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Autoreferát disertační práce

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

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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

TABLE OF CONTENTS

1. Introduction

Multiple Sclerosis (MS) is chronic autoimmune disease of the central nervous system, characterized by the immune system's attack on the myelin sheath surrounding nerve fibers. In the Czech Republic, the estimated number of patients is 23 thousand with yearly diagnostics of approximately 700 new cases (Havrdova, 2013). MS treatment encompasses symptomatic management to alleviate immediate symptoms, acute treatment of clinical relapses, and long-term Disease-Modifying Therapies (DMTs) aimed at reducing relapses, slowing disease progression, and minimizing disability accumulation. DMTs can be categorized into Low-Moderate Efficacy DMTs (LE-DMTs) and Moderate-High Efficacy DMTs (HE-DMTs). 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. Common LE-DMTs include interferon-beta (e.g., Avonex, Rebif, Betaferon), glatiramer acetate (Copaxone), dimethyl fumarate (Tecfidera) and teriflunomide (Aubagio). HE-DMTs are reserved for patients with more aggressive forms of MS or those who have inadequate responses to lower efficacy drugs. 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).

The main focus in the MS research nowadays is on biomarkers. Biomarkers for MS can be grouped into two main sections: clinical and paraclinical markers. The mostly used clinical marker in MS is the Expanded Disability Status Scale (EDSS), which measures the level of neurological disability (Kurtzke, 1983). 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). Most explored paraclinical markers are Magnetic Resonance Imaging (MRI) techniques, providing insights about brain and spinal cord lesions and volume changes. Meanwhile, serum laboratory markers are primarily used for disease monitoring, with a significant emphasis on Serum Neurofilament Light Chain (sNfL) as a biomarker of axonal damage and disease activity (Barro et al., 2020; Benkert et al., 2022; Disanto et al., 2017).

2. Goals and hypotheses

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 no evidence of disease activity 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

Relapsing-remitting MS (RRMS) patients from the Czech and Swedish national multiple sclerosis registries who initiated their first DMT between January 1st, 2013, and December 31st, 2016, were included in the analysis. 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 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). 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.

At first, we balanced the Swedish and the Czech patients in age, gender, duration of the disease, baseline EDSS and annualized relapse rate 12 months before the study baseline using propensity score overlap weighting (Mlcoch et al., 2019). 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. 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.

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

For the subsequent analysis of Czech patients on LE-DMT only, patients starting on interferon beta, glatiramer acetate and teriflunomide were included only. 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. The LE-DMT patients (excluding now dimethyl fumarate patients) were divided into 2 subgroups: patients that started after

the first clinical relapse and patients with at least 2 relapses before initiating DMT treatment.

Dependency between the percentage of patients with relapses within the first year after LE-DMT treatment initiation and the remaining covariates 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 (represented by 8 different brand names). The importance of each covariate was examined using the likelihood ratio test and was interpreted in terms of the Odds Ratio (OR).

3.2.sNfL as monitoring and predictive marker in MS

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). Serum samples for the sNfL 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). This study used brain MRI scans performed at baseline and at 12, 24, 36 and 48 months of follow-up. All MRI scans were performed on a single MRI scanner (1.5-T Gyroscan; Philips Medical Systems, Best, The Netherlands) in the General University Hospital in Prague. Semi-automated image analysis of the whole brain, brain parenchymal fraction, corpus callosum volume loss, T2 lesion volume and number, and T1 lesion volume was performed with the ScanView software (Uher et al., 2017). sNfL levels at month 1 were used as a baseline.

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 Gadolinium-enhanced (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). 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 significance of each variable was computed using t-statistic.

4. Results

4.1. The evaluation of outcomes of DMTs using ReMuS registry

4.1.1. Primary analysis of Czech and Swedish registries

The analysis included 2991 patients from the Czech registry and 1529 patients from the Swedish registry for whom the weights were calculated. 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.

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) in the probability of CDW with respect to patients from the Czech registry was not significant (p-value 0.2764, Figure 1). Sensitivity analysis comparing only patients on HE-DMT and LE-DMT highlighted the trends. The risk of relapse was significantly reduced by 66% in patients from the Swedish registry (p-value <0.001, HR 0.34) relative to patients from the Czech registry (Figure 1). 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, Figure 1). 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. Most patients from the Czech registry (54%) were switched due to the lack of efficacy of the treatment. 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. 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%).

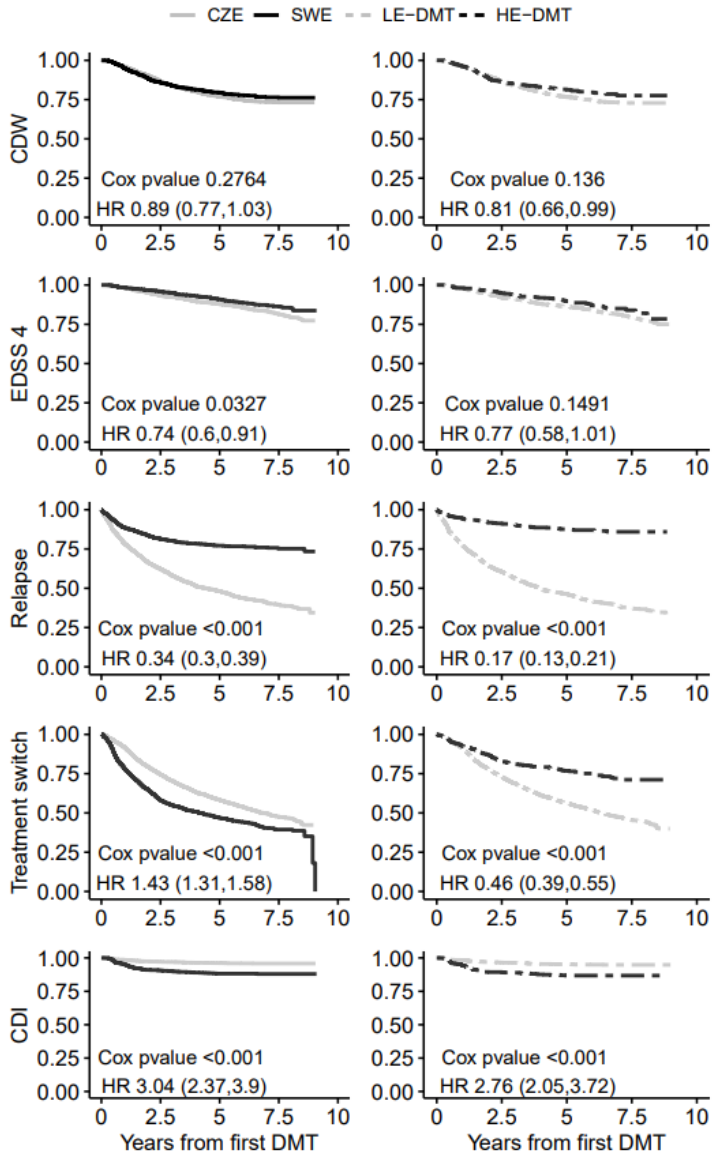

Figure 1 KM curves describing the proportion of patients who: remained CDW free (CDW), remained below EDSS 4 (EDSS 4), did not experience any relapse (Relapse), stayed on DMT with same mode of action (Treatment switch) and remained without CDI (CDI). Solid curves represent comparisons between entire 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).

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

For the purpose of our detailed analysis between cohorts starting their first LE-DMT between 2013–2016, we used data from 3203 patients. The probability of having a new relapse one year after the start of a LE-DMT was significantly associated with the sex (p-value 0.012), age at first visit (p-value <0.001), the time between disease onset and DMT initiation (p-value <0.001), the EDSS score one year before starting DMT treatment (p-value <0.001), and the number of previous relapses (p-value <0.001). 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).

4.2.sNfL as monitoring and predictive marker in MS

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). The only clinical parameter with significant, however weak association was found between sNfL change and cumulative number of relapses (p-value 0.036).

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. EDSS and percentage global and regional brain volume changes were not associated with percentage sNfL changes over the follow-up. 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.

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). 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%).

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). 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.

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.

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 did not have an effect on relapse activity, probably reflecting the comparative effectiveness of these drugs (Melendez-Torres et al., 2018).

5.2. sNfL as monitoring and predictive marker in MS

Univariate models showed the closest relationship between sNfL levels change and with T2 lesion volume absolute change, T1 lesion volume absolute change, increase of T2 lesion number, and number of GAD lesions (p-values <0.001). Clinical parameters were not significant except cumulative relapse number with weak significance (p-value 0.036). 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.

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 no evidence of disease activity 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 no evidence of disease activity 3 status.

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8. List of publications

8.1. 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

Horakova, D., Rockova, P., Jircikova, J., Dolezal, T., Vachova, M., Hradilek, P., Valis, M., Sucha, J., Martinkova, A., Ampapa, R., Grunermelova, M., Stetkarova, I., Stourac, P., Mares, J., Dufek, M., Kmetova, E., Adamkova, J., & Hrnciarova, T. (2019). Initiation of first disease-modifying treatment for multiple sclerosis patients in the Czech republic from 2013 to 2016: Data from the national registry ReMuS. *Multiple sclerosis and related disorders*, *35*, 196–202. https://doi.org/10.1016/j.msard.2019.08.003 IF 4,808

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Andelova, M., Vodehnalova, K., Krasensky, J., Hardubejova, E., Hrnciarova, T., Srpova, B., Uher, T., Menkyova, I., Stastna, D., Friedova, L., Motyl, J., Lizrova Preiningerova, J., Kubala Havrdova, E., Maréchal, B., Fartaria, M. J., Kober, T., Horakova, D., & Vaneckova, M. (2022). Brainstem lesions are associated with diffuse spinal cord involvement in early multiple sclerosis. *BMC neurology*, *22*(1), 270. https://doi.org/10.1186/s12883-022-02778-z IF 2,903

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