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Serum Neurofilament Heavy Chains as a Biomarker of Neuronal Damage in Multiple Sclerosis Relapse

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Summary. *Introduction.* One of the newest methods that could facilitate the diagnosis and treatment of multiple sclerosis is the measurement of neurofilament levels in the blood and spinal fluid. Neurofilament chains can be found not only in multiple sclerosis but also in other neurodegenerative diseases. Latest research findings have revealed that neurofilament levels in serum along with magnetic resonance imaging and clinical evaluation could help evaluate disease relapses and prognosis. No research on neurofilament levels in multiple sclerosis patients has been done in Lithuania so far.

Methods. Permit No. BE-2-73 was obtained from Kaunas Regional Biomedical Research Ethics Committee. Using random sampling we examined 28 patients with relapsing-remitting multiple sclerosis who were treated for relapse in the Multiple Sclerosis Centre of Klaipėda University Hospital. Demographic and clinical data and disease modifying therapies were evaluated. Neurofilament heavy chains levels in the blood were measured using ELISA immunoenzyme assay.

Results. The research involved 28 patients: 64.3% women and 25.7% men. Neurofilament heavy chains levels in the blood were higher in patients with 3 or more functional systems affected, compared with patients with only pyramidal or cerebellar systems damaged but the difference was not statistically significant. Significantly higher neurofilament heavy chains levels were found in patients with hyperintense magnetic resonance imaging T2 lesions in both brain and spinal cord areas and with contrast-enhanced lesions. Higher neurofilament heavy chains levels were associated with positive oligoclonal bands and prolonged visual evoked potentials. No significant correlation between disease duration, age, disability, and neurofilament heavy chains levels was found. Patients with no disease modifying treatment had higher serum neurofilament heavy chains levels but the difference was not statistically significant.

Conclusion. We found higher serum neurofilament heavy chains levels in patients with 3 and more functional systems affected, radiological disease activity, positive oligoclonal bands, and prolonged visual evoked potentials. These results support the hypothesis that neurofilaments could be a promising biomarker for evaluation of multiple sclerosis relapse and disease prognosis in clinical practice.

Keywords: neurofilament, multiple sclerosis, relapse, neurodegeneration.

INTRODUCTION

Multiple sclerosis (MS) is an autoimmune inflammatory neurodegenerative disease of the central nervous system (CNS) that usually manifests at a young age [1]. It is one of the most frequent neurological disorders causing severe disability, thus improvement in diagnosis and treatment of MS is in high demand [2]. One of the novel diagnostic methods is the measurement of a specific biomarker – neurofilament protein (NFL) – in blood serum and

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cerebrospinal fluid (CSF). Neurofilament chains are axonal proteins contributing to the overall structural integrity of neuronal cells. Neurofilament chains are divided into three types differing in molecular weight: low molecular weight (NFL-L), medium molecular weight (NFL-M), and high molecular weight (NFL-H). These NFL chains can be found not only in MS patients but also in patients with various other neurodegenerative disorders such as Alzheimer's disease, amyotrophic lateral sclerosis, and Creutzfeldt-Jakob disease [3]. Due to axonal demyelination and neuronal damage, neurofilaments can be detected in both serum and cerebrospinal fluid. A strong correlation between serum and cerebrospinal fluid neurofilament concentrations has been established so new NFL studies do not require repeated lumbar punctures [4]. According to the latest data, this method could be used not only as an additional tool for MS prognosis evaluation, but also for a more accurate assessment of relapses [5–7]. New studies have found a link between high molecular weight neurofilaments (NFL-H), disease relapses, and disability in patients with relapsing-remitting multiple sclerosis (RRMS) [8]. Furthermore, the relationship between NFL concentration and the number of lesions, disease activity and duration have been demonstrated [7]. Neurofilament levels in blood are exceptionally high in patients with amyotrophic lateral sclerosis or Creutzfeldt-Jakob disease. The main element of pathogenesis of these disorders is neurodegeneration which shows that elevated levels of NFL are a sign not only of active demyelination but also of the progression of neurodegeneration [9]. When comparing results of various studies with both low molecular weight and high molecular weight neurofilaments, NFL-L has shown more promising results; nevertheless NFL-H still plays an important role in MS research [10]. To date, no studies have been carried out in Lithuania investigating the relation between NFL-H and MS. The main goal of this study is to determine correlation between NFL-H and other clinical and demographic characteristics of MS.

Table. **Demographic and clinical data of patients with multiple sclerosis**

Patients	**n=28**
Gender	Female=18 (64.3%) Male=10 (25.7%)
Average age, years	41.4±2.3
Average duration of multiple sclerosis, years	8.8±1.3
Average EDSS score	4.2±0.2
Functional system impaired during a relapse	Pyramidal=9 (32.1%) Cerebellar=2 (7.1%) 3 functional systems 17 (60.8%)
MRI brain lesions MRI brain and spinal cord lesions	14 patients (50%) 14 patients (50%)
MRI contrast-enhanced lesions No MRI activity	12 patients (42.9%) 16 patients (57.1%)

METHODS

Permit No. BE-2-73 was obtained from Kaunas Regional Biomedical Research Ethics Committee. All patients signed an informed consent form. Using random sampling, we examined 28 patients with relapsing-remitting multiple sclerosis who were treated for relapse in the Multiple Sclerosis Centre of Klaipėda University Hospital from July 17, 2019 to December 31, 2019. Multiple sclerosis diagnosis was confirmed based on the 2017 McDonald diagnostic criteria [11].

Demographic (gender, age) and clinical (disability, number and activity of T2 hyperintensive lesions in magnetic resonance imaging (MRI), visual evoked potential (VEP), and oligoclonal immunoglobulin G-bands (OCBs) in cerebrospinal fluid) characteristics were evaluated. Disease modifying therapies (DMT) were also evaluated. First-line DMT: interferons, glatiramer acetate, teriflunomide, and dimethyl fumarate. Second-line DMT: fingolimod, natalizumab, ocrelizumab, alemtuzumab, and cladribine. Disability was assessed using Kurtzke Expanded Disability Status Scale (EDSS) [12]. The visual evoked potential test is a painless method to test the electrical activity of the brain and to determine the deceleration caused by demyelination [13]. Neurofilament heavy chains levels in the blood were measured in the Laboratory of immunology and allergology of Kaunas Clinics by the ELISA immunoenzyme assay using high-sensitivity reagents (manufacturer Euroimmun, Germany) according to the manufacturer's recommendations. Statistical analysis was performed using SPSS 23.0. Descriptive statistics were performed to assess the relationship between individual clinical factors and neurofilament concentrations, nonparametric criteria and Spearman correlation coefficient were used. P values below 0.05 were considered statistically significant.

RESULTS

Twenty-eight patients were involved in the research. Demographic and clinical characteristics of the patients are shown in the Table.

The average serum NFL-H level during a relapse was 22.2±5.9 pg/ml.

No statistically significant correlation was found between serum NFL-H levels and patient age (correlation coefficient 0.127, p=0.521), duration of multiple sclerosis (correlation coefficient 0.059, p=0.765), and EDSS scores (correlation coefficient 0.123, p=0.532).

NFL-H level in patients with impaired pyramidal system was 12.6±8.8 pg/mL, impaired cerebellar system 0.1±0.01 pg/mL, 3 or more functional neurological systems impaired 30.0±8.2 pg/mL; (p=0.062).

The average serum NFL-H levels were statistically significantly higher in patients with lesions in the brain and spinal cord (Fig. 1a). The average serum NFL-H levels were higher in patients with MRI activity (Fig. 1b).

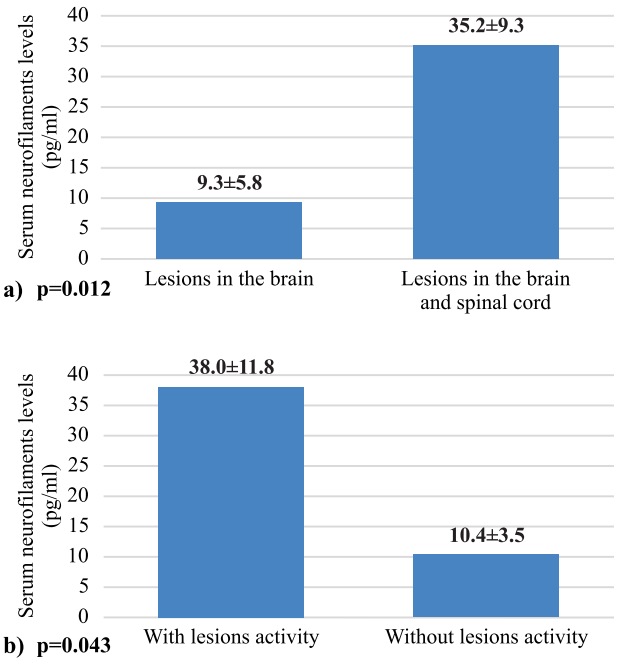

Fig. 1. **Comparison of neurofilament levels in patients with multiple sclerosis due to radiologic changes**
a) due to lesions in the brain with/without spinal cord; b) due to lesions activity in the MRI

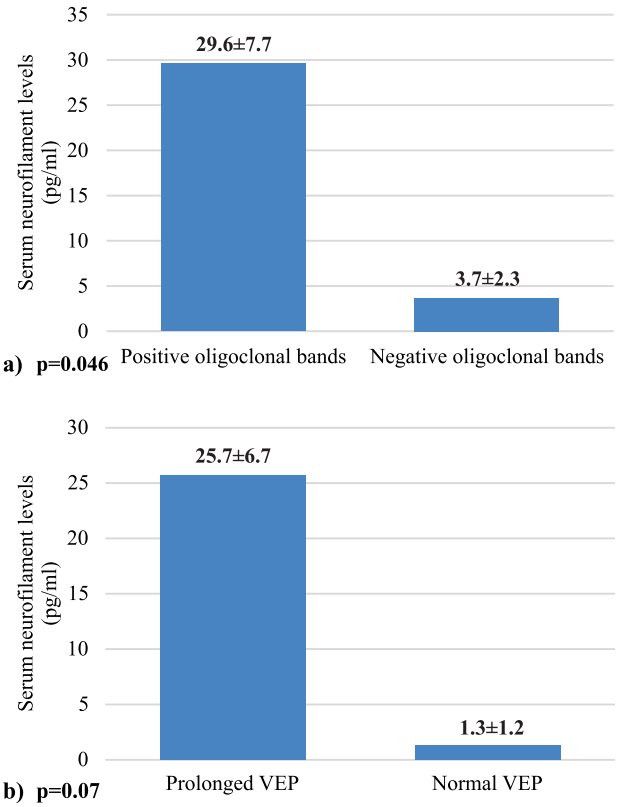

Fig. 2. **Comparison of neurofilament levels in patients with multiple sclerosis**
a) due to oligoclonal bands positivity, b) due to visual evoked potential changes

At disease onset, positive OCBs were found in 20 patients (71.4%), negative – in 8 patients (28.6%). Higher serum NFL-H levels were found in patients with positive CSF oligoclonal bands (p=0.046) (Fig. 2a).

Prolonged VEP P100 wave latency was found in 24 (85.7%) patients, VEP results were normal in 4 patients (4.3%). Higher serum neurofilament levels were found in patients with prolonged visual evoked potentials but the difference was not statistically significant (p=0.07) (Fig. 2b).

Eight patients (28.6%) were treated with first-line DMT, 17 patients (60.7%) with second-line DMT, 3 patients (10.7%) – no DMT. The average serum neurofilament levels in the different treatment groups were not significantly different (19.6±11.0 pg/mL vs. 22.3±7.6 pg/mL vs. 28.7±25.8 pg/mL; p=0.825).

DISCUSSION

Clinical studies in healthy individuals have shown that slightly elevated NFL concentrations can be also found in non-MS patients and that NFL levels are age-related. It is also established that NFL concentration usually increases by about 2.2% every year [14]. Consequently, before using NFL in clinical practice, clear NFL concentration limits should be set for target population of different age. According to our study results, 29% of patients had lower concentration of NFL than the standard normal range and 7% of patients had immeasurable NFL levels. These results show that the increase of NFL concentration is found not in all MS patients during a relapse. Because of these seronegative patients (concentration below standard norm or immeasurable), NFL levels in relapse differentiation should not be used alone, but in combination with clinical evaluation and MRI.

When comparing NFL and MS-related functional disability, previous studies have shown very weak links between NFL levels in serum, EDSS, and age [15]. Our study also didn't show a statistically significant connection between these values. The reasons explaining these results may lie in a small sample size, and also because NFL levels in blood serum indicate an ongoing process of axonal damage but not alterations from previous relapses.

The results of our research indicate that during a relapse, NFL concentration is higher in patients who are not treated with DMT compared with those who are treated with first- or second-line medications. Even though the difference is not statistically significant, it supports the findings and reinforces the hypothesis that neurodegeneration is slowed down and the process of demyelination is less expressed during a relapse in those patients who underwent specific treatment. According to the latest meta-analysis, not all NFL-L and NFL-H studies have shown a consistent reduction of NFL levels in patients treated with DMTs. Therefore, in order to use NFL concentration in blood serum as a treatment monitoring tool, additional large sample size, randomized controlled clinical studies are necessary [16].

Analysis of data of 271 patients with clinically isolated syndrome has revealed a high probability (97% sensitivity) of developing multiple sclerosis if high levels of NFL and positive oligoclonal bands are found in the cerebrospinal fluid [17]. Our study indicates that significantly higher levels of NFL are found in patients who presented with positive OCBs at disease onset. These results suggest that NFLs together with OCBs are valuable prognostic MS indicators. During a relapse, patients with contrast-enhancement lesions in the head or neck presented with considerably higher levels of NFL. These findings are supported by previous studies which show that NFL levels are associated with inflammatory activity manifested by clinical symptoms or MRI in patients with relapsing-remitting MS [5, 7, 14]. Our study also shows that higher NFL levels are found in patients with MRI lesions located in both the brain and the cervical part of the spinal cord, also in patients with elongated visual evoked potentials. These findings support the hypothesis that, during a relapse, higher levels of NFL in blood serum correlate with larger-scale MRI lesions and poorer electrophysiological test results, making NFL levels a viable additional method for evaluating MS relapses.

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NEUROFILAMENTAI KRAUJO SERUME: NEURONŲ PAŽEIDIMO BIOŽYMUO IŠSĖTINĖS SKLEROZĖS PAŪMĖJIMO METU

Santrauka

Įvadas. Vienas naujausių metodų, galinčių palengvinti išsėtinės sklerozės diagnostiką ir gydymą, yra specifinių biožymenų – neurofilamentų, koncentracijos matavimas kraujo serume ir likvore. Neurofilamentų grandinės gali būti randamos ne tik išsėtinės sklerozės, bet ir kitų neurodegeneracinių ligų metu. Remiantis naujausių mokslinių tyrimų duomenimis, neurofilamentų koncentracija kraujyje, kartu su magnetinio rezonanso tyrimu ir objektyviu ištyrimu, galėtų padėti įvertinti išsėtinės sklerozės paūmėjimus ir prognozę. Tyrimų, kurie nagrinėtų neurofilamentų koncentraciją, sergant išsėtine skleroze, Lietuvoje iki šiol nebuvo atlikta.

Metodika. Tyrimo protokolas patvirtintas Kauno regioninio biomedicininių tyrimų etikos komitete (leidimo Nr. BE-2-73). Atsitiktinės atrankos metodu Klaipėdos universitetinės ligoninės išsėtinės sklerozės centre ištirti 28 recidyvuojančia remituojančia išsėtine skleroze sergantys pacientai, kurie buvo hospitalizuoti dėl ligos paūmėjimo. Įvertinti šių pacientų demografiniai ir klinikiniai duomenys, taikomas ligos eigą modifikuojantis gydymas, neurofilamentų koncentracija kraujyje imunofermentiniu ELISA metodu.

Rezultatai. Tyrime dalyvavo 28 pacientai, sergantys išsėtine skleroze (64,3 % moterų, 25,7 % vyrų). Neurofilamentų koncen-

tracija kraujyje buvo didesnė pacientams, kuriems nustatytas 3 ar daugiau funkcinių nervų sistemų pažeidimas nei esant pažeistoms tik piramidinei ar smegenėlių sistemai atskirai, tačiau statistiškai reikšmingo ryšio tarp šių grupių nenustatyta. Reikšmingai didesnė neurofilamentų koncentracija buvo rasta pacientams, kuriems hiperintensiniai T2 režimo židiniai nustatyti galvos bei nugaros smegenyse ir rasti magnetinio rezonanso aktyvumo požymiai. Taip pat didesnė neurofilamentų koncentracija kraujyje rasta pacientams, kuriems nustatytos teigiamos oligokloninės juostos ir buvo sutrikę regos sukeltieji potencialai. Vertinant sąsajas tarp ligos trukmės, pacientų amžiaus, negalios ir neurofilamentų koncentracijos kraujyje, statistiškai reikšmingo ryšio nenustatyta. Gydomiems ir negydomiems ligos eigą modifikuojančiais vaistais pacientams neurofilamentų koncentracija kraujyje reikšmingai nesiskyrė.

Išvada. Nustatytos didesnės neurofilamentų koncentracijos kraujyje, esant 3 neurologinių funkcinių sistemų pažeidimams, radiologiniam židinių aktyvumui, teigiamoms oligokloninėms juostoms ir sutrikusiems regos sukeltiesiems potencialams, pagrindžia hipotezę, kad šis tyrimas galėtų būti diagnostinis paūmėjimo ir ligos prognozės vertinimo biožymuo, naudojamas ir klinikinėje praktikoje.

Raktažodžiai: neurofilamentai, išsėtinė sklerozė, paūmėjimas, neurodegeneracija.

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