BRIEF REPORT

Serum Neurofilament Light Chain as a Biomarker of Brain Injury in Wilson's Disease: Clinical and Neuroradiological Correlations

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ABSTRACT: Background: Clinical scales and neuroimaging are used to monitor nervous system injury in Wilson's disease, while data on serum markers are scarce.
Objective: To investigate whether serum concentrations of neurofilament light chain (sNfL) correlate with brain injury in Wilson's disease patients.
Methods: In 61 treatment-naïve patients, the Unified Wilson's Disease Rating Scale and a validated semi-quantitative brain magnetic resonance imaging scale were compared with concentrations of sNfL.
Results: Concentrations of sNfL were significantly higher in patients with neurological disease compared with patients presenting with other forms (39.7 ± 73.4 pg/mL vs. 13.3 ± 9.2 pg/mL; $P < 0.01$). Moreover, the sNfL concentration positively correlated with neurological severity scores and with acute and chronic brain damage based on the neuroimaging scale.
Conclusions: Neurofilament light chain concentrations may be used as a marker of brain injury in Wilson's disease, in addition to the clinical and neuroimaging disease severity scales. © 2022 International Parkinson and Movement Disorder Society

Key Words: Wilson disease; copper; biomarkers; magnetic resonance imaging; neurofilament light chain

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Introduction

Wilson's disease (WD) is an inherited disorder of copper metabolism with pathological copper accumulation in different organs, mainly the liver and brain.[1-4] The disease is caused by genetic defects in the copper-transporting ATPase, ATP7B, which is expressed mainly (but not only) in hepatocytes. The functions of ATP7B could be categorized as biosynthetic (in the trans-Golgi network), involving loading copper into newly synthetized copper-dependent proteins (mainly ceruloplasmin), and homeostatic, due to export of excess copper from the cells (mainly into the bile).[1-4] Inactivation of ATP7B leads to liver copper overload, with hepatocyte injury and necrosis, and release of free non-ceruloplasmin-bound copper into the circulation, which accumulates in different organs and results in the clinical manifestations of WD.[1-4]

The most frequent clinical symptoms of WD include hepatic (from a clinically asymptomatic increase in liver enzymes, to acute liver failure to compensated or decompensated liver cirrhosis) and neurological (particularly movement disorders).[1-4]

The recommendations for treatment of WD are based mainly on center experience and retrospective analyses.[1-12] The long-term prognosis in WD patients is good in around 85% cases[11]; however, about 10% of patients have paradoxical neurological deterioration, around 50% of neurological deficits remain despite treatment, and others require liver transplantation.[1-7,9-15] There is a need to perform treatment studies comparing current and new treatment methods,[12,15,16] and for this purpose, biomarkers of severity of liver and brain disease are required.[1,2,17-20]

Serum markers of liver disease are well characterized; moreover, they are used to calculate liver failure scales (eg, Model of End Stage of Liver Disease [MELD] and modified Nazer score), in parallel with structural assessment.[1-4,16-18]

The assessment of neurological injury in WD comprises scales describing activities of daily living and neurological deficits, such as the Unified Wilson's Disease Rating Scale (UWDRS) and the Global Assessment Scale (GAS) for WD.[1-3,19] A new semiquantitative scale for brain magnetic resonance imaging (MRI) changes in WD has also been proposed.[20] However, there is a lack of quantitative markers of brain injury in WD.

Neurofilament light (NfL) is a cytoskeletal light chain that is released during neuroaxonal injury.[21-26] NfL concentration can be measured in cerebrospinal fluid (CSF) as well as serum, and in many neurological diseases demonstrating significant neuroaxonal destruction.[21-26] These results led to the inclusion of serum NfL (sNfL) concentration as a relevant biomarker in clinical studies.[24] Shribman et al. described higher sNfL levels in WD patients with neurological presentation compared with a control group, as well as with WD patients with hepatic presentation,[27] warranting further analysis of sNfL in WD.

The aim of our study was to verify whether sNfL concentration may be useful as a marker of severity of neuronal injury in WD.

Methods

Patients

In total, 61 consecutive adult drug-naïve WD patients, newly diagnosed at the Second Department of Neurology at the Institute of Psychiatry and Neurology, Warsaw, Poland between June 2012 and June 2017, were included. The study was approved by the local Bioethical Committee. Clinical information and diagnostic procedure were performed at the time of diagnosis in our prospectively constructed database.

The diagnosis of WD was based on clinical presentation, copper metabolism assessment, and genetic examination and was confirmed using international criteria (Leipzig score).[1,2] Depending on the clinical manifestation, WD was classified as: (1) hepatic; (2) neurological; or (3) presymptomatic (as previously described and according to international recommendations[2]). Additionally, we further categorized patients with neurological WD based on their predominant neurological syndrome: (1) tremor (predominantly tremor and ataxia); (2) parkinsonism; (3) dystonia; or (4) discrete/unclassified symptoms (minimal signs or those not encompassed by the other classifications, such as slight dysarthria, drooling, occasional mild tremor, etc.).[3]

Assessments

Copper metabolism parameters (serum ceruloplasmin, total serum copper and 24-h urinary copper excretion) were measured before initiation of anti-copper treatment as described previously.[3] All patients were examined and their UWDRS score was determined for part II (activities of daily living) and part III (detailed neurological deficits) as described previously.[19] All patients underwent brain MRI in our institute using the Philips Achieva 1.5 T system (Philips Healthcare, Eindhoven, Netherlands), with sequences as described previously.[20] In addition to the standard description of the brain MRI at the time of diagnosis, all MRI examinations were further analyzed by a blinded neurologist (M.S.) using a semiquantitative scale for assessing brain MRI abnormalities (acute toxicity and chronic damage) in WD.[20]

sNfL was determined in plasma samples collected at disease diagnosis using the SimplePlex™ NfL Assay (ProteinSimple, CA, USA) and Ella™ instrument, according to the manufacturer's instructions. Ella™ was calibrated using the in-cartridge factory standard curve. All samples were measured in triplicate on the same day after a single thaw, with a 1:2 dilution for Ella™. The lower limit of quantification was 2.7 pg/mL.[28]

Statistical Analysis

The relationship between mean sNfL and clinical presentation of WD, duration of disease, copper metabolism parameters, severity of neurological deficits scored by the UWDRS (part II and part III), and brain injury scored by the brain MRI scale (acute toxicity and chronic damage) were analyzed using Spearman's rank correlations.

The statistical analyses were performed using Statistica 13.3 (Stat Soft Inc, 2020, Tulsa, OK, USA). Data are presented as number with percentage or mean with range and standard deviation (SD). Comparisons were made using two-tailed Fisher exact test or the Mann–Whitney U test as appropriate; $P < 0.05$ was considered statistically significant and $P < 0.1$ was considered borderline statistically significant.

Results

The characteristics of the 61 analyzed WD patients, particular groups and clinical forms are presented in Table 1. The WD patients with neurological presentation were older at the time of disease diagnosis than hepatic and presymptomatic cases. They also presented with more severe brain injury scored in brain MRI semiquantitative scale.

Mean sNfL was higher in WD patients with neurological presentation compared with non-neurological (hepatic form and presymptomatic patients) (39.7 ± 73.4 pg/mL vs. 13.3 ± 9.2 pg/mL; $P < 0.01$) and when compared separately with patients with the hepatic form (11.5 ± 9.1 pg/mL; $P < 0.01$) and presymptomatic cases (12.1 ± 6.4 pg/mL; $P < 0.05$) (Table 1). There was no statistically

TABLE 1 *Demographic, clinical, and laboratory characteristics with mean serum neurofilament light chain concentrations of Wilson's disease patients included in the analysis*

Characteristic	All WD patients (n = 61)	Hepatic form (n = 18)	Neurological form (n = 36)	Presymptomatic form (n = 7)	P*	P**	P***
Gender, male, n (%)	32 (52.4%)	8 (44.4%)	22 (61.1%)	3 (42.8%)	0.334	0.810	0.610
Age at onset, mean ± SD (y)	28.5 ± 10.9	25.4 ± 9.2	30.1 ± 11.5	n/a	0.173	n/a	n/a
Age at diagnosis, mean ± SD (y)	32.0 ± 11.7	27.5 ± 9.2	34.5 ± 11.8	31.0 ± 14.6	0.034	0.469	0.614
Diagnostic delay[a], mean ± SD (y)	3.0 ± 5.0	2.0 ± 2.4	4.1 ± 6.0	n/a	0.126	n/a	n/a
K–F rings presence, n (%)	43 (70.4%)	7 (38.8%)	34 (94.4%)	2 (28.5%)	**<0.001**	**<0.001**	0.406
UWDRS part II score, mean (range)	2.4 (0–38)	0 (0)	2.4 (0–38)	0 (0)	**<0.001**	0.005	n/a
UWDRS part III score, mean (range)	11.3 (0–96)	0 (0)	11.3 (0–96)	0 (0)	**<0.001**	**<0.001**	n/a
Acute toxicity score, mean (range)	1.81 (0–11)	0.05 (0–1)	3.05 (0–11)	0 (0)	**<0.001**	0.004	0.836
Chronic damage score, mean (range)	1.73 (0–10)	0.27 (0–2)	2.69 (0–10)	0.57 (0–4)	**<0.001**	0.014	0.882
Total score, mean (range)	3.57 (0–13)	0.33 (0–2)	5.77 (0–13)	0.57 (0–4)	**<0.001**	0.003	0.745
Serum ceruloplasmin concentration, mean ± SD (mg/dL)	12.8 ± 6.6	12.5 ± 6.3	12.7 ± 6.9	12.1 ± 6.4	0.820	0.373	0.495
Urinary copper excretion, mean ± SD (μg/24 h)	344.1 ± 1117.9	719.6 ± 2033.5	203 ± 171.1	102.4 ± 36.0	0.566	0.026	0.034
sNfL concentrations, mean ± SD (pg/mL)	28.2 ± 58.2	11.5 ± 9.1	39.7 ± 73.4	12.1 ± 6.44	**<0.001**	**0.041**	0.458

Serum ceruloplasmin concentrations, normal range = 15–25 mg/dL.
Urinary copper excretion, normal range = 0–50 μg/24 h.
*P** when comparing neurological and hepatic group.
*P*** when comparing neurological and presymptomatic group.
*P**** when comparing hepatic and presymptomatic group.
Significant results are shown in bold.
[a]The time between first symptoms onset and disease diagnosis.
Abbreviations: WD, Wilson's disease; SD, standard deviation; n/a, not applicable; K–F, Kayser–Fleischer; UWDRS, Unified Wilson's Disease Rating Score Scale; sNfL, serum neurofilament light.

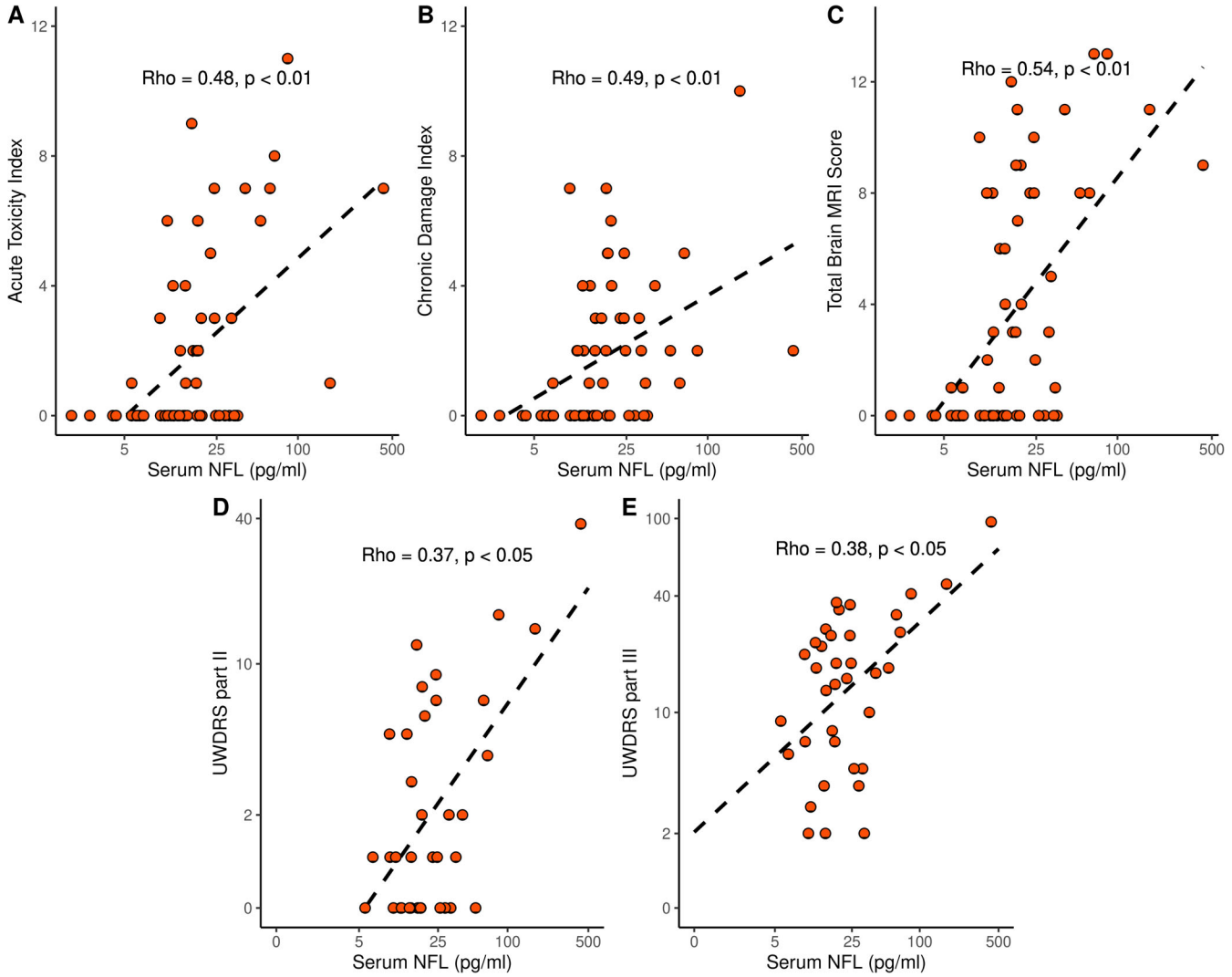

FIG. 1. Relationship between mean serum neurofilament light chain (sNfL) concentrations and brain magnetic resonance imaging (MRI) semiquantitative scale (**A** – acute toxicity score, **B** – chronic damage score, **C** – brain MRI total score; in all 61 patients) and severity of neurological disease scored with Unified Wilson's Disease Rating Score Scale (UWDRS) (**D** – part II and **E** – part III; in 36 neurological patients) analyzed with Spearman's rank correlations. [Color figure can be viewed at wileyonlinelibrary.com]

significant difference in sNfL between hepatic and presymptomatic WD patients.

In detailed analysis of the patients with neurological presentation, patients with predominant tremor (n = 13; mean UWDRS scores part II: 1.6 ± 2.7, part III: 14.3 ± 11.8) tended to have lower mean sNfL (16.2 ± 5.3 pg/mL) compared with neurological patients with the more severe presentation of parkinsonism (n = 12; mean UWDRS scores: part II: 4.0 ± 5.5; part III: 21.4 ± 13.5; sNfL: 44.9 ± 46.6 pg/mL) and with dystonia (mean UWDRS part II: 9.3 ± 12.2; part III: 29.7 ± 27.9; sNfL: 78.7 ± 142.9 pg/mL) ($P = 0.065$ for tremor vs. other neurological presentations).

In our analysis of 36 neurological WD patients, sNfL concentrations correlated positively with the severity of neurological manifestations of WD scored using UWDRS part II ($r = 0.37$; $P < 0.05$) and part III ($r = 0.38$; $P < 0.05$) (Fig. 1). As hepatic and presymptomatic WD patients present with brain MRI pathology,[4] the relationship between sNfL and brain MRI abnormalities was assessed in the total group of 61 patients. sNfL was positively correlated with the semiquantitative MRI scale based on acute toxicity score ($r = 0.48$; $P < 0.01$), chronic damage score ($r = 0.49$; $P < 0.01$), as well as with the total score ($r = 0.54$; $P < 0.01$) (Fig. 1).

There were no significant correlations between sNfL and copper metabolism parameters (serum ceruloplasmin level and urinary copper excretion) as well as duration of disease (data not shown).

Discussion

Results from our study in a large and homogenous drug-naïve group of WD patients indicate that sNfL seems to be an appropriate biomarker of neurological

injury in WD. Mean sNfL was significantly higher in neurological WD patients compared with hepatic and presymptomatic cases. In addition, patients with mild neurological presentation (tremor) tended to have lower sNfL concentrations compared with more severe forms (parkinsonism and dystonia). Positive correlations were seen between sNfL and the severity of neurological disease assessed using both parts of the UWDRS as well as the acute and chronic components of the semiquantitative brain MRI abnormalities scale.

Only one study, by Shribman et al, has previously investigated sNfL in WD patients.[2] This was the first-ever positive study looking for serum biomarkers of brain injury in WD. This study demonstrated that patients with neurological presentation of WD had higher sNfL compared with healthy controls as well as hepatic patients,[27] and led us to extend their observations in a more homogenous group of WD patients (drug-naïve and evaluated in single center by the same physicians), and with additional new tools, namely the brain MRI semiquantitative scale.

As a marker of neuroaxonal destruction involvement, sNfL is not specific for WD, but is elevated in many neurodegenerative and neuroinflammatory disorders.[21-26] Similarly, the scales and markers of hepatic injury used in WD are not specific for WD.[1,2,4,19] However, the concordance of our findings with the WD-specific UWDRS and brain MRI scale suggest that sNfL measures clinically relevant neurodestruction in WD.

Our study has some limitations. We did not analyze NfL from cerebrospinal fluid (CSF); however, there is a large body of evidence that serum and CSF are equivalent markers of neuronal injury.[29] We did not include healthy controls in the analysis and there is a lack of a reference norm for sNfL.

To conclude, we propose that sNfL could be a valuable marker of the severity of neurological WD involvement, complementary to UWDRS and brain MRI. sNfL concentration is a promising tool for monitoring WD patients in everyday clinical practice and clinical trials. ■

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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