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Hodnocení klinických a paraklinických markerů aktivity nemoci u autoimunitních onemocnění nervového systému

Evaluation of clinical and paraclinical markers of disease activity in autoimmune diseases of the nervous system

Disertační práce

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Abstrakt

Roztroušená skleróza (RS) je chronické zánětlivé autoimunitní a neurodegenerativní onemocnění centrálního nervového systému. V současnosti se klinické a paraklinické markery využívají jak pro monitoraci vývoje RS, tak pro vyhodnocení odpovědi na léčbu. V neposlední řadě je cílem využít markery pro predikci budoucího vývoje nemoci. V práci se zabýváme nejprve využitím klinických markerů nemoci (výskyt relapsů, hodnocení EDSS) pro zhodnocení různých strategií nasazení počáteční specifické léčby RS. Nejprve jde o porovnání konzervativního nasazování prvoliniových preparátů při počátku nemoci vůči okamžitému nasazení vysokoúčinné léčby, následně řešíme i časnost nasazení prvoliniové léčby. S využitím dat českého registru pacientů s RS jsme ukázali, že pacienti ve švédském registru, kde je okamžité nasazení vysokoúčinné léčby výrazně více zastoupeno než v České republice, mají lepší budoucí vývoj nemoci (např. pozdější nástup relapsu, zlepšení v EDSS). Dále jsme potvrdili důležitost rychlého nasazení specifické RS léčby při co nejnižším EDSS a po co nejnižším počtu relapsů. V druhé části se zabýváme lehkými řetězci neurofilament v séru (sNfL) jako možným prediktivním markerem pro budoucí rezonanční i klinickou aktivitu nemoci. Na kohortě 172 nově diagnostikovaných relaps-remitentních pacientů bylo ukázáno, že sNfL mohou být využity jako marker probíhajícího zánětu CNS, a také jako prediktor budoucí mozkové atrofie.

Klíčová slova: roztroušená skleróza, biomarkery, lehké řetězce neurofilament, léčebné strategie

Abstract

Multiple sclerosis (MS) is a chronic, autoimmune, and neurodegenerative disorder of the central nervous system. We currently utilize clinical and paraclinical markers for several purposes: to monitor the disease status, assess the response to MS specific treatments, and predict the future disease course. The first part of this work focuses on the use of clinical markers (such as relapse rate and EDSS) to evaluate different treatment strategies for the initial therapy. At first, we compare the initiation of treatment with first-line drugs to the direct initiation of treatment with high-efficacy drugs. Additionally, we investigate the importance of promptly starting first-line treatment immediately diagnosis. By a comparison of data from the Czech and Swedish MS registries, we have demonstrated better outcomes (future relapses, improvement in EDSS) in patients in Sweden, where high-efficacy therapy is initiated directly in a significantly larger proportion of patients compared to the Czech Republic. Furthermore, we have highlighted the importance of early treatment initiation for patients with minimal EDSS and low relapse rate. The second part of this work evaluates serum neurofilament light chain (sNfL) as a promising predictive marker of future clinical and magnetic resonance imaging disease activity. In a cohort of 172 newly diagnosed patients with relapsing-remitting MS, we have confirmed that sNfL serves as a marker of the ongoing disease activity and as a predictor of future brain atrophy.

Key words: multiple sclerosis, biomarkers, neurofilament light chain, treatment strategies

List of Abbreviations

AIC – Akaike Information Criterion

ARR – Annualized Relapse Rate

BICAMS – Brief International Cognitive Assessment for Multiple Sclerosis

BPF – Brain Parenchymal Fraction

CDI – Confirmed Disability Improvement

CDW – Confirmed Disability Worsening

CI – Confidence Interval

CIS – Clinically Isolated Syndrome

CNS – Central Nervous System

CSF – Cerebrospinal Fluid

DMT – Disease-Modifying Therapy

EBV – Epstein-Barr Virus

EDA – Evidence of Disease Activity

EDSS – Expanded Disability Status Scale

ELISA – Enzyme-Linked ImmunoSorbent Assay

FLAIR – Fluid-Attenuated Inversion Recovery

GAD – Gadolinium-Enhancing

HE-DMT – Moderate-High Efficacy Disease Modifying Therapy

HR – Hazard Ratio

IQR – Interquartile Range

KM – Kaplan-Meier

LE-DMT – Low-Moderate Efficacy Disease Modifying Therapy

LE-DMT 1R – Patients Starting Low-Moderate Efficacy Disease Modifying Therapy after the First Clinical Relapse

LE-DMT ≥2R – Patients Starting Low-Moderate Efficacy Disease Modifying Therapy after at Least 2 Clinical Relapses

MS – Multiple Sclerosis

MSFC – Multiple Sclerosis Functional Composite

MRI – Magnetic Resonance Imaging

NEDA – No Evidence of Disease Activity

NfL – Neurofilament Light Chain

OR – Odds Ratio

PASAT – Paced Auditory Serial Addition Test

PIRA – Progression Independent of Relapse Activity

PPMS – Primary-Progressive Multiple Sclerosis

RAW – Relapse-Associated Worsening

ReMuS – Czech National Registry of Multiple Sclerosis

RRMS – Relapsing-Remitting Multiple Sclerosis

RWD – Real-World Data

SD – Standard Deviation

SDMT – Symbol Digit Modalities Test

SET – Study of Early Interferon beta-1a Treatment

Simoa - Single Molecule Array

sNfL – Serum Neurofilament Light Chain

SPMS – Secondary-Progressive Multiple Sclerosis

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1. Introduction

1.1. Multiple sclerosis

Multiple Sclerosis (MS) is chronic autoimmune disease of the Central Nervous System (CNS), characterized by the immune system's attack on the myelin sheath surrounding nerve fibers. The disease affects mostly women with female:male ratio 2.8 (Wallin et al., 2019).

Worldwide, the number of people with multiple sclerosis has been estimated to 2.8 million in 2020, increasing the prevalence by 30% as compared to 2013. Mostly affected region is European, with 142.8 multiple sclerosis patients per 100 000 inhabitants (Walton et al., 2020). In the Czech Republic, the estimated number of patients is 23 thousand with yearly diagnostics of approximately 700 new cases (E. K. Havrdova, 2013). The increase in the incidence might be explained by better diagnostics of the disease.

The cause of the disease remains unknown, but various factors are suspected to contribute to its development. Among the potential causes are genetic predisposition, environmental triggers, and immune system dysregulation. Genome-wide association studies have identified several genetic variants associated with increased MS risk, indicating a hereditary component (Patsopoulos, 2018). Additionally, certain environmental factors, such as low vitamin D levels, smoking, and viral infections, especially Epstein-Barr Virus (EBV) infection, have been linked to an elevated risk of MS (Alfredsson & Olsson, 2019; Aloisi et al., 2023; Bjornevik et al., 2022). The maternal exposure to ultraviolet light and vitamin D deficiency also play a role in the development of the disease (Dobson et al., 2013).

MS can manifest with a wide spectrum of symptoms, ranging from mild to severe, and vary greatly among affected individuals. The demyelination leads to impaired nerve signal transmission, causing a wide range of neurological symptoms as impaired motor functions, muscle weakness, sensory disturbances, coordination difficulties, fatigue, cognitive deficits, and visual impairments.

MS treatment encompasses symptomatic management to alleviate immediate symptoms, acute treatment of clinical relapses, and long-term therapies aimed at modifying the disease course. Symptomatic treatments focus on addressing specific MS-related symptoms, such as muscle spasticity, pain, and fatigue, improving patients' quality of life. These may include muscle relaxants, analgesics, and physical therapy. Acute therapy usually consists of intravenous pulses of methylprednisone (Solumedrol), possibly followed by Medrol or Prednisone, to reduce inflammation and hasten recovery during relapse episodes. In contrast, long-term Disease-Modifying Therapies (DMTs) aim to reduce relapses, slow disease progression, and minimize disability accumulation.

1.2.Diagnostics and clinical phenotypes

Currently, the diagnosis of MS involves evaluation of clinical symptoms, neuroimaging findings, and laboratory tests. The diagnosis is based on revised McDonald criteria from 2017 (Thompson et al., 2018), Table 1.

Clinical attacks (relapses)	Objective clinical evidence (number of lesions)	Additional data needed for a diagnosis of multiple sclerosis
≥2	≥2	None
≥2	1 (as well as clear-cut historical evidence of a previous attack involving a lesion in a distinct anatomical location)	None
≥2	1	Dissemination in space demonstrated by an additional clinical relapse implicating a different CNS site or by MRI
1	≥2	Dissemination in time demonstrated by an additional clinical relapse or by MRI OR demonstration of CSF-specific oligoclonal bands
1	1	Dissemination in space demonstrated by an additional clinical relapse 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 oligoclonal bands)
Progression from onset		1 year of disability progression (retrospectively or prospectively determined) independent of clinical relapse AND at least two of the following criteria: One or more T2-hyperintense lesions characteristic of multiple sclerosis in one or more of the following brain regions: periventricular, cortical or juxtacortical, or infratentorial Two or more T2-hyperintense lesions in the spinal cord

		Presence of CSF-specific oligoclonal bands

Table 1 Revised McDonald criteria for MS diagnosis, MRI: Magnetic Resonance Imaging; CSF: Cerebrospinal Fluid

As seen from the criteria, Magnetic Resonance Imaging (MRI) plays a crucial role in identifying characteristic white matter lesions in the CNS, aiding in the diagnostic process of dissemination in space and in time (Filippi et al., 2016). Cerebrospinal Fluid (CSF) analysis is another essential tool, allowing an evaluation of inflammatory processes circumscribed to the CNS. The detection of the oligoclonal bands in CSF is used for laboratory diagnosis of MS (Lo Sasso et al., 2019).

The primary definition of different clinical phenotypes describing the disease's course and progression was initially presented by Lublin in 1996 but underwent an update in 2013 to incorporate MRI evaluation (Lublin et al., 2014). These clinically recognized phenotypes include Clinically Isolated Syndrome (CIS), Relapsing-Remitting MS (RRMS), Primary-Progressive MS (PPMS), and Secondary-Progressive MS (SPMS). CIS is identified as the first clinical presentation of a disease that shows characteristics of inflammatory demyelination that could be MS, but has yet to fulfill criteria of dissemination in time The RRMS is the most common form of MS at the time of diagnosis (Dobson et al., 2013) and is characterized by unpredictable episodes of symptom flare-ups (relapses) followed by periods of partial or complete recovery (remissions). PPMS demonstrates a gradual, steady worsening of neurological symptoms without distinct relapses or remissions. It accounts for a smaller proportion of MS cases and often has a later age of onset compared to RRMS. Many individuals with RRMS eventually transition to SPMS, where the disease progresses more steadily without prominent relapses. This progression may occur with or without periods of remission. The differences between the phenotypes satisfying the MS diagnosis are shown in Figure 1.

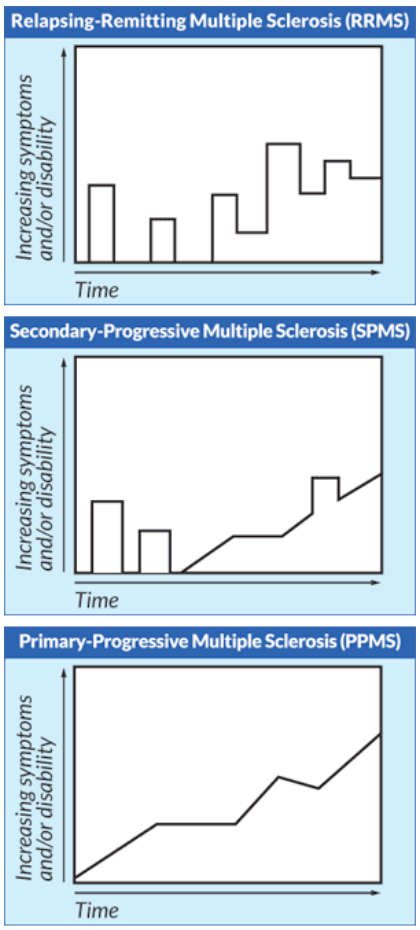

Figure 1 Evolution of different phenotypes over time, the figure source: (Kujawski, 2020)

Recently, the concept of four clinical phenotypes with active or not active form (as shown in Figure 2) has faced criticism for not entirely explaining the disease course. Despite the suppression of focal disease activity, some patients may continue to experience a progressive accumulation of disability that cannot be fully accounted by MRI or relapse activity (Giovannoni et al., 2022). This has led to introduction of the concept of Progression Independent of Relapse Activity (PIRA) or "smouldering MS", describing an ongoing damage process within the CNS that doesn't align entirely with Relapse-Associated Worsening (RAW). Per PIRA, the progression of MS can occur not only due to RAW but also because of delayed relapse-associated neurodegeneration, subsequent pos-inflammatory neurodegeneration (including viral infections), and aging, as suggested in Figure 3.

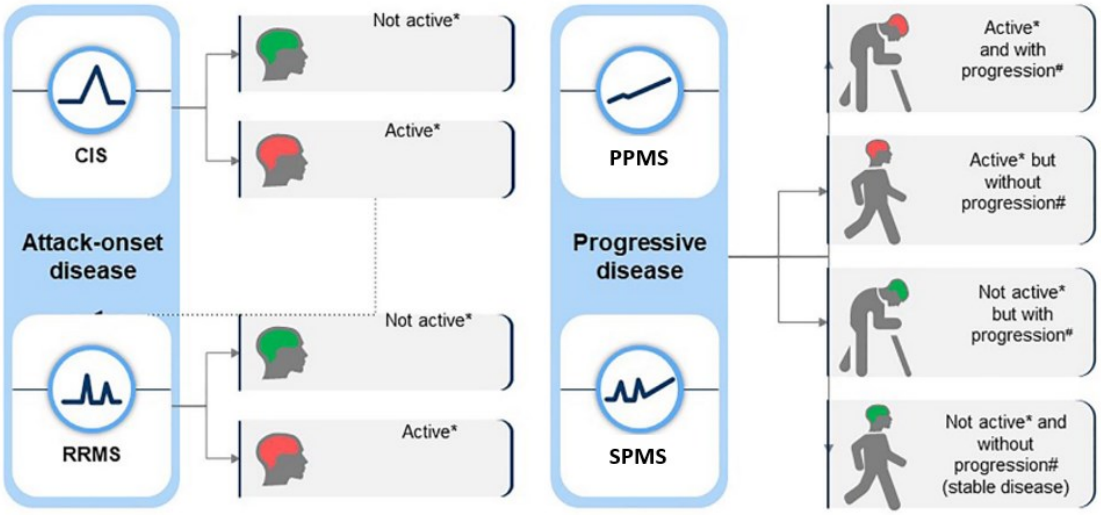

Figure 2 Lublin four MS phenotypes description for relapsing and progressive disease, *Activity determined by relapses and/or MRI activity, # Progression measured by clinical evaluation. the figure source (Giovannoni et al., 2022)

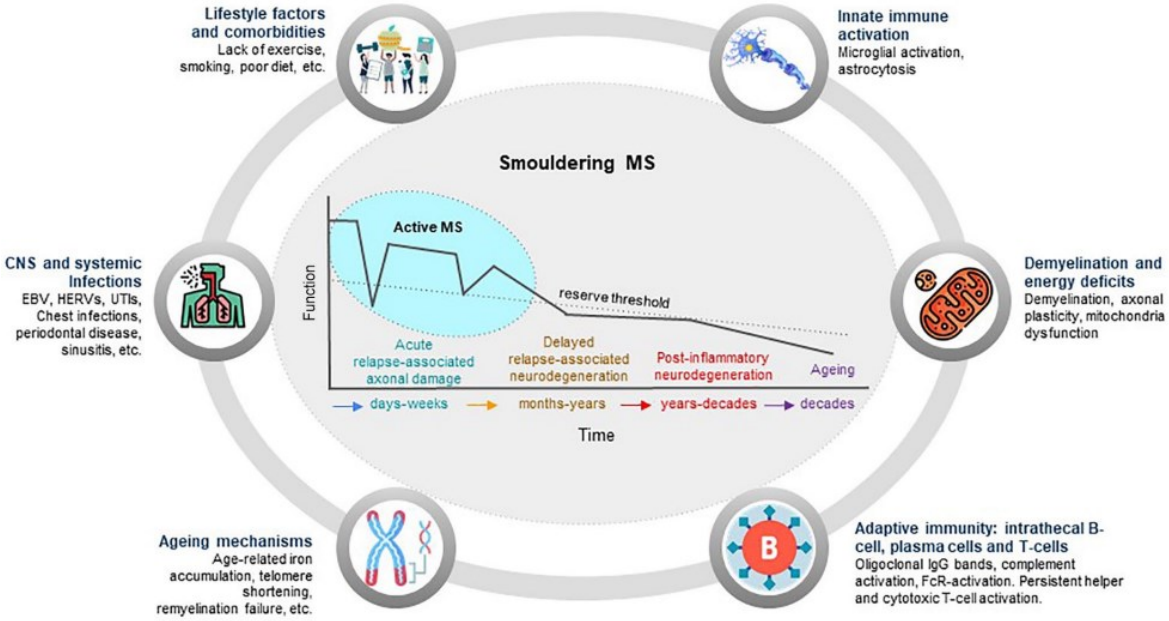

Figure 3 The pathological drivers of smouldering MS, presented by (Giovannoni et al., 2022), HERV: Human Endogenous Retroviruses, UTI: Urinary Tract Infection

Since the introduction of the new PIRA concept, its application to the classification of clinical phenotypes is still a topic of discussion. Therefore, for the purposes of clinical trials and general patient classification in various studies, the 2016 four-phenotype classification by Lublin is still commonly used.

1.3. Biomarkers

Biomarkers in MS are essential tools used to facilitate disease diagnosis, prognosis, and monitoring of disease activity. These are measurable indicators that provide valuable insights into various aspects of the disease's pathophysiology and progression. Also, biomarkers play a vital role in monitoring treatment responses. They can help assess the efficacy of DMTs and guide treatment adjustments. By tracking changes in biomarker levels over time, clinicians can evaluate disease activity, treatment response, and the potential need for therapeutic modifications.

Biomarkers can serve as surrogate markers, predictive markers, and be associated with various endpoints and outcomes. Surrogate markers are measurable indicators that stand in for a clinical outcome and are used to assess the information about disease status or treatment effects more rapidly than traditional clinical endpoints. For example, neuroimaging measures, such as brain lesion burden or volume, can serve as surrogate markers, providing insights into the treatment's impact on disease progression or remyelination (Sormani et al., 2011). Predictive markers, on the other hand, help with prediction of the disease evolution or the treatment response of particular patient. Endpoints and outcomes may include reductions in relapse rates, disability progression, cognitive function, or quality of life, and are used to assess the disease course and the overall benefit of a therapeutic intervention.

Biomarkers for multiple sclerosis can be grouped into two main sections: clinical markers and paraclinical markers.

1.3.1. Clinical markers

Clinical markers in MS are essential tools used to evaluate disease progression, treatment response, and overall patient outcomes. These markers are derived from clinical assessments and observations, helping clinicians monitor the disease course and make decisions regarding treatment strategies. The mostly used clinical marker in MS is the Expanded Disability Status Scale (EDSS), which measures the level of neurological disability (Kurtzke, 1983).

Due to the lack of sufficient evaluation of detailed hand function and cognition by EDSS, additive measure widely used is the MS Functional Composite (MSFC), a composite measure evaluating walking speed, hand function, and cognitive processing speed (Rudick et al., 2001). The sub-tests used for MSFC calculation are 9-hole peg test, 25-foot walk test and Paced Auditory Serial Addition Test (PASAT). The 9-hole peg test evaluates fine motor skills and hand dexterity by measuring the time taken to insert and remove pegs. The PASAT examines cognitive processing speed and working memory by assessing the ability to add numbers presented audibly (Gronwall, 1977). The 25-foot walk test quantifies ambulation and gait speed, providing insights into mobility and physical disability, since EDSS focuses solely on the ability to walk certain longer distance.

As additional measures of cognition, Symbol Digit Modalities Test (SDMT) assesses cognitive function, particularly information processing speed (Smith, 1982), while the Brief International Cognitive Assessment for MS (BICAMS) combines the SDMT, the California Verbal Learning Test, and the Brief Visuospatial Memory Test-Revised to comprehensively evaluate cognitive impairment (Langdon et al., 2012).

Additionally, relapse rates, time to disability progression, and cognitive assessments serve as crucial clinical markers in determining disease severity and treatment efficacy (Lublin et al., 2014).

1.3.2. Paraclinical markers

Paraclinical markers include various imaging techniques, laboratory tests, and other diagnostic procedures that assist in confirming and monitoring MS-related abnormalities. The main focus nowadays is on MRI techniques, providing insights about brain and spinal cord lesions and volume changes. For the diagnostic purposes, CSF laboratory markers play a crucial role, with the presence of oligoclonal bands being included in the McDonald revised criteria for MS diagnosis. Meanwhile, serum laboratory markers are primarily used for disease monitoring, with a significant emphasis on Neurofilament Light Chain (NfL) as a biomarker of axonal damage and disease activity (Barro et al., 2020; Benkert et al., 2022; Disanto et al., 2017).

1.3.2.1. Magnetic resonance imaging

MRI is a crucial imaging modality in MS that allows non-invasive visualization of the CNS, aiding in diagnosis, monitoring disease activity, and assessing treatment response (Wattjes et al., 2021).

Various MRI techniques are employed in MS imaging to provide comprehensive insights into the disease. Conventional MRI sequences, such as T1-weighted, T2-weighted, and Fluid-Attenuated Inversion Recovery (FLAIR) images, help to identify MS lesions as areas of abnormal signal intensity in the brain and spinal cord. Contrast-enhanced MRI, using gadolinium-based contrast agents, highlights active inflammatory lesions, indicating ongoing disease activity.

The main markers that we obtain with these sequences are the number and volume of lesions in the brain and spinal cord, and furthermore their changes over time, especially the so-called "active lesions" (new or enlarged lesions on follow-up MR scans). Although lesional pathology is important for prognosis and monitoring disease activity, its correlation with clinical status is not sufficient. This so-called “clinico-radiological paradox” and its reasons were described already in 1999 by Barkhof (Barkhof, 1999). Thus, there is a long-term effort to obtain additional information that would help in disease monitoring. Measurement of brain and spinal cord volume change (total and regional atrophy) is one marker that is related to disease activity (Matthews et al., 2023).

Unfortunately, the results so far correlate well at the group level, but it is difficult to use them to monitor an individual patient. This is due to the relatively large variability of volume changes even in healthy subjects, the need to use special software that measures volume with some inaccuracy, and the influence of aging and other comorbidities that distort volume changes (Sastre-Garriga et al., 2020).

Currently, efforts are being made to use so-called non-conventional MRI techniques that allow better imaging of microstructural changes. Examples include monitoring of chronic active lesions (Preziosa et al., 2021), microglial activations (Airas & Yong, 2022), changes in normal-appearing white matter (Vaneckova et al., 2022), measurement of spinal atrophy, and others.

1.3.2.2. Serum neurofilament light chain

Serum NfL (sNfL) is emerging as a promising biomarker in MS due to its potential to provide valuable insights into disease activity and neurodegeneration. Neurofilaments are structural proteins that are primarily found in neurons, and their levels in the blood increase when there is neuronal damage or axonal degeneration. NfL measured in CSF has been repeatedly studied for its predictive potential of disease progression (Teunissen & Khalil, 2012). The commonly used ELISA (Enzyme-Linked ImmunoSorbent Assay) method allows standardized measurement of NfL levels in CSF, but it requires invasive procedure of lumbar puncture. Thus, it cannot be used for continuous monitoring of the disease progression in common practice.

However, the collection of serum samples for NfL measurement is relatively non-invasive and can be performed through standard blood draws. Once collected, the serum samples are processed to separate the blood cells from the liquid component, and the sNfL levels are quantified using sensitive laboratory assays. The methodology used for sNfL measurement is crucial to ensure accuracy and reproducibility, and standardized protocols are being established to facilitate comparability across different studies. The ELISA method does not allow the evaluation of sNfL levels, as they are usually below the detection limit (32 pg/ml) of the method. The breakthrough occurred in 2015 when the new Single Molecule Array (Simoa) method was developed and used for sNfL levels detection (Gisslén et al., 2016).

sNfL levels can be stored at low temperatures and preserve their stability up to four freeze-thaw cycles (Keshavan et al., 2018). This enables researchers and clinicians to establish longitudinal data, allowing the monitoring of disease progression and the assessment of treatment efficacy in MS patients.

The analysis of sNfL levels can serve as a valuable marker of neurodegeneration and axonal damage in MS. Higher sNfL levels have been associated with increased disease activity, a higher risk of disability progression, and a greater likelihood of relapses in MS patients (Barro et al., 2018; Benkert et al., 2022; Disanto et al., 2017). As a marker of neuroaxonal injury, sNfL complements other established clinical and imaging markers, enhancing the understanding of disease pathology and progression. However, the widespread adoption of

sNfL as a standalone marker in routine clinical practice remains unvalidated. This is primarily because sNfL is not exclusive to MS but another neurological conditions and can be influenced by various physiological processes (Barro et al., 2020).

1.4. Disease modifying therapies for multiple sclerosis

DMTs for MS play a crucial role in managing the disease by reducing relapse rates, delaying disability progression, and improving the quality of life of patients. Over the past two decades, disease management has seen significant advancements, with the availability of more than 10 different DMTs, each having distinct efficacy and safety profiles (Giovannoni, 2018; Šťastná et al., 2023). DMTs can be categorized into Low-Moderate Efficacy DMTs (LE-DMTs) and Moderate-High Efficacy DMTs (HE-DMTs), as summarized in Table 2.

1) Low-moderate Efficacy Drugs:

LE-DMTs are generally considered as first-line treatments for relapsing forms of MS and are often prescribed for patients with less aggressive disease or those who have milder symptoms. These medications work by modulating the immune system and reducing inflammation. Common LE-DMTs include interferon-beta (e.g., Avonex, Rebif, Betaferon), glatiramer acetate (Copaxone), dimethyl fumarate (Tecfidera) and teriflunomide (Aubagio). Although they have a more modest impact on relapse rates and disease progression compared to higher efficacy drugs, they are well-tolerated and can be suitable for many patients.

2) Moderate-high Efficacy Drugs:

HE-DMTs are reserved for patients with more aggressive forms of MS or those who have inadequate responses to lower efficacy drugs. These medications have a more potent impact on immune system modulation, leading to a more substantial reduction in relapse rates and disability progression. HE-DMTs include monoclonal antibodies such as natalizumab (Tysabri), anti CD20 group (rituximab, ocrelizumab (Ocrevus), ofatumumab (Kesimpta)), and alemtuzumab (Lemtrada). HE-DMTs also include S1P agonists (fingolimod (Gilenya), ozanimod (Zeposia), Siponimod (Mayzent), ponesimod

(Ponvory)), and cladribine (Mavenclad). These drugs are associated with a higher risk of certain side effects, and patients receiving them require closer monitoring.

Generic name	Brand name	Category of efficacy
Dimethyl fumarate	Tecfidera	Low-moderate
Glatiramer acetate	Copaxone	Low-moderate
Interferon beta-1a	Avonex, Rebif 22, Rebif 44	Low-moderate
Interferon beta-1b	Betaferon, Extavia	Low-moderate
Pegylated interferon beta-1a	Plegridy	Low-moderate
Teriflunomide	Aubagio	Low-moderate
Alemtuzumab	Lemtrada	Moderate-high
Cladribine	Mavenclad	Moderate-high
Fingolimod	Gilenya	Moderate-high
Mitoxantrone	Novantrone	Moderate-high
Natalizumab	Tysabri	Moderate-high
Ocrelizumab	Ocrevus	Moderate-high
Ofatumumab	Kesimpta	Moderate-high
Ozanimod	Zeposia	Moderate-high
Ponesimod	Ponvory	Moderate-high
Rituximab	Mabthera, Rituxan	Moderate-high
Siponimod	Mayzent	Moderate-high

Table 2 The overview of DMTs and their classification

There are currently two treatment strategies: the escalation approach and the induction approach. The escalation approach involves initiating treatment with LE-DMTs initially and switching to HE-DMTs if disease activity persists or progresses. This strategy allows physicians to evaluate the response to initial treatments and reserve the use of more potent therapies for cases where they are truly necessary. On the other hand, the induction approach takes a more aggressive stance by initiating treatment with HE-DMTs early in the disease course. This strategy aims to rapidly control disease activity and inflammation to achieve a better long-term outcome.

In recent years, the prevailing strategy has been to initiate treatment with LE-DMTs and escalate to a more efficacious DMT in patients presenting breakthrough in disease activity.

However, the early initiation of HE-DMTs has been suggested as a better approach (Stankiewicz & Weiner, 2020). This hypothesis is currently being evaluated by multiple research studies (He et al., 2020; Simonsen et al., 2021).

1.5. No evidence of disease activity evaluation

The concept of No Evidence of Disease Activity (NEDA) has emerged as a crucial treatment goal in the management of MS (E. Havrdova et al., 2009; Havrdová et al., 2018). NEDA represents a comprehensive approach to disease management, aiming to achieve a state in which patients show no clinical or radiological signs of disease activity over a defined period. This innovative measure considers various aspects of disease activity, including clinical relapses, disability progression, and new or enlarging lesions observed on brain MRI.

Several studies have shown that achieving NEDA status is associated with better long-term outcomes (Rotstein et al., 2015) and is used as an outcome for evaluating the treatment efficacy (Alonso et al., 2023).

NEDA-3 refers to the achievement of a state where patients show no clinical relapses, no disability progression, and no new or enlarging lesions on MRI over a defined period. It represents a robust measure of treatment success, indicating a reduction in acute inflammatory attacks, preservation of neurological function, and a lack of ongoing inflammatory activity. NEDA-3 has been criticized for overly emphasizing focal inflammatory activity (Gasperini et al., 2019). That was the reason for introducing NEDA-4 in 2016 (Kappos et al., 2016). NEDA-4 involves achieving NEDA-3 status along with the absence of brain atrophy, as determined by MRI, which is indicative of neurodegeneration and loss of brain tissue. NEDA-4 represents a more comprehensive treatment goal, highlighting the importance of not only controlling inflammatory disease activity but also preserving brain health and preventing long-term disability in individuals with MS.

1.6. ReMuS registry

The Czech National Registry of MS (ReMuS) was established in 2013 and is operated by the independent organization, Endowment Fund IMPULS, in collaboration with the Czech

Neuroimmunological Society. ReMuS gathers data on patients with MS from all 15 specialized MS centers in the Czech Republic, encompassing approximately 85% of all MS patients in the country (Horakova et al., 2019; Stastna et al., 2023).

Table 3 outlines the primary parameters collected and analyzed within the ReMuS registry. Additionally, certain voluntary characteristics are not consistently collected across all MS centers, including cognitive tests, the 25-foot walk test, the 9-hole peg test, comorbidities, and mild adverse events. These parameters are not routinely recorded in the registry.

Demographic parameters	Birth Date, Gender, Region of permanent residence, Date of death, Pregnancy, Breastfeeding
MS-related parameters	Date of MS onset, Type of MS onset, MS phenotype, EDSS, including functional subsystems, Relapses including severity and form of treatment, Selected laboratory parameters (Oligoclonal bands etc.)
Treatment-related parameters	DMT medication, Symptomatic treatment, Adverse events related to MS treatment
Socio-economic parameters	Individual healthcare insurance company, Employment status, Social benefits
Covid-19 parameters	Symptoms, Severity, Therapy, Vaccination, Relevant Comorbidities

Table 3 Parameters collected in the Czech national registry ReMuS

Data is collected using standardized software, iMed, and exported from each center every six months. To ensure data accuracy and integrity, a multiple-level quality control process is applied. Quality control reports are sent back to the MS center, prompting validation and correction of any suspicious, invalid, or missing information at the local level. The complete dataset then undergoes comprehensive analysis, which is summarized into semi-annual, descriptive reports giving an overview of the current MS situation. These reports are publicly accessible at www.multiplesclerosis.cz.

Until 2015, the registry's semi-annual reports focused exclusively on data from patients treated using DMTs that could only be prescribed by the 15 specialized MS centers. Since 2015, the registry has expanded its scope to encompass data from all treated and untreated MS patients monitored by the MS centers (Horakova et al., 2019).

This project received approval from the designated ethics committees in all participating hospitals, ensuring adherence to ethical guidelines. Furthermore, all patients provided

informed consent by signing the necessary consent form before their data was included in the registry.

1.7. Usage of statistical methods in the clinical research

Statistical methods are integral to clinical research, enabling researchers to extract valuable insights from complex medical data. These methods play a pivotal role in understanding disease dynamics, treatment efficacy, and patient outcomes. In clinical research, various statistical techniques are employed to address specific research questions and analyze diverse types of data. The selection of the appropriate statistical approach depends on the specific research context and goals, with the ultimate aim of advancing our understanding of diseases and improving patient care.

In the realm of medical research, Real-World Data (RWD) has emerged as a valuable resource, offering insights beyond the controlled settings of clinical trials (Sherman et al., 2016). When it comes to understanding and managing complex diseases such as MS, RWD plays a pivotal role. However, harnessing the potential of RWD comes with its own set of aims and challenges for statisticians and researchers. The primary reasons for using RWD in MS research are: capturing the real-world heterogeneity, longitudinal analysis and long-term follow-up.

First, RWD is instrumental in the study of diverse patient populations. Unlike clinical trials that typically involve carefully selected patient cohorts, RWD encompasses a broad spectrum of patients. The statistical aim here is to account for the inherent heterogeneity in patients' demographics, disease manifestations, and treatment responses. Additionally, RWD allows the inclusion of patients with non-standard comorbidities and treatment sequences, who are often excluded from clinical trials. This diversity is essential for comprehensive clinical research.

Secondly, RWD offers the advantage of extended patient follow-up over many years, providing valuable data on the long-term development of MS and the outcomes of initial treatments. It also helps identify rare adverse events that may not become apparent in the limited duration of clinical trials. Events such as pregnancy and childbirth, which can impact disease progression, are captured through RWD unlike clinical trials. Long-term

follow-up enables in-depth survival (time-to-event) analysis of the MS course, revealing insights that may take years to observe in shorter-term clinical trials.

However, the utilization of RWD presents its own set of challenges, some of which cannot be fully addressed through statistical methods alone.

As RWD come from the clinical practice, often sourced from electronic health records, administrative databases, or patient registries, can contain missing or erroneous data. While some registries implement data quality checks to prevent typographical errors, further data discrepancies may surface during analysis. Statisticians must decide whether to correct or exclude such records from the analysis. Addressing data completeness is also critical. While various statistical methods can fill in missing data, researchers should exercise caution, as these techniques can introduce artificial data. It is advisable to explore methods that can handle partially missing data without fabrication.

The second big challenge with RWD is selection bias. Unlike clinical trials, RWD does not involve randomized patient selection. Consequently, selection bias can arise, leading to overrepresentation or underrepresentation of specific patient groups. For example, if a comparative group consists mainly of healthy volunteers who are often relatives of MS patients, it may not provide a comprehensive view of the non-MS population. Researchers must interpret comparative studies involving such groups carefully.

However, the major challenge with RWD remains in the potential of confounding. The absence of randomization in RWD introduces the risk of unmeasured or unknown factors influencing disease outcomes. While techniques like balancing methods can reduce confounding and control for selected covariates, complete elimination of confounding is unattainable through statistical means alone. Researchers must approach RWD analysis with an awareness of potential confounding effects.

In this work, we employ several statistical methods, which we introduce below.

Simple models, such as linear and logistic regression (Harrell, 2015), are widely used in clinical research, mostly for their good interpretability. Linear regression helps explain one continuous variable by possibly multiple all-type variables, while logistic regression is suitable for binary outcomes, like disease presence or absence. These models provide

straightforward interpretations of associations, are implemented in most of the statistical software and therefore are widely used in clinical investigations.

Survival analysis is a complex yet an essential statistical tool in clinical research (Harrell, 2015). It is employed to analyze time-to-event data, such as the time until a patient experiences a relapse, recurrence, or death. Survival analysis accounts for censoring (incomplete follow-up or follow-up without the occurrence of the analyzed event) and provides valuable insights into patient prognosis and treatment effectiveness. In the context of MS, survival analysis is commonly applied to study outcomes such as time-to new relapse occurrence or time to EDSS worsening.

Among the most frequently used survival analysis techniques is the Kaplan-Meier (KM) curve. This graphical representation serves as a visual tool to illustrate the probability of surviving up to a particular time point, offering a snapshot of survival probabilities over time. This not only helps researchers estimate the likelihood of surviving (i.e., not experiencing the event, such as a relapse) up to specific time intervals (e.g., 2 years from treatment initiation) but also aids in conveying complex information to patients using measures like the median survival time.

In survival analysis, the most used model is the Cox Proportional Hazards Model. It allows researchers to examine how various factors, such as treatment type or demographic characteristics, influence the hazard rate—a measure of the risk of experiencing the event of interest. The hazard ratio (HR), derived from this model, is a crucial statistic that quantifies the effect of these factors on survival outcomes. An HR greater than 1 suggests a higher risk of experiencing the event, while an HR less than 1 indicates a lower risk. This facilitates clear communication with patients, explaining which factors can potentially affect their prognosis either negatively or positively.

In addition to these methods, the log-rank test is commonly employed to compare survival between two or more groups. It assesses whether there are significant differences in the survival curves of these groups. Moreover, researchers may explore more advanced techniques like competing risks analysis when multiple types of events can occur, or parametric survival models when the distribution of survival times is assumed to follow a

specific mathematical form. These models are less commonly used due to their complex interpretation.

For longitudinal data, where repeated measurements are collected from the same individuals over time, more complex models are necessary (Diggle, 2002). Patient registries often encompass such data, where the same patient is observed over an extended period with multiple clinical and paraclinical measurements. Mixed-effects models, also known as hierarchical or multilevel models, are favored for such data. These models account for the correlation among repeated measurements within the same subject and allow for the analysis of within-subject and between-subject variation. They are particularly valuable when assessing the impact of interventions or treatments over time. However, these models may require complex interpretation, as they do not yield straightforward results.

The last used technique in this work are balancing methods. Balancing methods (Austin, 2011), like propensity score matching or weighting, are increasingly popular in clinical research to address confounding variables and create balanced treatment groups. These methods are primarily used for comparing two cohorts of patients, where these cohorts were not selected at random (i.e., it was not randomly generated if the patient was in cohort A or cohort B). In such scenarios, there is a high risk of selection bias and confounding, as mentioned above, and balancing methods are employed to mitigate the confounding effects of selected variables. However, it's important to note that balancing methods do not substitute for the randomization process. While they can reduce confounding, there remains a risk of "left-over" confounding variables that may still influence outcomes despite matching or weighting efforts.

2. Goals and hypotheses

The thesis is structured into two main sections, one divided into two subsections, each focusing on a different topic and employing specific statistical methodologies tailored to its objectives.

2.1. The evaluation of outcomes of DMTs using ReMuS registry

The aim of the registry data analysis is to assess various approaches to DMT initiation in MS. The primary goal is to compare patients who begin treatment directly with HE-DMTs to those who start with LE-DMTs with potential later escalation. The secondary goal focuses mainly on LE-DMT initiation, comparing early DMT initiation (after the onset of the first relapse) to later DMT initiation (after experiencing two or more relapses).

2.1.1. Primary analysis of Czech and Swedish registries - hypotheses

1. Patients who initiate treatment directly with HE-DMT exhibit better long-term outcomes compared to those who initiate with LE-DMT and potentially escalate later.

2.1.2. Secondary analysis – LE-DMT patients from Czech registry - hypotheses

1. Early initiation of LE-DMT after the onset of the first relapse is associated with better outcomes compared to initiating LE-DMT after experiencing multiple relapses.
2. The probability of experiencing a new relapse after the initiation of LE-DMT increases with the delay of treatment initiation.
3. The treatment strategy in the Czech Republic demonstrates improvement from 2013 to 2016.

2.2. sNfL as monitoring and predictive marker in MS

The goal of the analysis of sNFL is to determine whether sNfL measured over the treatment period can be used as predictive and monitoring marker of the MS disease activity.

2.2.1. Hypotheses

1. sNfL levels show a corresponding trend with the clinical disease activity.
2. sNfL levels show a corresponding trend with the radiological disease activity.
3. sNfL can be used as predictor of the clinical disease activity.
4. sNfL can be used as predictor of the brain volume loss.
5. the evolution patterns of sNfL correspond with maintaining NEDA-3 status.

3. Methods

3.1. The evaluation of outcomes of DMTs using ReMuS registry

3.1.1. Primary analysis of Czech and Swedish registries

To evaluate if the direct initiation of treatment with HE-DMTs is better to achieve the best therapeutic results or the initiation of LE-DMT and later escalation comes with similar outcomes, multiple studies (Brown et al., 2019; Iaffaldano et al., 2021) based on registry RWD have been conducted. Although the results have in general favored an early HE-DMT strategy, more studies are needed to confirm these conclusions, as differences in population characteristics and statistical methodology, as well as selection bias, may have impacted the outcome.

Recently, the Danish and Swedish registries conducted a comparison of their large cohorts of RRMS patients (Spelman et al., 2021). The MS patient populations of these two countries are very similar, which makes a comparison of treatment strategies plausible. In Sweden, the initial HE-DMT strategy is preferred by a significant part of physicians. In contrast, almost all patients in Denmark initiated treatment with LE-DMTs. This comparison showed better outcomes in patients with early initiation of HE-DMT. To confirm the conclusions derived from the Denmark-Sweden comparison, we decided to compare the data from the Czech and Swedish MS registries. In the Czech Republic, the situation was similar to that of Denmark up to 2022, when the reimbursement criteria changed. The escalation strategy has been highly supported based on the reimbursement criteria, and therefore the vast majority of MS patients included in the Czech national registry received LE-DMTs as initial therapy. Furthermore, the threshold for treatment switch to a more efficacious DMTs was higher than in Sweden, as the criteria for escalation of DMT in the Czech Republic required at least one clinical relapse. In this study, we aimed to evaluate the same outcomes assessed in the Denmark-Sweden comparison, performing the comparison on similar cohorts of patients but with the added advantage of a longer follow-up period.

3.1.1.1.Patients and data collection

The Swedish MS registry has been active since 2000, and has a coverage of more than 80% of the estimated MS population in the country (Hillert & Stawiarz, 2015). Relapsing-remitting MS (RRMS) patients who initiated their first DMT between January 1st, 2013, and December 31st, 2016, were included in the analysis.

The LE-DMT vs HE-DMT study focused on adults aged 18 to 55 years. Older patients were excluded to minimize the influence of comorbidities on outcomes. Patients with progressive MS at the time of the initiation of the first DMT were excluded. In total, 3487 patients from the Czech registry and 2923 patients from Swedish registry were included.

3.1.1.2. Definitions and outcomes

The initiation of the first DMT was considered the study baseline. All EDSS measurements recorded within 90 days from relapse were excluded from the analysis of EDSS outcomes. The primary endpoint was time to Confirmed Disability Worsening (CDW), defined as an increase from baseline EDSS by 1 point (or by 1.5 points when baseline EDSS was 0, and by 0.5 when baseline EDSS was 5.5 or above). CDW should have been confirmed by two consecutive visits, with a minimum interval of at least six months (Kalincik et al., 2015). Secondary endpoints included Annualized Relapse Rate (ARR), time to first relapse, time to EDSS 4 (evaluated only for patients with baseline EDSS below that value) and time to treatment switch (to any other DMT with a different mechanism of action). The reasons for the treatment change were unified between the registries and described. As a secondary endpoint, Confirmed Disability Improvement (CDI) was also analyzed for patients with baseline EDSS values of 2 or above. CDI was defined as an improvement from baseline EDSS by at least 1 point, or at least 0.5 point when the baseline EDSS value was 6 or above. This improvement should have been sustained for at least two consecutive visits, separated by at least six months.

3.1.1.3. Statistical analysis

All continuous baseline characteristics were described as mean plus Standard Deviation (SD). The discrete variables were quantified by counts and percentages. EDSS values were described also as median.

As the two compared cohorts originate from different registries, the balancing methodology was deemed suitable for mitigating confounding variables. The variables selected for the final propensity score model were then balanced between the groups, ensuring that the subsequent analysis remains unaffected by the initial differences in these variables. However, any imbalance or confounding stemming from unmeasured variables or variables not included in the propensity score model may still introduce bias into the analysis results.

At first, we balanced the Swedish and the Czech patients in terms of the most important baseline characteristics using propensity score overlap weighting (Mlcoch et al., 2019). The weighting was used over a propensity score matching to include all patients from both registries, but to assure balance in the most important factors. Using matching instead of weighting, only subgroups of patients would be analyzed. The propensity score overlap weighting assures the mean values of characteristics selected for the model are equal between the two groups. The propensity score model consisted of age, gender, duration of the disease, baseline EDSS and ARR 12 months before the study baseline.

Only patients with calculated weights (all available characteristics for the models) were included in the primary analysis.

Initially, we focused on the comparison of entire registries, to make our results comparable with those reported in previous studies. However, since not all the patients included in the Swedish registry received HE-DMT as initial therapy, we performed an additional comparison between patients from the Swedish registry that received HE-DMT as initial therapy versus patients from the Czech registry that received LE-DMT as initial therapy. Therefore, two independent propensity score models were fitted. In the first case, weights for all Czech vs. Swedish patient comparisons were calculated, whereas in the second case weights for the Swedish patients that received HE-DMT and the Czech patients that received LE-DMTs were estimated. Time-to-event outcomes were analyzed using a weighted Cox proportional hazards model, resulting in a Hazard Ratio (HR) estimate with a 95% Confidence Interval (CI) and a p-value for the likelihood ratio test. KM curves were used for the graphic presentation.

The sensitivity analysis was performed considering only the patients from the Swedish registry that received HE-DMTs as initial therapy and those from the Czech registry that received LE-DMTs as initial therapy. An additional sensitivity analysis considered only patients who started in the years 2015 and 2016, since the proportion of the patients commencing HE-DMT in Sweden during these particular years was higher. For this comparison, the weights for the overall population comparison were used.

The primary statistical analysis was performed using R version 4.2.1 (http://www.R-project.org).

3.1.2. Secondary analysis – LE-DMT patients from Czech registry

3.1.2.1. Patients and data collection

For the subsequent analysis of Czech patients on LE-DMT only, patients starting on interferon beta, glatiramer acetate and teriflunomide were included only, see the patient flowchart on Figure 4. Since the analysis was performed early in 2017, dimethyl fumarate was classified as HE-DMT in the Czech Republic based on the Czech reimbursement criteria. Due to this, in the secondary analysis, dimethyl fumarate patients are excluded from later comparison.

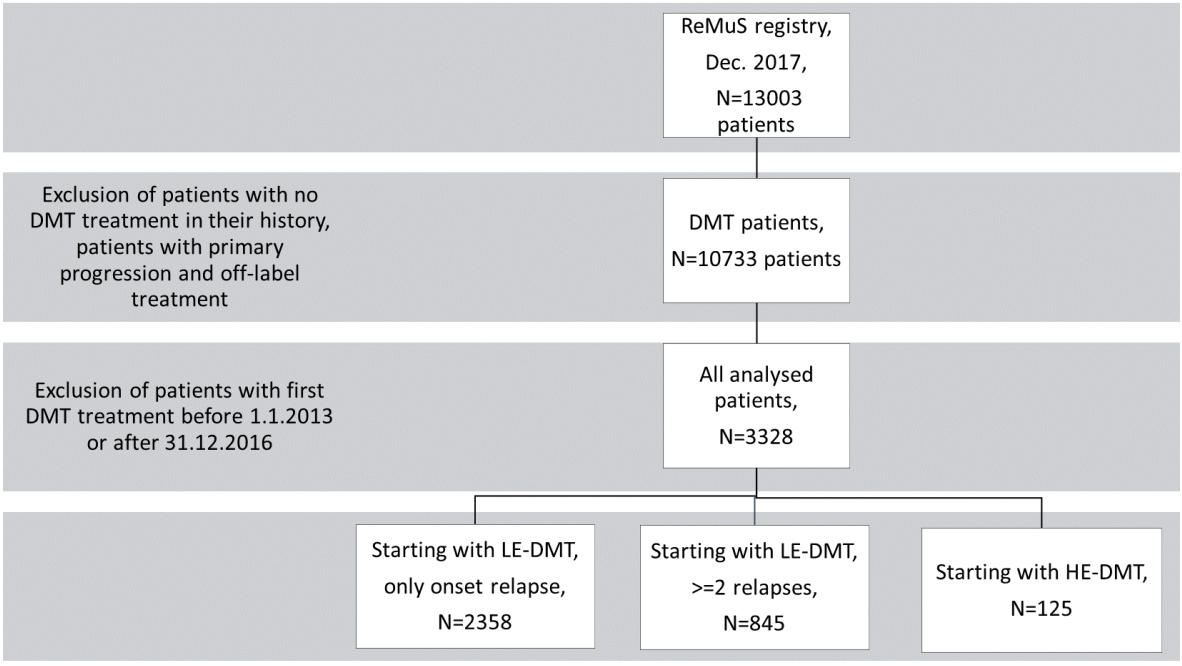

Figure 4 Flowchart of patient disposition for the secondary analysis, figure source (Horakova et al., 2019)

3.1.2.2.Definitions and outcomes

For the secondary analysis, the LE-DMT patients (excluding now dimethyl fumarate patients) were divided into 2 subgroups: (1) Patients that started after the first clinical relapse (LE-DMT 1R) and (2) Patients with at least 2 relapses before initiating DMT treatment (LE-DMT ≥2R).

3.1.2.3.Statistical analysis

All continuous baseline characteristics were described as mean plus Standard Deviation (SD). The discrete variables were quantified by counts and percentages. EDSS values were described also as median. In the secondary analysis of LE-DMT patients from the Czech registry only, described LE-DMT 1R group and LE-DMT ≥2R group, no balancing was done. In the next step, we examined the relationship between the percentage of patients with relapses within the first year after LE-DMT treatment initiation and the remaining covariates. Dependency was modelled using multivariate logistic regression, with the relapse event as an outcome and the following as predictive variables: the age at the first recorded visit, time between disease onset and initiating LE-DMT (i.e., groups < 3, 3–12, and > 12 months), sex, the average EDSS score value 1 year before initiating LE-DMT, the number of previous relapses (i.e., binary, only at onset, or more), and the LE-DMT type (i.e. interferon beta, glatiramer acetate, and teriflunomide represented by 8 different brand names). The model fit was evaluated using the Hosmer and Lemeshow goodness of fit test. The importance of each covariate was examined using the likelihood ratio test and was interpreted in terms of the Odds Ratio (OR). We also explored differences in covariates affecting the relapse rate between patients starting treatment in specific years (e.g., 2013 vs. 2014). For each year, we described patients starting treatment and compared them with cohorts from other years. Descriptive statistics were constructed in the same manner as for the whole population. Moreover, tests comparing measures between years were performed. P-values were derived from an analysis of variance for continuous variables and the Chi-squared test for binary variables. Changes of patients' characteristics between years were confirmed using KM curves for time to the first relapse after LE-DMT initiation. Curves were compared using the log-rank test.

The secondary statistical analysis was performed using R version 3.4.0 (http://www.R-project.org).

3.2. sNfL as monitoring and predictive marker in MS

3.2.1. Patients and data collection

From the original SET cohort (Study of Early Interferon beta-1a Treatment), 172 MS patients after first demyelinating event (according to McDonald criteria 2017) were included (Kalincik et al., 2012). The SET study was an investigator-initiated, observational, prospective multicenter clinical study in the Czech Republic. Patients were enrolled between October 2005 and July 2009. Inclusion criteria included age between 18 and 55 years, enrolment within 4 months from the first demyelinating event, EDSS score at baseline at maximum 3.5, at least 2 T2-hyperintense lesions on diagnostic MRI (before corticosteroid treatment) and at least 2 CSF-restricted oligoclonal bands obtained at the screening prior to corticosteroid treatment (all patients were treated with 3–5g methylprednisolone). Baseline brain MRI was acquired at least 30 days after steroids and prior DMT initiation. All patients started intramuscular interferon beta-1a once a week (30 mg; Biogen-Idec, Cambridge, MA, USA).

Clinical follow-up of patients was each 3-6 months for 48 months from DMT initiation.

Serum samples for the sNfL levels determination were collected on the same day as the clinical visits and stored at −80°C. Sampling procedures were performed according to the standard protocol (Teunissen et al., 2009). Serum samples were assembled from screening (i.e. before corticosteroid treatment), at baseline (i.e. on a day of initiation of interferon beta-1a), at month 1 and then annually over the next 36 months (i.e. at 12, 24 and 36 months). sNfL concentration was measured using a sensitive immunoassay on the Simoa platform at the University Hospital Basel as described previously (Barro et al., 2018; Disanto et al., 2017). Interassay coefficients of variation for three native serum samples were below 10%, the mean intra-assay coefficients of variation of duplicate determinations for concentration was 6.4%. One patient's samples showed a sNfL value below 1.3 pg/mL (i.e. the lower limit of quantification). This patient was excluded from the analysis. Measurements were performed on coded samples. All laboratory personnel had no access to clinical data and remained blinded to treatment allocation and diagnosis.

This study used brain MRI scans performed at baseline and at 12, 24, 36 and 48 months of follow-up. A standardized protocol was performed on a 1.5-T MRI scanner (Gyroscan; Philips Medical Systems, Best, The Netherlands) and consisted of two sequences: FLAIR and T1-weighted three-dimensional turbo field echo. In addition, patients underwent post-contrast T1 spin echo scans 5 minutes after contrast injection of a single dose of 0.1 mmol/kg of Gd-DTPA. All MRI scans were performed on a single MRI scanner in the General University Hospital in Prague. MRI scans were performed at least 30days after corticosteroid treatment. Semi-automated image analysis of the whole brain, Brain Parenchymal Fraction (BPF), corpus callosum volume loss, T2 lesion volume and number, and T1 lesion volume was performed with the ScanView software (Uher et al., 2017). The presence and number of Gadolinium-Enhancing (GAD) lesions was established on post-contrast images by visual inspection of experienced neuroradiologist. Grey matter volume and white matter volume were analyzed using SIENAX (http://www.fmrib.ox.ac.uk/analysis/research/siena/). Regional brain volumes were normalized with respect to the total intracranial brain volume.

3.2.2. Statistical analysis

All analyses were performed using the R statistical system (http://www.R-project.org).

Baseline characteristics were described using median and Interquartile Range (IQR). Relationships between sNfL levels at different time points as well between sNfL and baseline parameters (MRI and clinical) were evaluated using Spearman correlation test. The longitudinal relationship between percentage changes of sNfL levels (change between months 1 and 12, months 1 and 24, months 1 and 36) and changes of MRI and clinical parameters (change between months 0 and 12, months 0 and 24, months 0 and 36) were explored. sNfL levels at month 1 were used as a baseline instead of sNfL levels at month 0 or sNfL levels at screening due to a high proportion of missing sNfL data at month 0 and strong linear relationship between sNfL levels at months 0 and 1 (Figure 5).

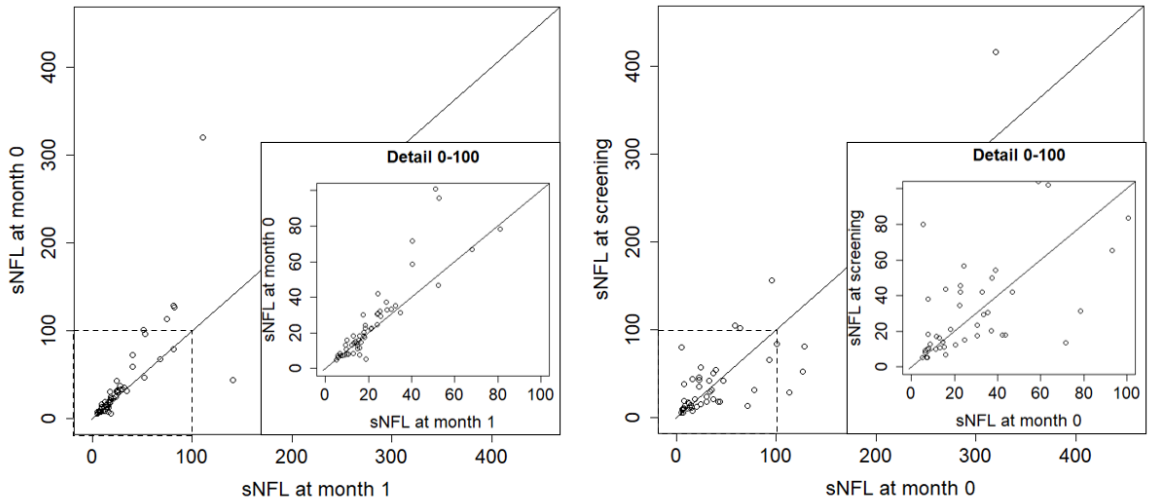

Figure 5 Linear relationship between the sNfL levels at screening, month 0 and month 1 with detail of the values up to 100

Moreover, we observed highly variable sNfL levels at screening measured at the time or shortly after the first clinical event (Figure 5). This together with a longer time from baseline and weaker correlation with month 0 sNfL levels argued against their use instead of the month 0 levels. In validation analysis, sNfL level at screening was used as a baseline measure.

As the data were collected over a long period, it was necessary to apply a mixed-effects model to account for the fact that several measurements originate from the same patient. Given the multitude of potential explanatory variables (including clinical and MRI markers) for modeling sNfL development over time, it was essential to initially select the most relevant explanatory variables for the final multivariable model. These crucial variables for explaining sNfL development over time were chosen based on the results of univariate models, where sNfL was modeled using only one selected explanatory variable at a time. Each relevant variable underwent testing in this manner, and based on the results, the variables for the multivariate model were selected. The multivariate model then considers sNfL not based on a single explanatory variable but rather on all the included explanatory variables collectively.

We applied adjusted log-linear mixed effect models with random intercept per patient fitted by maximum likelihood method. First, univariate models were conducted using logarithmically transformed relative change of sNfL from month 1 as the dependent variable and time from baseline, change of EDSS from baseline, cumulative number of

relapses from baseline, absolute change of T1 lesion volume and T2 lesion volume from baseline, cumulative number of T2 lesions from baseline, number of GAD lesions at particular time points and percentage changes of whole brain, grey matter and corpus callosum volumes from baseline as explaining variables (one by one). Absolute changes of lesion volumes over time were used to prevent overestimation of relative increase in patients with low lesion load and marginal lesion volume accumulation. Akaike Information Criterion (AIC) was calculated for each univariate model.

Based on the results from the univariate models, taking into account the clinical importance and degree of collinearity between the above-mentioned explanatory variables, we defined a final multivariate loglinear mixed-effects model with random intercept per patient. In the final model, time from baseline, change of EDSS from baseline, cumulative number of relapses from baseline, cumulative number of T2 lesions from baseline, absolute change in T1 lesion volume and percentage change in whole brain volume from baseline were used as independent variables. The model fit was assessed via AIC and significance of each variable was computed using t-statistic.

To investigate a predictive role of sNfL levels in comparison with lesional pathology (number and volume), we also analyzed the relationship between sNfL levels at month 1 and the evolution of MRI volumetric parameters over 48 months using multivariate linear regression adjusted for age and sex. We also analyzed predictive role of sNfL levels from various time points.

Finally, we investigated the evolution of sNfL levels in patients with NEDA-3 status over the whole follow-up and compared them with patients who lost their NEDA-3 status (i.e. had Evidence of Disease Activity – EDA-3) between different timepoints.

4. Results

4.1. The evaluation of outcomes of DMTs using ReMuS registry

4.1.1. Primary analysis of Czech and Swedish registries

In total, 3487 patients from the Czech registry whose data was exported in December 2021, and 2923 patients from the Swedish registry whose data was exported in March 2022 were included in the study. Out of all the patients included, 3327 of those from the Czech registry

(95.41%) and 1771 of those from the Swedish registry (60.59%) had initiated their treatment with LE-DMT. The remaining patients had received HE-DMT as initial therapy.

The analysis included 2991 patients from the Czech registry and 1529 patients from the Swedish registry for whom the weights were calculated. The patients differed in age and duration of the disease, with those from the Swedish registry having a duration of disease that was more than one year longer at baseline. ARR prior to DMT initiation was almost twice as high in patients from the Czech registry (1.08 vs. 0.54). The patients were followed in average for 6.64 years in the Czech Republic, and for 5.9 years in Sweden (Table 4).

		All analyzed patients		
		Czech registry	Swedish registry	p-value
N		2991	1529	
Age*, mean±SD, years		35.5±9.05	36.92±9.86	<0.001
Disease duration*, mean±SD, years		2.63±4.93	3.87±6	<0.001
EDSS*	mean±SD	2.01±1.01	1.66±1.53	<0.001
	median	2	1.5	
ARR 12 months prior baseline*, mean±SD		1.08±0.66	0.54±0.68	<0.001
ARR 24 months prior baseline, mean±SD		0.62±0.38	0.33±0.38	<0.001
Follow-up, mean±SD, years		6.64±1.31	5.9±1.67	<0.001
Annualized number of follow-up visits, mean±SD		3.24±0.87	1.37±0.51	<0.001
Gender*	Female	2103 (70.31%)	1040 (68.02%)	0.113
	Male	888 (29.69%)	489 (31.98%)	
Baseline year	2013	673 (22.5%)	336 (21.98%)	0.672
	2014	728 (24.34%)	398 (26.03%)	
	2015	800 (26.75%)	400 (26.16%)	
	2016	790 (26.41%)	395 (25.83%)	
DMT group	Low-moderate	2877 (96.19%)	887 (58.01%)	<0.001
	Moderate-high	114 (3.81%)	642 (41.99%)	
DMT	Dimethyl fumarate	35 (1.17%)	348 (22.76%)	
	Glatiramer acetate	822 (27.48%)	63 (4.12%)	
	Interferon beta-1a	1453 (48.58%)	302 (19.75%)	
	Interferon beta-1b	431 (14.41%)	93 (6.08%)	
	Pegylated interferon beta-1a	0 (0%)	45 (2.94%)	
	Teriflunomide	136 (4.55%)	36 (2.35%)	
	Alemtuzumab	1 (0.03%)	10 (0.65%)	
	Fingolimod	50 (1.67%)	61 (3.99%)	
	Natalizumab	36 (1.2%)	170 (11.12%)	
	Ocrelizumab	19 (0.64%)	2 (0.13%)	

	Ponesimod	2 (0.07%)	0 (0%)	
	Rituximab	6 (0.2%)	399 (26.1%)	

Table 4 Baseline characteristics of patients in the Czech and Swedish national registries, only patients for whom weight values were available were included in the analysis, * Variables used in the propensity score model, hence exactly balanced between the Czech and Swedish registries during the analysis

The HE-DMT initiation strategy was dominant in the Swedish registry (41.99%) compared to the Czech registry (3.81%).

The sensitivity analysis included 642 patients that received HE-DMT as initial therapy in Sweden and 2877 patients that received LE-DMTs as initial therapy in the Czech Republic (Table 5). The differences between the two groups were more pronounced than in the comparison of all patients included in the study. The groups differed mainly in age (mean 35.49 years and 37.04 years for LE-DMT and HE-DMT, respectively) and duration of the disease (mean 2.46 years and 4.46 years for LE-DMT and HE-DMT, respectively). The number of patients starting with HE-DMT in Sweden increased over the years (102 in 2013, 146 in 2014, 174 in 2015, and 220 in 2016). The proportion of patients for whom the escalation strategy was used in the Czech registry remained stable during the entire period of the study.

		Analyzed patients		
		LE-DMT (Czech registry)	HE-DMT (Swedish registry)	p-value
N		2877	642	
Age*, mean±SD, years		35.49±9.06	37.04±10.3	<0.001
Disease duration*, mean±SD, years		2.46±4.78	4.46±6.58	<0.001
EDSS*	mean±SD	1.97±0.97	2.07±1.72	0.127
	median	2	2	
ARR 12 months prior baseline*, mean±SD		1.08±0.64	0.57±0.72	<0.001
ARR 24 months prior baseline, mean±SD		0.62±0.37	0.33±0.4	<0.001
Follow-up, mean±SD, years		6.64±1.32	5.63±1.66	<0.001
Annualized number of follow-up visits, mean±SD		3.24±0.87	1.39±0.5	<0.001
Gender*	Female	2028 (70.49%)	419 (65.26%)	0.009
	Male	849 (29.51%)	223 (34.74%)	
Baseline year	2013	656 (22.8%)	102 (15.89%)	<0.001
	2014	689 (23.95%)	146 (22.74%)	
	2015	780 (27.11%)	174 (27.1%)	
	2016	752 (26.14%)	220 (34.27%)	

Table 5 Baseline characteristics of patients who initiated LE-DMT in the Czech registry and HE-DMT in the Swedish registry, * Variables used in the propensity score model, hence exactly balanced between the Czech and Swedish registries during the analysis

The impact of the balancing approach is demonstrated in Table 6 and Table 7. The variables utilized for the propensity score model exhibit precise balance between the Czech and Swedish registries. The remaining variables do not exhibit substantial alterations to the original data or any selection of a subgroup of patients.

		All analyzed patients	
		Czech registry	Swedish registry
N		2991	1529
Age*, years		36.12±9.02	36.12±9.85
Disease duration*, years		3.31±5.73	3.31±5.42
EDSS*		1.84±0.97	1.84±1.61
ARR 12 months prior baseline*		0.75±0.56	0.75±0.74
ARR 24 months prior baseline		0.47±0.31	0.44±0.41
Follow-up, years		6.64±1.30	5.96±1.65
Annualized number of follow-up visits		3.16±0.85	1.39±0.50
Gender*	Female	68.46%	68.46%
	Male	31.54%	31.54%
Baseline year	2013	22.26%	22.68%
	2014	23.73%	25.80%
	2015	27.8%	26.16%
	2016	26.21%	25.35%
DMT group	Low-moderate	95.65%	55.92%
	Moderate-high	4.35%	44.08%
DMT	Dimethyl fumarate	1.72%	21.27%
	Glatiramer acetate	27.43%	3.76%
	Interferon beta-1a	47.03%	19.15%
	Interferon beta-1b	13.76%	6.22%
	Pegylated interferon beta-1a	0%	3.31%
	Teriflunomide	5.7%	2.21%
	Alemtuzumab	0.01%	0.80%
	Fingolimod	2.07%	4.21%
	Natalizumab	0.84%	12.92%
	Ocrelizumab	1.14%	0.14%
	Ponesimod	0.03%	0%
	Rituximab	0.26%	26.00%

Table 6 Balanced characteristics of patients in the Czech and Swedish national registries. For continuous variables, mean±SD is shown, for the rest only percentages are shown., * Variables used in the propensity score model, hence exactly balanced between the Czech and Swedish registries during the analysis

		Analyzed patients	
		LE-DMT (Czech registry)	HE-DMT (Swedish registry)
N		2877	642
Age*, years		36.16±9.05	36.16±10.16
Disease duration*, years		3.69±6.18	3.69±5.78
EDSS*		1.97±1.00	1.97±1.64
ARR 12 months prior baseline*		0.71±0.57	0.71±0.76
ARR 24 months prior baseline		0.45±0.32	0.40±0.42
Follow-up, years		6.64±1.32	5.69±1.63
Annualized number of follow-up visits		3.15±0.84	1.40±0.50
Gender*	Female	66.44%	66.44%
	Male	33.56%	33.56%
Baseline year	2013	23.16%	16.43%
	2014	22.35%	22.35%
	2015	28.64%	26.87%
	2016	25.86%	34.34%

Table 7 Balanced characteristics of patients who initiated LE-DMT in the Czech registry and HE-DMT in the Swedish registry. For continuous variables, mean±SD is shown, for the rest only percentages are shown. * Variables used in the propensity score model, hence exactly balanced between the Czech and Swedish registries during the analysis

When the countries were compared in terms of CDW outcomes, patients from the Swedish registry showed slightly better results, mainly during longer follow-up. However, the 11% reduction (HR 0.89, 95% CI 0.77 to 1.03) in the probability of CDW with respect to patients from the Czech registry was not significant (p-value 0.2764, Figure 6). Sensitivity analysis comparing only patients on HE-DMT and LE-DMT highlighted the trends (HR 0.81, 95% CI 0.66 to 0.99). However, the early crossing of the curves might have prevented the p-value from becoming significant.

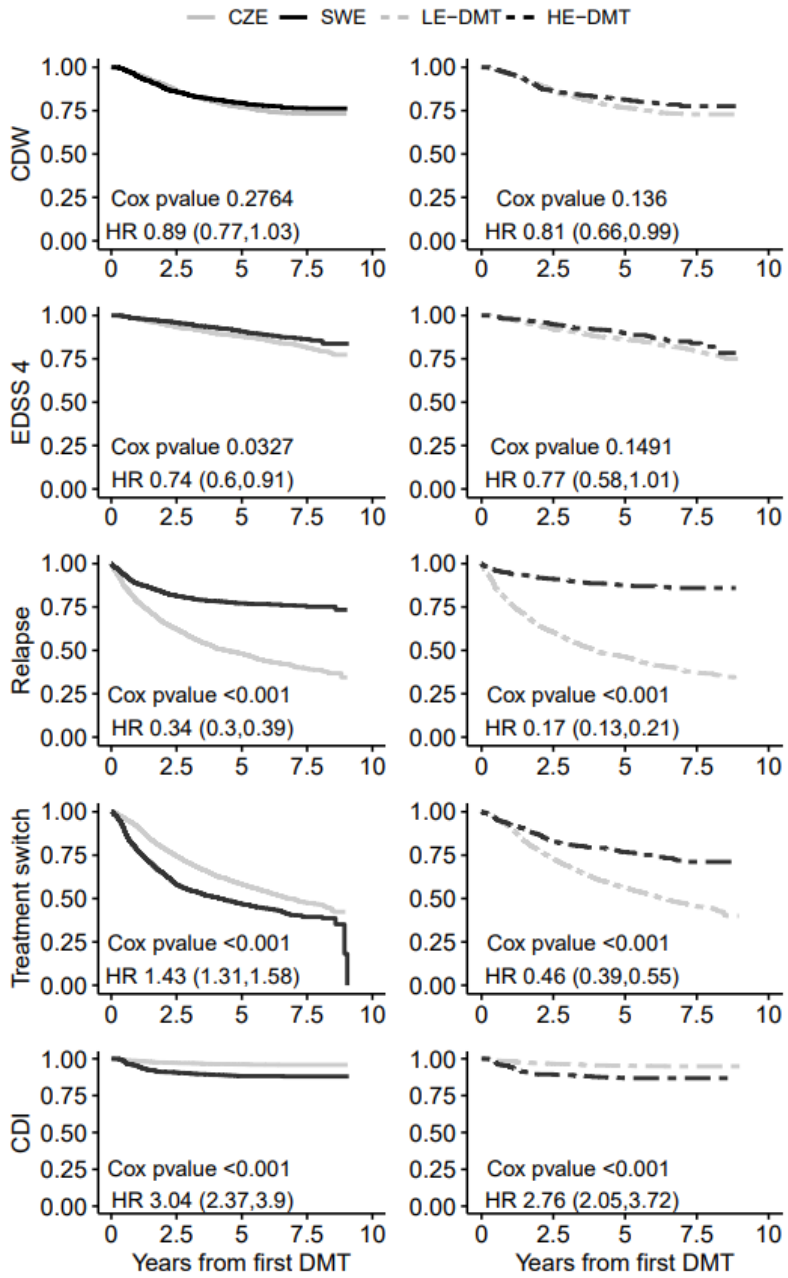

Figure 6 KM curves describing the proportion of patients who: remained CDW free (CDW), remained below EDSS 4 (EDSS 4), did not experience any relapse (Relapse), stayed on DMT with same mode of action (Treatment switch) and remained without CDI (CDI). Solid curves represent comparisons between entire registries (CZE vs. SWE), dashed curves represent comparisons between patients from the Swedish registry on HE-DMT (HE-DMT) and patients from the Czech registry on LE-DMT (LE-DMT).

A sensitivity analysis that considered only patients with baseline in the years 2015 and 2016 showed an even more pronounced differences between patients from each registry. Patients from the Swedish registry were associated with a 23% reduction in the probability of CDW relative to patients from the Czech registry (p-value 0.0946, HR 0.77, 95% CI 0.62 to 0.96; Figure 7).

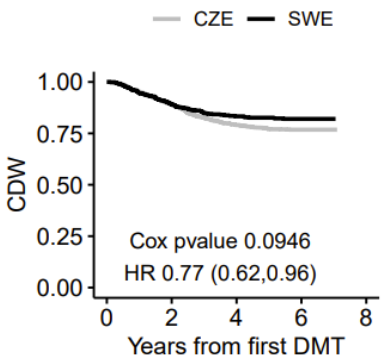

Figure 7 Proportion of CDW-free patients (CDW) who initiated the first DMT in 2015 or 2016

The risk of relapse was significantly reduced by 66% in patients from the Swedish registry (p-value <0.001, HR 0.34, 95% CI 0.3 to 0.39) relative to patients from the Czech registry (Figure 6). This was supported by the results of the sensitivity analysis of HE-DMTs vs LE-DMTs, which revealed a 83% reduction in the risk of relapse for patients receiving HE-DMTs as initial therapy (p-value <0.001, HR 0.17, 95% CI 0.13 to 0.21).

The average ARR for patients in the Czech registry was 0.199 with 0.266 SD, whereas the value was considerably lower for patients in the Swedish registry (mean 0.056, SD 0.141). When the results in patients on HE-DMT and LE-DMT alone were compared, the difference was even more evident (mean 0.208 and 0.268 SD in Czech registry patients receiving LE-DMT as initial therapy versus mean 0.033 and 0.135 SD in Swedish registry patients receiving HE-DMT as initial therapy).

Patients from the Swedish registry were switched to DMT with a different mechanism of action sooner than those from the Czech registry (p-value <0.001, HR 1.43, 95% CI 1.31 to 1.58, Figure 6). However, this was not the case when only those patients receiving HE-DMT versus LE-DMT as initial therapy were considered. In this sensitivity analysis, the trend was the opposite: patients from the Czech registry switched DMTs earlier (p-value <0.001, HR 0.46, 95% CI 0.39 to 0.55). The median time to treatment switch was 6.34 years for patients with LE-DMT in the Czech registry, and it was not reached for patients with HE-DMT in Sweden.

Most patients from the Czech registry (54%) were switched due to the lack of efficacy of the treatment (Table 8). The second main reason for treatment switch in patients from the Czech registry was the presence of side effects (28.84%). As almost all patients from the

Czech registry received LE-DMT as initial treatment, the sensitivity analysis provided similar results. The main reason for treatment switch in patients from the Swedish registry was also a lack of efficacy (37.82%), followed by the presence of side effects (34.85%). However, when only patients from the Swedish registry that received HE-DMT were considered, the main reason for the discontinuation of treatment was another reason (52.6%), followed by lack of efficacy (23.77%) and side effects (14.96%).

		Main analysis		Sensitivity analysis	
		Czech registry	Swedish registry	LE-DMTs (Czech registry)	HE-DMTs (Swedish registry)
First DMT Reason for discontinuation	Another reason	14.18%	22.13%	13.33%	52.6%
	Lack of efficacy	54%	37.82%	55.79%	23.77%
	Pregnancy	2.98%	5.2%	2.7%	8.68%
	Side effects	28.84%	34.85%	28.17%	14.96%

Table 8 Reasons for first DMT discontinuation. Group "Another reason" includes the following categories: Schedule Stop (used mainly for end of the therapy with no further treatment, discontinuation of a single dose for a drug dosed in pulses, detection of JC virus antibodies), Non adherence and Patient Choice/Convenience (for patients from the Czech registry), Stable condition, Another reason and Antibodies detected (for patients from the Swedish registry). The reasons could not be unified, as the available options for explaining DMT discontinuation differed between the registries.

The patients were switched mainly to the dimethyl fumarate, fingolimod, glatiramer acetate and teriflunomide in the Czech registry, and dominantly to the rituximab in the Swedish registry (Table 9). Most of the patients in the Czech registry were not escalated to HE-DMT as a second treatment option.

	Main analysis		Sensitivity analysis	
Second DMT preparate	Czech registry	Swedish registry	LE-DMT (Czech registry)	HE-DMT (Swedish registry)
Dimethyl fumarate	20.47%	17.66%	20.26%	4.4%
Glatiramer acetate	18.43%	6.08%	17.58%	3.44%
Interferon beta-1a	4.74%	0.91%	4.75%	1.79%
Interferon beta-1b	0.12%	0%	0.12%	0%
Pegylated interferon beta-1a	1.53%	1.04%	1.6%	0%
Teriflunomide	18.39%	5.56%	17.41%	0%
Alemtuzumab	0.29%	1.26%	0.4%	4.78%
Cladribine	3.87%	1.02%	3.84%	1.33%
Fingolimod	18.26%	8.04%	19.07%	7.92%
Mitoxantrone	0.16%	0%	0.13%	0%
Natalizumab	6.18%	13.5%	6.8%	12.07%
Ocrelizumab	6.11%	0.23%	6.39%	0%
Ponesimod	0.24%	0%	0.27%	0%

Rituximab	0.23%	44.18%	0.13%	64.27%
Siponimod	0.99%	0%	1.24%	0%
Study medication	0%	0.51%	0%	0%

Table 9 The DMTs patients were switched to from the initial treatment

In the Czech Republic, a minimum of patients experienced CDI. In contrast, in the Swedish registry patients significantly improved three times more often (p-value<0.001, HR 3.04, 95% CI 2.37 to 3.9, Figure 6). This strong trend was confirmed by the sensitivity analysis as well (p-value<0.001, HR 2.76, 95% CI 2.05 to 3.72).

4.1.2. Secondary analysis – LE-DMT patients from Czech registry

The total number of patients included in the Czech registry as of the December 2017 export, used for the secondary analysis, was 13003 (Figure 4). Of these patients, 10733 were treated with DMTs, and 2270 were without DMT treatment. For the purpose of our detailed analysis between cohorts starting their first LE-DMT between 2013–2016, we used data from 3203 patients. Table 10 presents an overview of baseline characteristics, together with a description of disease progression.

		LE-DMT 1R	LE-DMT ≥2R
N of patients		2358	845
Age at onset of MS, mean±SD, years		33.39±9.96	32.15±9.66
Gender, % of males		30.3	26.3
EDSS score at first recorded visit	mean±SD	1.94±0.93	2.21±1.03
	median	2.0	2.0
EDSS score at start of first DMT	mean±SD	1.91±0.93	2.43±1.03
	median	2.0	2.5
Time from onset to start of DMT, mean±SD, years		1.20±3.19	5.61±6.59
N relapses before start of DMT (without post-onset relapses), mean±SD		0±0	2.10±1.52
Relapses 0–12 months after start of DMT	ARR	0.288	0.475
	sum of relapses	665	397
	% of people with relapse*	21.1	34.9
	N of patients	486	291
Confirmed progression in EDSS score 0–12 months after start of DMT	% of patients*	5.6	6.9
	N of patients	112	51

Table 10 Summary characteristics for different number of prior relapses for patients starting their first LE-DMT between the years 2013 and 2016, * out of the number of patients observed at least one year after DMD treatment initiation

The probability of having a new relapse one year after the start of a LE-DMT was significantly associated with the sex, age at first visit, the time between disease onset and DMT initiation, the EDSS score one year before starting DMT treatment, and the number of previous relapses (Table 11). Men were 23% less likely to have a relapse in the first year (OR 0.77) than women. Similarly, with every one year older a patient was at the first recorded visit the chance of having a relapse, in the first year, was 3% lower (OR 0.97); each EDSS point increased the chance of having a relapse during the first year by 39% (OR 1.39), and patients without relapse activity after disease onset were 63% less likely to have a relapse during the first year of treatment than patients with multiple relapses (OR 0.37). The Hosmer and Lemeshow test for model fit was non-significant (p-value 0.320).

Covariate		Coefficient	OR (95% CI)	P-value
Gender	Men vs Women	−0.266	0.77 (0.62;0.94)	0.012
Age at first visit		−0.027	0.97 (0.96;0.98)	< 0.001
Months to DMT from onset	3-12 vs <3	−0.214	0.81 (0.64;1.02)	< 0.001
	>12 vs <3	−0.774	0.46 (0.33;0.64)	
EDSS score 1 year before DMT		0.328	1.39 (1.25;1.54)	< 0.001
Relapses (R) prior DMT	1R vs >=2R	−0.986	0.37 (0.29;0.48)	< 0.001
DMT medication name*				0.479

Table 11 The probability of having a new relapse one year after the start of LE-DMT, p-value indicates the significance of covariates in the final logistic regression model, *variable is not significant and contains too many levels to present results of estimates

The number of patients starting their first LE-DMT in each year did not differ in terms of demographic characteristics (i.e., the age at first visit and sex proportion remained the same, meaning that there was no change in the epidemiology characteristics of MS). However, there was a significant improvement in terms of early diagnoses and early treatment (i.e., EDSS scores when starting the first LE-DMT and the number of previous relapses), as shown in Table 12.

Year first LE-DMT started	2013	2014	2015	2016	P-value
N of patients	745	744	754	746	
Gender, % of males	30.9	27.6	27.9	29.4	0.467
Age at first visit, mean±SD, years	34.71± 9.86	35.08± 10.13	35.23± 10.11	35.14± 9.98	0.755

Time from onset to start of DMT, mean±SD, years	2.41± 4.60	2.24± 4.80	2.39± 4.70	2.29± 4.94	0.889
EDSS score 1 year before DMT, mean±SD	2.15± 1.02	2.08± 0.98	1.97± 0.95	1.97± 0.91	0.002
N of previous relapses (without onset relapse), mean±SD	0.66± 1.33	0.58± 1.25	0.65± 1.29	0.48± 1.10	0.018

Table 12 Comparison of characteristics of patients starting first LE-DMT in each of the years 2013–2016, p-values evaluate the difference in covariates between different starting years. For this analysis, only patients with complete follow-up data were used.

There were also differences in the time to first relapse after starting LE-DMT between patients starting therapy in 2013 and 2016 (Figure 8), with a trend toward lower risks of further relapses.

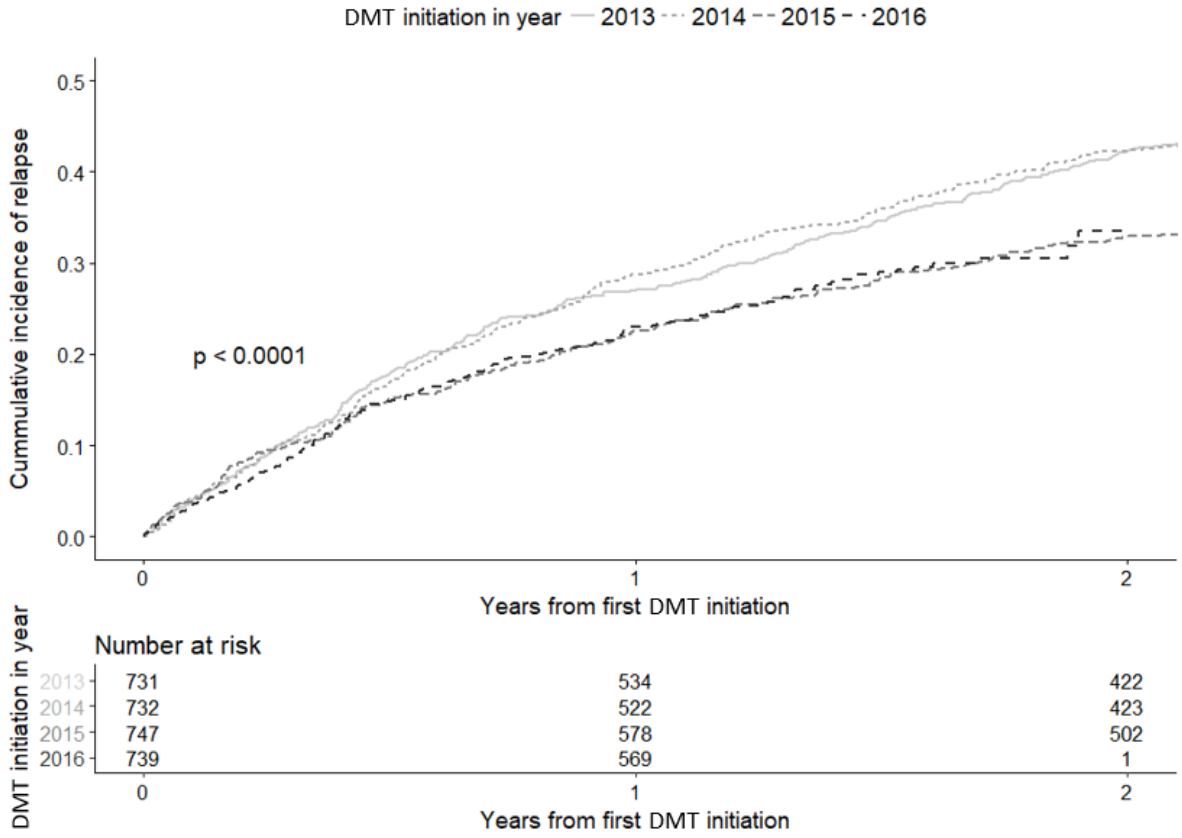

Figure 8 KM curves for time to first relapse after LE-DMT initiation for cohorts of patients starting in different years, p-value from log rank test is presented

4.2. sNfL as monitoring and predictive marker in MS

Table 13 provides basic demographic, clinical and MRI characteristics at baseline. Mean age of patients was 29 years (median 28 years) with the female:male ratio being 2:1. The mean time between disease onset and treatment initiation was 82 days (median 79 days).

Variable	Median (IQR)
Age at onset	28.00 (23.50 – 33.50)
Gender (Female/Male)	115/57
Time between onset and baseline (days)	79.50 (64.00 – 99.75)
EDSS	1.50 (1.5– 2.0)
Whole brain volume (cm3)	1184.00 (1117.40 – 1249.30)
BPF (%)	87.09 (85.83 –88.10)
Corpus callosum volume (cm3)	4.42 (3.94 – 4.70)
Grey matter volume (cm3)	609.80 (569.70 – 640.40)
T2 lesion volume (cm3)	0.63 (0.21 – 2.01)
T1 lesion volume (cm3)	0.48 (0.28 – 0.92)
T2 lesion number	6.00 (3.00 – 15.00)
GAD lesion number	0.00 (0.00 – 1.00)

Table 13 Demographic, clinical and MRI markers at baseline

All investigated cross-sectional atrophy MRI parameters, including BPF (rho 0.08, p-value 0.338), grey matter (rho 0.1, p-value 0.212) and corpus callosum (rho -0.07, p-value 0.422), were not associated with sNfL levels at month 1. The strongest relationship was found between sNfL levels and T2 lesion volume (rho 0.46; p-value <0.001). There was a weak association between sNfL and baseline EDSS (rho 0.21, p-value 0.01) (Figure 9, Table 14).

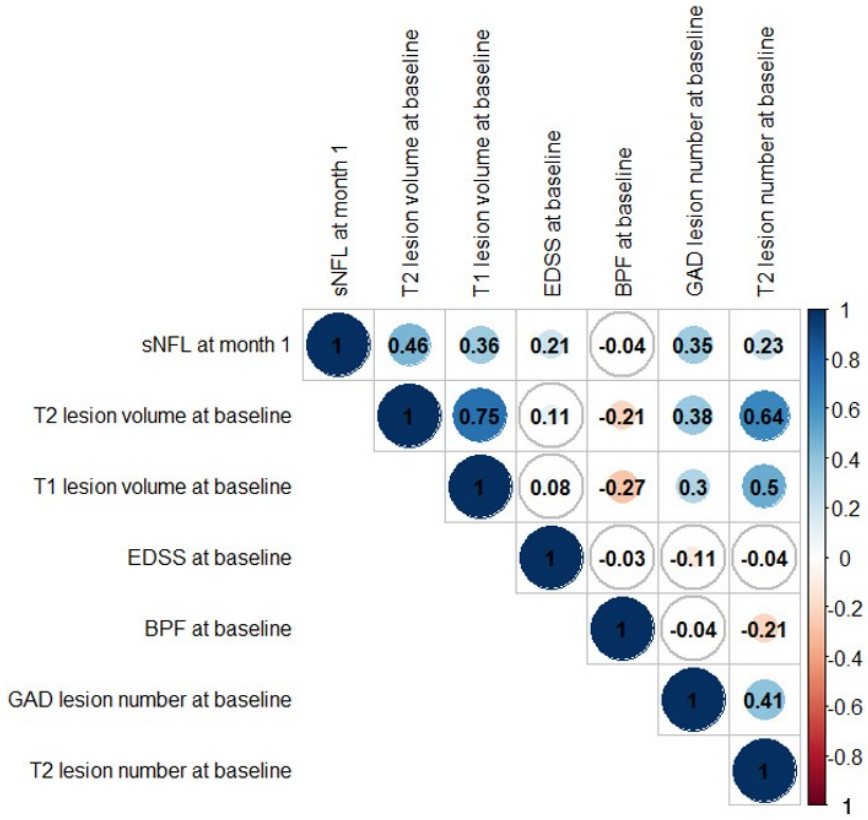

Figure 9 Correlogram of the relationships among serum neurofilament light chain levels at month 1, clinical and MRI parameters at baseline

Variable	Rho	p-value
Age at onset	-0.09	0.281
Time between onset and baseline	-0.09	0.27
EDSS	0.21	0.01
T2 lesion volume (cm3)	0.46	<0.001
T1 lesion volume (cm3)	0.36	<0.001
T2 lesion number	0.23	0.006
GAD lesion number	0.35	<0.001
Grey matter volume (cm3)	0.1	0.212
Corpus callosum volume (cm3)	-0.07	0.422
BPF (%)	0.08	0.338

Table 14 Spearman cross-sectional correlations between sNfL at month 1 and baseline clinical and MRI parameters

The highest sNfL levels were found at baseline (median 22.68 pg/ml, IQR 12.62 – 39.89 pg/ml) and the lowest sNfL levels at 36 months (median 12.24 pg/ml, IQR 8.96 – 16.49 pg/ml) (Table 15).

	N	Mean	Median	Minimum	Maximum	IQR
sNfL screening	156	38.66	20.71	1.39	416. 21	13.84 - 42.30
sNfL month 0	64	43.99	22.68	4.97	475.78	12.62 - 39.89
sNfL month 1	157	32.23	17.70	0.44	268.94	10.99 - 31.05
sNfL month 12	155	19.00	13.86	1.69	147.57	9.51 - 21.29

sNfL month 24	135	17.46	12.48	0.54	171.73	8.61 - 18.00
sNfL month 36	126	15.17	12.24	3.11	137.26	8.96 - 16.49

Table 15 Serum NfL levels at different timepoints, sNfL levels are in pg/ml

However, early sNfL levels were only weakly associated with sNfL levels at later timepoints (Table 16).

sNfL	Screening	Month 0	Month 1	Month 12	Month 24	Month 36
Screening	1.00	0.71***	0.79***	0.47***	0.41***	0.23*
Month 0	0.71***	1.00	0.93***	0.39**	0.21	0.10
Month 1	0.79***	0.93***	1.00	0.41***	0.31***	0.18
Month 12	0.47***	0.39**	0.41***	1.00	0.58***	0.58***
Month 24	0.41***	0.21	0.31***	0.58***	1.00	0.68***
Month 36	0.23*	0.10	0.18	0.58***	0.68***	1.00

Table 16 Spearman correlations among sNfL levels at different timepoints, *** are Spearman correlations with p-value <0.001, ** are p-value <0.01, * are p-value <0.05

At the group level we observed a linear decrease of sNfL levels over time (Figure 10).

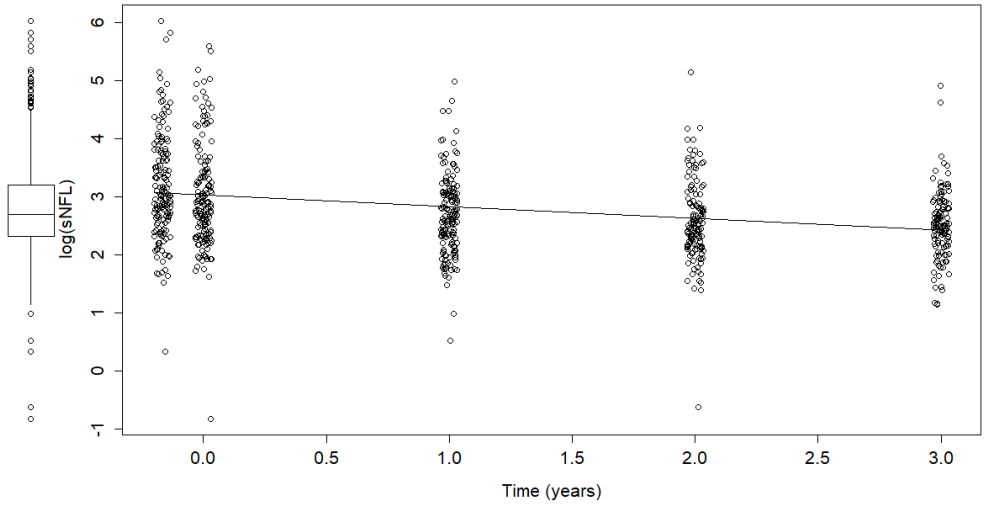

Figure 10 Log-linear trend of decrease in level of sNfL within 3-year follow-up, the line represents the linear regression line

The percentage sNfL level changes over time were most closely associated with T2 lesion volume absolute change (p-value <0.001), T1 lesion volume absolute change (p-value <0.001), increase of T2 lesion number (p-value <0.001) and number of GAD lesions (p-value <0.001, Table 17). The only clinical parameter with significant, however weak association was found between sNfL change and cumulative number of relapses (p-value 0.036).

Independent variable	Regression coefficient	AIC	p-value
Time from baseline	-0.044	612	0.094
EDSS absolute change	-0.03	609	0.454
Cumulative relapse number	0.058	606	0.036
T2 lesion volume absolute change	0.104	549	<0.001
T1 lesion volume absolute change	0.256	557	<0.001
Cumulative number of T2 lesions	0.062	548	<0.001
Number of GAD lesions	0.07	578	<0.001
Whole brain volume % change	4.273	603	0.148
Grey matter volume % change	1.314	603	0.183
Corpus callosum volume % change	0.654	605	0.557

Table 17 Longitudinal univariate mixed effect models explaining the percentage change of sNfL from baseline by change (relative or absolute) in each of subsequent variables. Changes taken into consideration are between baseline and month 12, month 24 and month 36

In the multivariate model taking into the account all selected variables based on the results of univariate model, T1 lesion volume absolute change, T2 lesion number change and time from baseline were the best independent predictors of sNfL percentage change over follow-up (Table 18). EDSS and percentage global and regional brain volume changes were not associated with percentage sNfL changes over the follow-up. AIC of this model was 504.5.

Variable	Regression coefficient	p-value
Intercept	1.701	<0.001
Time from baseline	-0.083	0.003
EDSS absolute change	-0. 046	0.290
Cumulative relapse number	0.046	0.179
T1 lesion volume absolute change	0.241	<0.001
Cumulative number or T2 lesions	0.051	<0.001
Whole brain volume % change	5.231	0.094

Table 18 Longitudinal multivariate mixed-effects model explaining the percentage change in level of sNfL from baseline by changes in all subsequent variables together. Changes taken into account are between baseline and month 12, month 24 and month 36.

We found a strong relationship between cross-sectional log-transformed sNfL levels at month 1 and percentage change of whole brain (p-value <0.001), corpus callosum (p-value <0.001) and grey matter volume loss (p-value <0.001) over 48 months. Percentage or absolute change of sNfL between screening and month 1 was not associated with imaging measures at 48 months.

We did find a trend for stronger association between early sNfL levels (at screening, month 1 or 12) and whole brain volume loss but not BPF or T2 lesion volume at 48 months compared with later sNfL levels (month at 24 or 36) and MRI measures (Table 19).

sNfL timepoint	BPF	Log T2 lesion volume	Whole brain volume % change
Screening	-0.27***	0.45***	-0.36***
Month 1	-0.24**	0.40***	-0.33***
Month 12	-0.16	0.42	-0.26**
Month 24	-0.29**	0.49***	-0.15
Month 36	-0.27**	0.29**	-0.16

Table 19 Spearman cross-sectional correlations between sNfL levels at different timepoints and MRI parameters at 48 months, *** are Spearman correlations with p-value <0.001, ** are p-value <0.01, * are p-value <0.05

In the multivariate models, sNfL was a stronger and independent predictor of brain volume loss than T1LV, T2LV, T2 lesion number or GAD lesion number. Results from the predictive models are summarized in Table 20.

Variable	Predictors at baseline	Regression coefficient	p-value
Whole brain volume %	Intercept	0.007	0.210
	sNfL month 1	0.005	0.001
	T2 lesion volume	0.001	0.106
	Gender	0.001	0.701
	Age	<0.001	0.031
Corpus callosum %	Intercept	-0.064	0.012
	sNfL month 1	0.031	<0.001
	T2 lesion volume	0.004	0.016
	Gender	0.012	0.208
	Age	<0.001	0.46
Gray matter %	Intercept	0.004	0.828
	sNfL month 1	0.014	0.001
	T2 lesion volume	<0.001	0.816
	Gender	0.005	0.418
	Age	-0.001	0.021
Whole brain volume %	Intercept	0.007	0.259
	sNfL month 1	0.005	<0.001
	T1 lesion volume	0.002	0.163
	Gender	0.001	0.726
	Age	<0.001	0.033
Corpus callosum %	Intercept	-0.068	0.008
	sNfL month 1	0.033	<0.001
	T1 lesion volume	0.010	0.038
	Gender	0.012	0.219
	Age	<0.001	0.464
Gray matter %	Intercept	0.004	0.794
	sNfL month 1	0.013	0.001
	T1 lesion volume	0.002	0.597
	Gender	0.005	0.399
	Age	-0.001	0.019
Whole brain volume %	Intercept	0.008	0.158

	sNfL month 1	0.004	0.003
	GAD lesion number	0.002	0.023
	Gender	-0.001	0.760
	Age	<0.001	0.104
Corpus callosum %	Intercept	-0.047	0.032
	sNfL month 1	0.023	<0.001
	GAD lesion number	0.020	<0.001
	Gender	-0.002	0.836
	Age	<0.001	0.895
Gray matter %	Intercept	0.009	0.611
	sNfL month 1	0.010	0.008
	GAD lesion number	0.004	0.061
	Gender	0.002	0.798
	Age	-0.001	0.073
Whole brain volume %	Intercept	0.009	0.114
	sNfL month 1	0.004	0.002
	T2 lesion number	<0.001	0.004
	Gender	<0.001	0.948
	Age	<0.001	0.011
Corpus callosum %	Intercept	-0.062	0.009
	sNfL month 1	0.030	<0.001
	T2 lesion number	0.001	0.002
	Gender	0.008	0.355
	Age	<0.001	0.388
Gray matter %	Intercept	0.009	0.606
	sNfL month 1	0.011	0.004
	T2 lesion number	<0.001	0.069
	Gender	0.002	0.714
	Age	-0.001	0.028

Table 20 Multivariate linear regression models (adjusted for sex and age) showing the best predictors (comparison of MRI lesional parameters at baseline and sNfL at month 1) of global or regional brain volume loss over 48 months.

Patients who lost NEDA-3 status within 36 months showed higher sNfL levels over follow-up than patients with NEDA-3 status. All patients with sNfL levels >25pg/ml (n=5) at screening (or baseline due to missing screening data) and NEDA-3 status after 36 months, lost their NEDA-3 status between 36 and 48 months (Figure 11). No patient with NEDA-3 status over 48 months had sNfL levels at screening over 25 pg/ml. Results remained identical whether or not GAD lesions were considered in NEDA status definition.

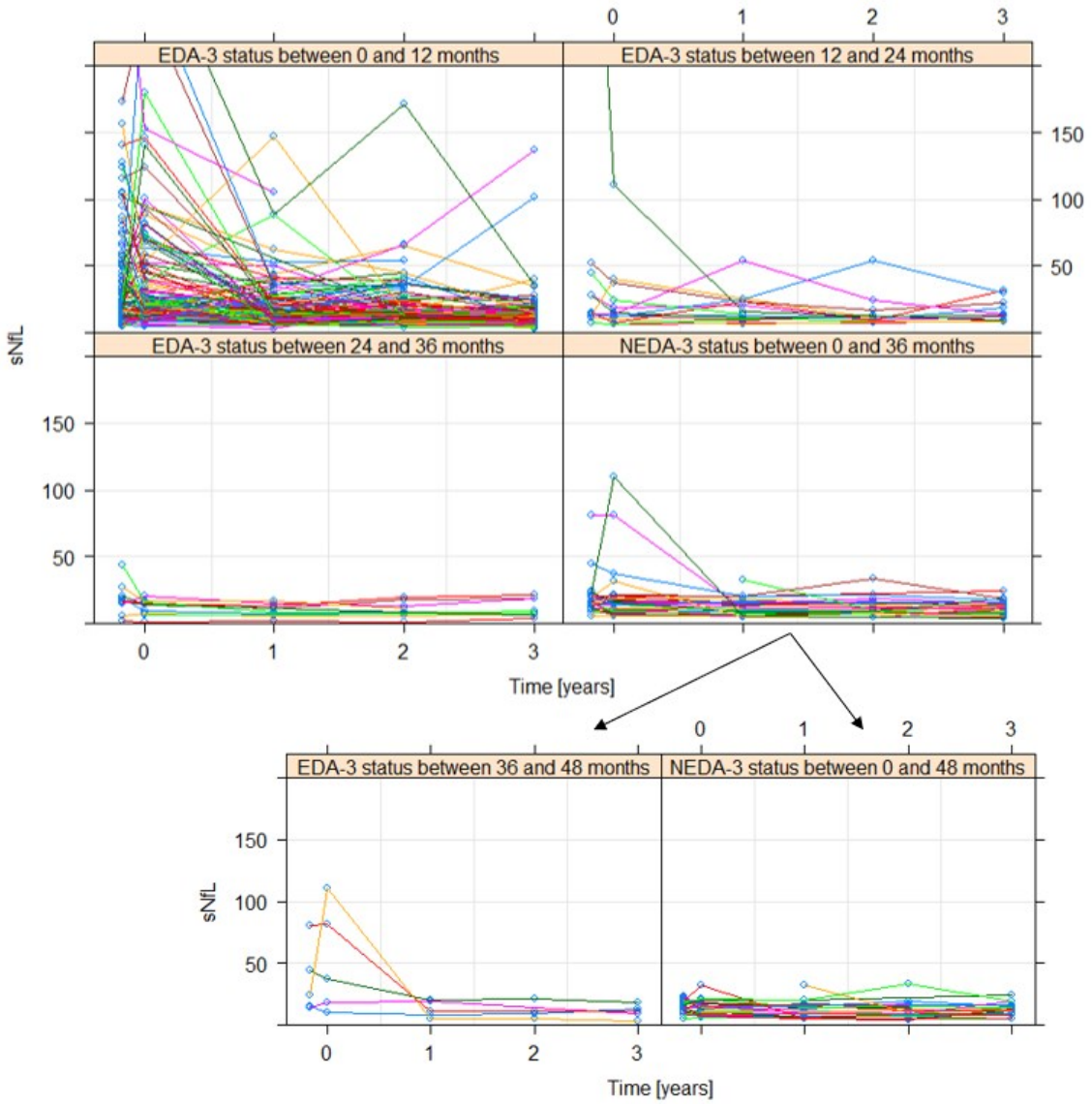

Figure 11 sNfL levels in patients with EDA-3 and with NEDA-3 status over follow up.

5. Discussion

5.1. The evaluation of outcomes of DMTs using ReMuS registry

5.1.1. Primary analysis of Czech and Swedish registries

By using data from the Czech and Swedish national MS registries of RRMS patients starting their first DMT between the years 2013 and 2016, we aimed to confirm the results of a previous comparison of the effect of different treatment strategies on long-term disability outcomes (Spelman et al., 2021). The objective of the previous study was to investigate whether receiving HE-DMT as initial therapy results in a better long-term disability outcome compared to starting patient treatment with LE-DMT (despite an eventual switch to HE-DMT later on). The previous study found a significantly lower risk of CDW in a Swedish cohort compared to that in a Danish cohort, where much smaller percentage of RRMS patients receive HE-DMT as initial treatment. As the treatment strategy preference in the Czech Republic was similar to that in Denmark, we repeated the comparison against the Swedish cohort, but this time with the Czech RRMS population replacing the Danish population. In the Czech Republic, only 4.59% of RRMS patients initiated the treatment directly with HE-DMT within the years 2013 and 2016, whereas in Sweden this strategy was substantially more frequent in the RRMS population (39.41%).

Despite the similarities between the Swedish and Czech RRMS populations, the patients differed slightly but significantly in several baseline characteristics, which may be related to differences in the timing of diagnosis. To minimize potential biases deriving from these differences, the outcomes were balanced using propensity score overlap weights. For the propensity score model, age, gender, duration of the disease, baseline EDSS and ARR calculated 12 months before the initiation of the first DMT were balanced. The model differed from the previous study in several variables. ARR values from 24 months prior to the study baseline were not considered, as they were highly correlated with the values recorded 12 months prior to the study baseline. The number of follow-up visits differed between the registries but was also not considered for the propensity score model. This variable differed between the registries consistently (patients from the Swedish registry had a lower frequency of visits in general), and therefore it was considered as too deterministic and not appropriate to be incorporated in the modeling. Neuroimaging data is not included in the Czech registry to the same extent as in the Swedish registry,

preventing us from analyzing MRI measures in the present study. The overlap weight method was selected because it avoids the multiplication of patients in the analysis, is very consistent, and assures equality of the mean values between the cohorts of selected variables.

In contrast to the previous comparison between the Swedish and Danish cohorts (Spelman et al., 2021), the primary outcome (CDW) did not show a significant difference in favor of the Swedish cohort (HR 0.89, p-value 0.2764). Even when only the patients receiving HE-DMTs as initial therapy in Sweden were considered, the difference remained non-significant (HR 0.81, p-value 0.136). However, in the second case, the insignificance might be caused by the early crossing of the survival curves: the curves diverged after the first 2.5 years of follow-up, and the prognosis was more favorable for patients with HE-DMT from the Swedish registry.

In contrast to what was observed for the primary outcome, all the remaining time-to-event outcomes considered showed significant differences between the registries. For patients from the Swedish registry, the risk of reaching EDSS 4 was reduced by 26% (HR 0.74, p-value 0.0327), the risk of relapse was reduced by 66% (HR 0.34, p-value <0.001) and the probability of CDI was three times higher (HR 3.04, p-value <0.001). Thus, it is reasonable to ask why such a significant reduction in the risk of relapse did not translate into a change in the long-term outcome of CDW. Our hypothesis is that the evaluation methods of EDSS might differ between the countries, as EDSS cannot be considered a hard endpoint, especially for the lower part of the scale (Amato et al., 1988; Noseworthy et al., 1990). This is supported by the fact that hard outcomes such as time to EDSS 4, which is characterized by restricted walking ability, and time to relapse were significantly better for patients from the Swedish registry. Moreover, as previously mentioned, different variables were used for weighting between the present and the previous study due to reasons related to data availability. For instance, the MRI status of the patients could not be balanced between the Swedish and Czech cohorts, which could have affected the results for the primary outcome.

Treatment switch has particular characteristics in different countries, not just in terms of the initial choice of DMT but also in the approach adopted for later escalation. The patients in Sweden were switched much sooner than in the Czech Republic (HR 1.43, p-value

<0.001). This would suggest that patients starting on LE-DMTs in Sweden were quickly escalated to HE-DMTs, which seems to be confirmed by the sensitivity analysis. According to the sensitivity analysis, when only patients from Sweden receiving HE-DMT as first therapy and patients from the Czech Republic receiving LE-DMT as first therapy are considered, the treatment switch trends were the opposite compared to those in the main analysis (HR 0.46, p-value <0.001). After eight years of follow-up, 68% of patients from the Swedish registry on HE-DMT were still without the need for a switch, compared to only 40% of patients from the Czech registry on LE-DMT. This means that patients on HE-DMT stayed on therapy much longer compared to the rest of the patients. The higher efficacy and good tolerance demonstrated by HE-DMT were also confirmed by the analysis of the reasons provided for the switch of treatment: more than half of the patients (54%) in the Czech registry were switched due to the lack of efficacy of the treatment, whereas in Sweden only 37.82% of the patients mentioned this as a reason. The possibility that an early switch of HE-DMTs may increase the incidence of side effects was not confirmed, as only 14.96% of patients receiving HE-DMT switched therapies due to side effects.

The primary limitation of the primary analysis revolves around the baseline disparities observed between the two national study populations. Although expected to be similar due to similar ethnicity, diagnostics and general clinical practice, some differences were apparent, including variances in disease duration, baseline EDSS scores, and particularly ARR measurements taken 12 months prior to baseline. To address these baseline differences, we employed propensity score weighting to moderate the imbalances. This method assured to balance the baseline characteristics between the two cohorts. Nevertheless, it is important to note that no statistical method can guarantee complete elimination of bias in the analysis, as this is a real-world evidence study.

The statistical methodology in the primary analysis aimed at possibility of comparison two different cohorts from two different countries. The aim was to use a method, that would help balance the observed differences between the registries. While propensity score weighting using overlap weights is not extensively utilized, it serves the purpose more effectively than alternative weighting methods like the inverse score weighting method. After the method is applied, it is possible to compare the outcomes as if the characteristics used for the weighting are balanced, meaning it decreases potential bias and confounding.

However, it is crucial to acknowledge that any statistical methodology, including this one, cannot fully substitute for a randomized trial. Consequently, the outcomes of the analysis must be interpreted cautiously, recognizing that residual biases and confounding factors might still influence the results.

5.1.2. Secondary analysis of Czech registry

In the secondary analysis using the Czech ReMus registry data only, we further aimed to investigate the timing of LE-DMT initiation, comparing patients who started their first LE-DMT immediately after their first relapse and those with later LE-DMT initiation.

The chance of having a relapse within one year after commencing treatment was significantly influenced by both the EDSS score 1 year before the start of LE-DMT treatment and the number of relapses in the previous year, with both parameters increased the chance of relapse after starting treatment. This is in line with several other studies (Capra et al., 2017; Cerqueira et al., 2018; Chalmer et al., 2018) supporting the concept that early treatment initiation of patients with a lower disease burden may result in early disease stabilization. In contrast, the time between disease onset and treatment initiation was inversely associated with the risk of relapse, i.e., the shorter the time, the higher the risk of a future relapse. This may be explained by the heterogeneity of our sample, where patients with severe relapses and more aggressive disease tended to start LE-DMT treatment earlier. The choice of LE-DMT treatment type (i.e., interferons, glatiramer acetate and teriflunomide represented by 8 different commercial brands) did not have an effect on relapse activity, probably reflecting the comparative effectiveness of these drugs (Melendez-Torres et al., 2018).

A comparison of demographic and clinical parameters of subgroups of patients starting LE-DMT in the years 2013–2016 showed a positive trend in the reduction of EDSS score and the number of relapses before the start of treatment. This suggests that management of MS improved in the Czech Republic over the 2013–2016 period. This improvement could also be responsible for the decreased relapse-rate one year after LE-DMT treatment initiation.

The secondary analysis did not need balancing, as it involved the comparison of patients solely from one registry. The straightforward implementation of logistic regression

facilitated the estimation of potential covariates that could impact the risk of future relapse. However, it's important to note that any factor omitted from the final model (such as MRI parameters that were unavailable) could potentially be important in the covariate relationship. As a result, caution should be exercised when interpreting the results, and exaggeration of the findings must be avoided.

Our secondary analysis has several limitations. Since our stratification of patients depends on the number of relapses before starting LE-DMT treatment, it may be affected by an incomplete record of relapses. Another potential limitation is that our statistical model, investigating the relationship between relapses one year after LE-DMT initiation and the remaining covariates, might not include all possible confounders of these relationships. However, this weakness affects all exploratory analyses of registry data and cannot be avoided.

5.2. sNfL as monitoring and predictive marker in MS

sNfL is a promising biochemical biomarker of disease activity in MS. Although previous studies have associated sNfL with delayed but not immediate brain volume loss, suggesting critical impact of early axonal damage on long-term neurodegenerative processes, as shown in previous research (Barro et al., 2018; Chitnis et al., 2018; Håkansson et al., 2018; Jakimovski et al., 2019). On the other hand, early relative or absolute sNfL change (between screening and month 1) was not associated with imaging outcomes. Surprisingly, there was no relationship between sNfL levels and previous or ongoing global and regional brain volume loss. In this respect, we suggest that sNfL levels in early disease stages reflect neuropathological processes driven mainly by ongoing neuroinflammatory activity. This is in an agreement with neuropathological studies showing very close association between inflammation and neurodegeneration in MS patients (Frischer et al., 2009). Hence, we hypothesize that the findings from our study provide indirect evidence that sNfL in early disease stages of MS is to the greater extent a marker of inflammation-driven than non-inflammatory-driven neurodegeneration. At a group level, we observed a decrease in sNfL levels over time, which is in agreement with recent studies and can be explained by treatment effects, and possibly regression to the mean (Disanto et al., 2017; Kuhle et al., 2019; Novakova et al., 2017; Piehl et al., 2018; Sormani et al., 2019). Finally, we also

showed lower sNfL levels in patients with NEDA-3 status over follow-up compared with active MS patients, which is in the line with recent results (Håkansson et al., 2018).

Taken together, considering clinical relevance of early sNfL levels for future clinical and radiological disease activity, sNfL may in future qualify as a biomarker of disease activity and endpoint for clinical trials (Sormani et al., 2019).

The methodology used for the analysis needed to account for multiple observations of a single patient over time. The mixed-effect models with random intercept provide such possibilities, although they present a challenge regarding the interpretation of the results. The random intercept allows each patient to have certain personal variability of the sNfL levels, however, such variability cannot be changed with time or other covariates. More complex model with random time and other factors would lead to very complicated interpretability of the results. The drawbacks of using this "simpler" is that the random intercept might not fully capture the patient variability in sNfL.

A limitation of the present study was the sample composition consisted of early stage MS patients treated mostly with interferons, which limits generalizability of our results to the whole MS population. Therefore, further research investigating MS cohorts on different treatments and with various disease phenotypes is warranted. In addition, future studies on patients in progressive phases of disease are needed to confirm an anticipated and more important role of non-inflammatory-driven neurodegeneration in the later disease stages.

Due to a lack of sNfL data at baseline, we re-baselined our sNfL levels data to the first month of the study, showing strong correlation (rho 0.93) with the baseline sNfL levels. In other words, for statistical purposes, sNfL levels at month 1 were considered as baseline sNfL levels. Considering a decrease in sNfL levels following treatment initiation, slightly lower levels of sNfL after 1 month of treatment were expected. Given that we analyzed percentage changes of sNfL, lower absolute sNfL levels at re-baselined month 1 should not play an important role in our longitudinal analysis.

Importantly, it is well known that sNfL levels reflect only recent or ongoing neuropathology and are not sensitive to the neuroaxonal injury occurring before more than 6–9 months (Bergman et al., 2016; Kuhle et al., 2019). In addition, fluctuation of sNfL

levels over time due to dynamic disease activity may occur (Barro et al., 2018; Varhaug et al., 2018). In this respect, a more frequent sNfL sampling would have the potential to increase a strength of association between sNfL levels and measures of ongoing neuroinflammation and also provide more relevant information for clinical practice.

Finally, given that MRI measures assessed in the study provide only indirect evidence to distinguish between inflammatory and non-inflammatory processes, confirmation of our results warrants further investigation.

Strengths of the present study were the large sample size, relatively long follow-up duration and the clinical homogeneity of the cohort. All patients were newly diagnosed with MS after first demyelination event, had ⩾2 oligoclonal bands in the CSF, ⩾2 T2-hyperintense lesions on diagnostic brain MRI and initiated the same DMT. Importantly, the observation that increased levels of sNfL are associated especially with ongoing neuroinflammation rather than recent accelerated brain volume loss could not be proven using only two longitudinal time points as available in previous studies.

6. Conclusion and evaluation of goals and hypotheses

6.1. The evaluation of outcomes of DMTs using ReMuS registry

6.1.1. Primary analysis of Czech and Swedish registries

6.1.1.1. Conclusions

- In Sweden in years 2013 to 2016, more patients initiated their treatment directly with HE-DMT as compared to the Czech Republic.
- Swedish patients performed significantly better long-term clinical outcomes as time to EDSS 4, time to first relapse and time to CDI as compared to Czech cohort.
- Time to CDW was not statistically significantly different between the compared registries.
- The differences in the outcomes were highlighted comparing only Swedish patients starting on HE-DMT and Czech patients starting on LE-DMT.
- Patients from Sweden on LE-DMT are quicker switched to another treatment regimen as compared to the Czech patients.
- HE-DMT does not show higher prevalence of intolerance or side-effects that would lead to early treatment switch.

6.1.1.2. Evaluation of goal and hypothesis

1. It was confirmed that patients treatment initiation directly with HE-DMT leads to better long-term outcomes as compared to initiation with LE-DMT, except outcome of CDW.

6.1.2. Secondary analysis – LE-DMT patients from Czech registry

6.1.2.1. Conclusion

- Timing of initiation of LE-DMT plays a role in the disease stabilization: patients starting immediately after the first relapse tend to have lower risk of next relapse.

6.1.2.2. Evaluation of goals and hypotheses

1. It was confirmed that initiation of LE-DMT only after onset relapse leads to better outcomes.
2. It was not confirmed that the risk of a new relapse is directly associated with prolonged time between the LE-DMT initiation and time from the diagnosis.
3. It was confirmed there was an improvement in the treatment strategy in the Czech Republic between 2013 to 2016.

6.2. sNfL as monitoring and predictive marker in MS

6.2.1. Conclusions

- sNfL in early disease stages reflects mainly ongoing neuroinflammatory activity.
- sNfL are not associated with previous or ongoing brain atrophy but can predict the future brain atrophy.
- sNfL levels are associated with T1 and T2 lesions.
- Patients satisfying NEDA-3 criteria demonstrated stable low sNfL levels during the whole observation period.

6.2.2. Evaluation of goals and hypotheses

1. It was not confirmed that sNfL levels correspond to clinical disease activity.
2. It was confirmed that sNfL levels correspond to radiological disease activity.
3. It was confirmed that sNfL can be used as predictor of clinical disease activity.
4. It was confirmed that sNfL can be used as predictor of the brain volume loss.
5. It was confirmed that the sNfL patterns correspond with maintaining NEDA-3 status.

7. Summary

7.1. Souhrn

Předmětem této práce bylo zkoumání využití klinických a paraklinických markerů pro posouzení vhodnosti léčebné strategie, dále jako markerů budoucího vývoje nemoci a také prozkoumání vztahu a využitelnosti markerů, dosud nepoužívaných rutinně v běžné klinické praxi.

První část práce porovnávala různé léčebné strategie – počátek léčby méně agresivní terapií versus vice účinnou terapií a počátek léčby časně po prvním relapsu versus po vice relapsech. K hodnocení efektivity různých strategií byly použity klinické markery (EDSS, relapsy). Druhá část práce se věnovala paraklinickému biomarkeru lehkých řetězců neurofilament naměřených v séru, jeho vztahu s ostatními klinickými, ale i MRI markery a možností jeho využití pro predikci budoucího vývoje nemoci.

Dle porovnání dat švédského a českého národního registru pacientů s roztroušenou sklerózou jsme prokázali celkově pomalejší vývoj nemoci švédských pacientů. Jelikož je ve Švédsku nepoměrně vyšší zastoupení iniciace právě vice efektivní léčbou než v České republice, naše výsledky zpochybňují pomalou iniciaci nízko efektivní léčbou jako vhodnější léčebnou strategii. Dále naše analýza již pouze českých dat ukázala, že pokud již dochází majoritně k iniciaci nízko efektivní léčby, je důležité tuto léčbu zahajovat časně po prvním prodělaném klinickém relapsu. Tato zjištění by měla podpořit budoucí směřování léčebných strategií pro nově diagnostikované pacienty s roztroušenou sklerózou.

V druhé části práce jsme ukázali, že vysoké hladiny sNfL u pacientů v rané fázi onemocnění reflektují hlavně probíhající zánětlivou aktivitu a mohou sloužit jako prediktor budoucí mozkové atrofie. Nízké a stabilní hladiny sNfL byly spojeny s minimálním zhoršením rezonančních parametrů, a tedy se zachování statusu NEDA-3. Toto podporuje zavedení sNfL jako markeru pro sledování aktivity nemoci u pacientů s roztroušenou sklerózou.

7.2. Summary

The aim of this work was the investigation of using clinical and paraclinical markers for assessing the suitability of treatment strategies, as well as markers for predicting future disease progression, and exploring the relationships and usability of markers not routinely employed in standard clinical practice.

The first part of the work compared different treatment strategies - initiating less aggressive therapy versus more effective therapy, and initiating treatment shortly after the first relapse versus after multiple relapses. Clinical markers (EDSS, relapses) were employed to assess the effectiveness of various strategies. The second part of the study focused on the paraclinical biomarker of serum neurofilament light chains, its correlations with other clinical and MRI markers, and its potential for predicting future disease progression.

Through comparing data from the Swedish and Czech national registries of patients with multiple sclerosis, we demonstrated an overall slower disease worsening in Swedish patients. As there is a proportionally higher representation of initiation of more effective treatment in Sweden compared to the Czech Republic, our results challenge the notion of slow initiation of low-efficacy treatment as a preferable treatment strategy. Furthermore, our analysis of Czech data alone revealed that if initiation of low-efficacy treatment is predominant, it is crucial to begin this treatment early after the first experienced clinical relapse. These findings should support the future direction of treatment strategies for newly diagnosed patients with MS.

In the second part of the study, we showed that high levels of sNfL in patients in the early stages of the disease mainly reflect ongoing inflammatory activity and may serve as a predictor of future brain atrophy. Low and stable levels of sNfL were associated with minimal deterioration of resonance parameters and thus the preservation of NEDA-3 status. This supports the incorporation of sNfL as a marker for monitoring disease activity in patients with MS.

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9. List of publications

9.1. Publications in extenso, used as primary for the thesis

Hrnciarova, T., Drahota, J., Spelman, T., Hillert, J., Lycke, J., Kubala Havrdova, E., Recmanova, E., Adamkova, J., Mares, J., Libertinova, J., Pavelek, Z., Hradilek, P., Ampapa, R., Stetkarova, I., Peterka, M., Martinkova, A., Stourac, P., Grunermelova, M., Vachova, M., Dufek, M., … Horakova, D. (2023). Does initial high efficacy therapy in multiple sclerosis surpass escalation treatment strategy? A comparison of patients with relapsing-remitting multiple sclerosis in the Czech and Swedish national multiple sclerosis registries. *Multiple sclerosis and related disorders*, *76*, 104803. Advance online publication. https://doi.org/10.1016/j.msard.2023.104803 IF 4,808

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