups in the propensity matched comparisons, but 1.7 years in the meDMT group and 0.5 years in the heDMT group in the unmatched cohorts. This suggests that in clinical practice, patients with normal rather than high disease activity may have a delay in the DMT initiation negatively impacting their outcome.

We only included patients diagnosed and treated with first DMTs after the year 2006, since the first high-efficacy therapy natalizumab became available for clinical use in Finland that year. We did not include fingolimod in the heDMT group but included rituximab similarly as in the study by He et al. (He et al, 2020). Inclusion of fingolimod into the heDMT group in our study changed the results such, that 5-year disability progression and time to 6-month CDP no longer significantly differed between the heDMT and meDMT groups. However, the risk of 3-year disability progression and time to first relapse remained significantly lower in the heDMT group also when fingolimod was included.

Recent observational studies have shown evidence that early initiation of highly effective therapy may decrease the risk of developing disability accrual and secondary progression, at least in 5- to 6 years of follow-up (Brown et al., 2019; Merkel et al., 2017; Prosperini et al., 2020). No randomized controlled trials have yet directly compared the effects of early intensive and escalation strategies, but such studies are already recruiting participants (Ontaneda et al., 2019, 2020).

6.2 Limitations and methodological considerations

There are several confounding factors limiting clinical applicability of brain volume measurement, including the physiological variables of the patient, variables related to MRI acquisition and scanner differences, and MS-related variables such as pseudo atrophy (Enzinger et al., 2005; Pérez-Miralles et al., 2013). We aimed to minimize the effect of confounding patient variables and all MRIs were acquired with the same Siemens MRI scanner and acquisition protocol. Unfortunately, normative data from the exact same scanner model was not available. However, we analysed our data using normative data from more than 400 cases from 8 different Siemens scanner models from the OASIS data base (Marcus et al., 2010) showing relatively consistent results: the 95 % confidence interval of the average thalamus volume for each scanner contains the average thalamus volume computed for all 8 scanners. If enough normative data were available, it would be ideal to compute the z-scores using data from the same scanner model. The numbers of patients in the brain volume studies were small. Most of the patients in the isolated thalamus atrophy group were treatment-naïve RRMS patients, treated with either interferon beta or glatiramer acetate. Although our results suggest that isolated thalamus atrophy could serve as a subclinical prognostic factor associated with NEDA-3 on the injectable platform therapies, the results are not necessarily generalizable to other DMTs and different DMT sequences. The results need to be confirmed in a larger patient series and the prognostic value in individual patients needs to be further studied.

The number of patients was small also in the NfL study, but the strength was the RCT setting of the study and stringent MRI protocol and MRI analyses performed

in a specialized MS imaging centre in Milan. We did not have serum samples prior to IFN therapy from our patients.

The limitations of the treatment strategy study include the retrospective study design, a small sample size in the propensity-matched cohorts, and possible selection bias attributable to exclusion from the analysis of a greater proportion of patients in the meDMT group than in the heDMT group. MRI data was not available for all of the patients and therefore propensity matching based on MRI parameters was not feasible. Safety parameters are not a compulsory element in the Finnish MS register, which has to be taken into consideration when interpreting the safety results. Longer follow-up would have been needed to reveal long-term risks of potent immunosuppression, such as malignancies.

Our study period was from the beginning of the year 2006 to the end of 2020. The majority of the patients in the heDMT group were using natalizumab, which had become available in Finland in 2006. Therefore, our results cannot be generalized and extended to all current high-efficacy treatments, such as cladribine, which was not available in Finland until 2018. Fingolimod was not included, based on previous comparative data and reimbursement by the social insurance institution of Finland instead of the hospital budget. However, we also performed additional analyses with fingolimod included**.** The strengths of the study include separate complete case matching performed for all outcome analyses, and high population- and clinical-data item coverage of the Finnish MS register in the hospitals participating in the study.

6.3 Clinical implications

Brain volume measurement is not in routine clinical use and measuring brain volume changes between time points is technically challenging in clinical practice (Wattjes et al., 2015). For example, it may be challenging to always scan the patient in the same scanner in clinical practice. We found that it is possible to detect isolated atrophy of thalamus only, by MRI-based brain volumetry performed at 1 time point. Automated methods enable detection of the earliest signs of brain atrophy before more widespread neural tissue damage has occurred. Performing brain volume measurement at a single time point could be more feasible in practice than the technically challenging measurement of brain volume changes in time. Brain volume measurement at a single time point before DMT start could help identify patients who have a more severe disease and a need for more effective therapy from early on.

Measurement of sNfL correlated with MRI activity and could serve as a serum marker of inflammatory disease activity in patients initiating a DMT. The sNfL analyses were performed in a Finnish laboratory. Availability of a domestic service provider would naturally assist the applicability of sNfL measurement also in clinical practice. There are, however, still a number of hurdles to overcome before sNfL can

be implemented in routine clinical decision making, including analytical validity issues and the challenge of interpretation of individual sNfL concentrations (Thebault et al., 2021).

The treatment strategy study supports the emerging evidence of the prognostic importance of early high-efficacy therapy in RRMS. No randomized controlled trials have yet directly compared the effects of escalation and early intensive treatment strategies, but such studies are needed and are currently recruiting patients.

6.4 Future directions

The ultimate goal in MS therapy needs to be prevention of not only relapses but also long-term physical and cognitive disability. To achieve this, more sensitive and specific surrogates of both inflammatory and degenerative disease processes are needed. The key biomarker needs in MS are a blood biomarker for monitoring of disease activity or severity enabling improved treatment decisions in individual patients and a biomarker that is prognostic for physical and cognitive worsening.

In this thesis, we studied the utility of automated brain volume measurement in assessing neurodegeneration in a clinical cohort of Finnish MS patients. The atrophic changes were determined using an automated imaging tool and z-scores obtained from a data-base originally collected for the purposes of brain volume measurement in memory disorders. Collection of a large database of Finnish MS patients and controls and standardization of the MS patients' imaging protocols to allow implementation of brain volume measurements in clinical practice is a future goal.

We have shown in a small cohort of clinically stable interferon beta-treated Finnish MS-patients a correlation of MRI-activity with higher sNfL levels. The use of sNfL is not yet implemented in Finnish Current Care Guidelines of MS. Serum NfL is used as a treatment response marker in clinical practice only in a few centres located mainly in Sweden and Switzerland with in-house tests. It is, however, the serum biomarker with the most published data to support becoming the first blood biomarker to enter clinical use as an additive tool in the monitoring and evaluation of treatment responses in MS patients (Thebault et al., 2021). Recent developments in creating large normative data bases and z-scores rendering sNfL values independent of age-related increases are likely to be the breakthrough that speeds clinical applicability (Benkert et al., 2022).

Development of a prognostic biomarker in MS is challenging, considering the variability of the disease course and the impact of so many baseline, early-treatment-related, and individual uncontrollable factors on the prognosis. The development of a predictive biomarker likely requires use of combinations of predictive factors. Serum NfL has been shown to be associated with brain volume and is predictive of reaching EDSS 6 (Kuhle et al., 2020). Combining sNfL with clinical and MRI

measures has shown increased sensitivity and specificity when predicting long-term disease outcomes (Häring et al., 2020). Combining sGFAP and sNfL measurements is another potential future avenue (Niiranen et al., 2021). The applicability of clinical laboratory values such as vitamin D and CSF IgG index measured at the diagnostic setting may also prove to be useful in the prognostics of MS. Perhaps in the future a combination of multiple clinical variables could serve as a tool in prognostic use. Collection of all the prognostic factors in the MS register is important for the development of potential prognostic models.

Accumulating data, including ours, suggests superiority of early highly effective therapy in RRMS to the conventional escalation strategy in preventing future disability. The DMT treatment strategies in MS are likely to undergo a change into a more proactive and aggressive treatment approach with the aim to suppress the inflammatory activity as long as the therapeutic window of inflammatory phase of the disease process is open. We have shown that infusion therapies as the first treatment choice lead to a better 5-year prognosis both in relapse rate and disability accumulation in propensity-matched patients. However, the highest efficacy infusion therapies will not be the best option to initiate with for many patients, implicating the importance of future development in precision medicine for selecting the right treatment for each patient (Bose & Freedman, 2020). It is important to develop more sensitive tools for detection of disease activity to allow change of DMT before neurodegeneration takes place, if the first therapeutic choice is not effective. The currently most-used clinical disability marker, EDSS, has been criticised for its reliance on walking as the main measure of disability, and its weakness in reliability and sensitivity to change. Digital monitoring tools, such as smartphone-sensor-based remotely administered digital tests, enable remote monitoring of physical and cognitive disability, for example by use of SDMT, gait and balance tests, and an upper extremity function test. These digital monitoring tools represent a promising new avenue to capture disability burden with quantitative accuracy (Montalban et al., 2021). Another potental novel method under research is extracting information from real-world clinical data sources, such as MS registers, and using machine-learning methods to predict future disability based on the register data (de Brouwer et al., 2021).

7 Conclusions

The purpose of this thesis was to study early prognostic radiologic, clinical, and serum biomarkers in Finnish MS patients. The more specific aims were to study which brain volume measures correlate with disability and cognition and to evaluate whether brain volume measurement at a single time-point could predict disease activity and disability outcomes at 2 years and at 5 years in a clinical cohort of Finnish MS patients. Further, we studied whether serum neurofilament light chain concentrations were affected by supplementation of vitamin D and correlated with disease activity in interferon-beta-1b-treated Finnish MS patients. A further aim was to compare outcomes of initial treatment with high efficacy infusion therapies vs starting therapy with medium-efficacy therapy in a propensity-matched cohort of Finnish RRMS patients. The conclusions based on the results presented in this thesis are as follows:

1. In line with previous studies, we detected significant differences in total and regional brain volumes between newly diagnosed RRMS patients and SPMS patients. The most significant differences between relapsing and progressive patients were found in thalamic volume and cerebellar WM volume. Worse performance in SDMT test and higher EDSS significantly correlated with smaller deep grey matter and total brain volumes at both time points.

2. Even very early brain atrophy, such that atrophy was detected in thalamus only, was a negative prognostic marker for disability progression. More specifically, patients with isolated thalamic atrophy were at a higher risk for not reaching 2-year NEDA-3 and for EDSS increase at 2 and at 5 years than patients with no identified brain atrophy. Based on EDSS, SDMT, and relapse activity, the RRMS patients with isolated thalamus atrophy and no detectable brain atrophy were clinically not distinguishable. Brain volume measurement at one time point could help predict disability progression in MS and complement clinical and routine MRI evaluation in therapeutic decision-making.

3. In a small cohort of clinically stable IFN-treated Finnish MS patients, subclinical disease activity in MRI was associated with higher sNfL levels.

Serum NfL levels were similarly low in patients supplemented with high-dose vitamin D or placebo. In clinically stable patients, elevated sNfL levels may assist detection of subclinical inflammatory disease activity.

4. Initiating MS therapy with high-efficacy infusion therapies significantly reduced the risk of 5-year disability progression and relapse, compared to using moderate-efficacy DMT as the first DMT choice in propensity-matched groups of Finnish MS-patients. Randomized clinical trials are needed to directly compare these different treatment strategies in relapsing MS.

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Katariina Kuutti

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