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BRIEF COMMUNICATION

Serum neurofilament light chain is increased in hereditary spastic paraplegias

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Abstract

Blood biomarkers are still largely missing in hereditary spastic paraplegias (HSPs). We here explored Neurofilament light chain (NfL) as a biomarker in HSP. Serum NfL was assessed in 96 HSP (63 genetically confirmed), 96 healthy control, and 33 ALS subjects by single molecule array (Simoa). Compared to controls, NfL was increased in HSP ($P < 0.001$), correlating with cross-sectional disease progression ($\rho = 0.28$). Levels were lower than in ALS ($P < 0.001$), allowing to differentiate HSP from ALS (AUC = 0.91). Serum NfL might serve as a biomarker in HSP indicating neuronal damage and, if confirmed longitudinally, disease progression. It might also support differentiating HSP from ALS.

Introduction

Hereditary spastic paraplegias (HSPs) share the hallmark of progressive axonopathy of upper motor neurons, frequently complicated by degeneration of nonpyramidal neuronal systems. Currently, blood biomarkers are still largely missing in HSP. However, biomarkers indicating neuronal damage in HSP are warranted, as disease-modifying treatment options are coming within reach, necessitating objective treatment-outcome parameters. For example, genotype-specific treatment trials have come into reach for HSP subtypes SPG5[1,2] and SPG4,[3] warranting the development of easily accessible peripheral biomarkers.

Blood concentrations of neurofilament light chain (NfL) have recently been demonstrated to serve as biomarker of neuronal damage across several neurodegenerative diseases,[4–6] including amyotrophic lateral sclerosis (ALS).[4,7–10] NfL might hereby be particularly sensitive to axonal destruction of long fiber tracts[11,12] and could possibly reflect particularly upper motor neuron degeneration, as suggested, for example for ALS.[11,13] Thus, NfL might be suited as a peripheral biomarker of neuronal damage in HSP, which is defined by axonal degeneration of upper motor neurons. We here aimed to provide first proof-of-concept that serum NfL might serve as a biomarker of neuronal damage in HSP and explored NfL as a biomarker of disease progression in HSP. Moreover, we hypothesized that NfL might support the diagnostic differentiation of HSP from the more rapidly progressive motor neuron disease ALS. To test these hypotheses, we measured serum levels of NfL in HSP patients, ALS patients, and age-matched healthy controls, using an established single molecule array (Simoa) assay.[14–16]

Methods

Subjects

We recruited a total of 225 subjects from the Department of Neurodegenerative Disorders, Hertie Institute for Clinical Brain Research, University Hospital Tübingen (sampling interval: 2014–2017). Our patient cohorts comprised of consecutive series of 96 HSP patients and 33 ALS patients, all diagnosed according to established criteria.[17,18] The HSP cohort consisted of 63 genetically confirmed patients (65.6%), comprising of patients with SPG4 ($n = 35$), SPG7 ($n = 11$), SPG11 ($n = 5$), SPG5 ($n = 4$), SPG3 ($n = 3$), SPG15 ($n = 2$), SPG10 ($n = 1$), mutations in *ABCD1* ($n = 1$) and *GAN* ($n = 1$), and 33 genetically unconfirmed patients (34.4%). Within all HSP patients, 43 (44.8%) presented with a complicated HSP phenotype according to the Harding criteria.[19] We assessed clinical disease severity with the Spastic Paraplegia Rating Scale (SPRS)[17] and defined cross-sectional disease progression as the cross-sectional quotient of disease severity and disease duration, as established previously.[20] The control cohort comprised of 96 age-matched healthy volunteers. All controls were assessed by neurologists with special expertise in neurodegenerative diseases, ascertaining that none of them had any history or clinical signs of neurodegenerative disease or of any other major neurological disorder. We matched controls to patients using propensity score matching (in the mode "nearest neighbor matching", following: Thoemmes, F. (2012). Propensity score matching in SPSS. arXiv:1201.6385). The university's ethics committee approved the study, the methods were carried out in accordance with the relevant guidelines and regulations and all subjects gave written informed consent prior to participation.

Biomaterial

Serum samples were frozen at −80°C within 1 h after collection, stored in the local biobank and analyzed without any previous thaw-freeze cycle.

Measurements

Serum NfL levels were measured in duplicates with an ultrasensitive Simoa assay, as established previously.[15] Between-run precision was 6.4% (for a concentration of 8.3 pg/mL), 6.1% (for 20.1 pg/mL) and 2.9% (for 97.0 pg/mL), within-run precision was 6.6 % (for samples with a mean concentration of 64 pg/mL).

Statistical Analysis

We used nonparametric procedures throughout the study. Data were reported as median and interquartile range, unless stated otherwise. We tested group effects using Wilcoxon test for matched data and Mann–Whitney test for independent data. To take into consideration the age-dependency of NfL levels, which has been reported previously[15] and was also observed here for both HSP and control subjects (see Appendix S2), we calculated each individual HSP patient's NfL increase as the difference between the individual NfL level of each patient and the NfL level of the a priori age-matched control subject. We then compared these NfL increases between the patient subsets with complicated HSP (cHSP) and pure HSP (pHSP) (Mann–Whitney test, two-sided), and assessed the correlation of the NfL increase with clinical disease progression (Spearman correlation). All analyses were performed with IBM SPSS (Version 24).

Results

NfL as a neuronal damage biomarker in HSPs

To explore the hypothesis that serum NfL might serve as a peripheral neuronal damage biomarker in HSP, we compared serum NfL levels between the overall cohort of HSP patients and age-matched healthy controls (Fig. 1A, Table 1). The values of the NfL levels of our control group (19.7 pg/mL (12.3–26.6), median (interquartile range)) fell into the same range as the values of the NfL levels of control groups measured also by Simoa by other groups,[15,21] corroborating the fact that our controls indeed did not have any neurological disease.

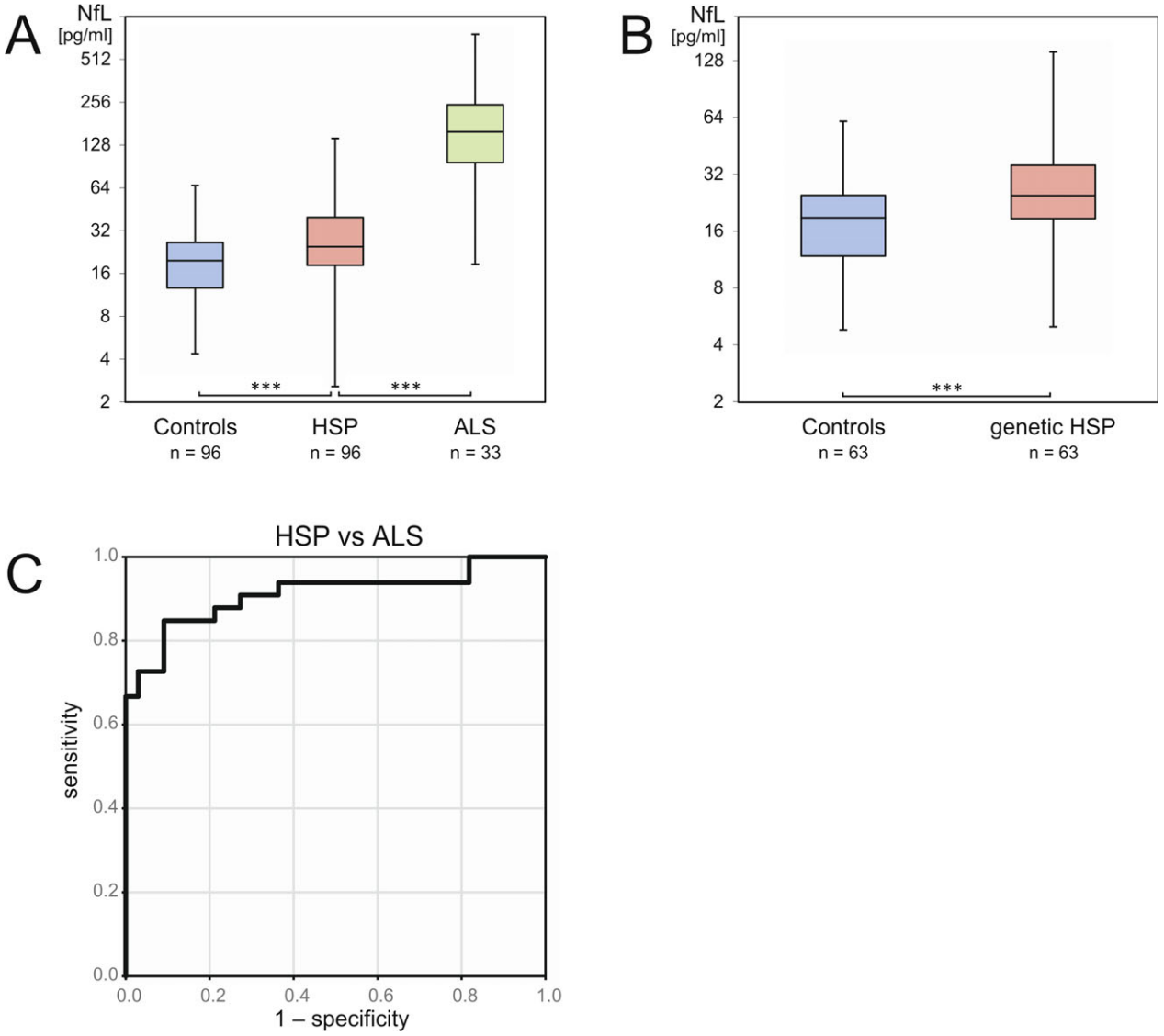

Figure 1. Serum neurofilament light chain (NfL) levels are increased in hereditary spastic paraplegias (HSPs), in an intermediate range still distinguishable from amyotrophic lateral sclerosis (ALS). Panel (A) illustrates serum NfL concentrations (pg/mL) in the overall HSP patient group, their age-matched healthy controls, and a cohort of ALS patients. Panel (B) illustrates NfL levels in the subset of genetically confirmed HSP patients in comparison to their a priori age-matched controls. In the boxplots, central horizontal lines indicate median values, boxes illustrate the ranges between lower and upper quartiles, and error bars represent the full ranges of data. ***$P < 0.001$. Please note the logarithmic scale of the *y*-axis. The receiver operating characteristics (ROC) curve illustrates sensitivity and specificity of serum NfL for differentiating HSP patients from ALS patients (C). The area under the curve (AUC) was used as a parameter to summarize the biomarker performance (AUC = 0.91 (0.84–0.99), mean and 95% confidence interval of the mean).

Table 1. Subject characteristics and neurofilament light chain (NfL) concentrations.

	HSP	Controls	ALS
Sample size (female rate)	96 (41.7%)	96 (42.7%)	33 (33.3%)
Age [years]	51.4 (41.2–55.0)	53.0 (34.5–60.4)	58.4 (52.0–62.4)
Disease duration [years]	15.8 (8.9–29.7)	NA	1.4 (1.0–2.5)
Age of onset [years]	30.0 (11.5–43.5)	NA	56.0 (50.0–61.5)
SPRS score	17.0 (11.0–24.0)	NA	NA
Cross-sectional annual disease progression (SPRS score divided by disease duration in years)	1.1 (0.7–1.7)	NA	NA
Serum NfL [pg/mL]	24.7 (18.1–40.0)	19.7 (12.3–26.6)	158.4 (93.2–246.3)

Disease severity was captured by the Spastic Paraplegia Rating Scale (SPRS).[17] We estimated the annual disease progression from our cross-sectional data by the quotient of each subject's SPRS score and their disease duration. The values of age, disease duration, age of onset, SPRS score, disease progression, and serum NfL levels are reported as median and interquartile range.

Compared to these NfL levels of controls, NfL levels were significantly increased in HSP patients (24.7 pg/mL (18.1–40.0)) ($z = 3.90$, $P < 0.001$, $r = 0.28$, two-sided Wilcoxon test). This NfL increase was confirmed, with comparable effect size, if only the subset of genetically confirmed HSP patients (24.7 pg/mL (18.5–36.4)) was compared to their a priori age-matched controls (18.9 pg/mL (11.6–25.5)) ($z = 3.52$, $P < 0.001$, $r = 0.31$, two-sided Wilcoxon test) (Fig. 1B). This suggests that the NfL increase observed in the overall HSP cohort was not merely caused by putative patients with HSP phenocopy syndromes.

NfL increases correlate with disease progression in HSP

We next performed a preliminary explorative analysis of factors which might drive the NfL increase in HSP patients. The NfL increase correlated significantly with patients' cross-sectional disease progression ($\rho = 0.28$, $P = 0.017$, Spearman correlation, two-sided test), as defined[20] by the quotient of the Spastic Paraplegia Rating Scale[17] (SPRS) score and disease duration in years. The correlation between NfL increase and disease progression was also observed in the subset of genetically confirmed HSP patients as a trend with comparable effect size ($\rho = 0.24$, $P = 0.087$, Spearman correlation, two-sided test). The NfL increase did not differ significantly between patients with pHSP (23.7 pg/mL (18.1–39.5)) and cHSP (29.4 pg/mL (17.3–43.6)) ($U = 979$, $z = 1.18$, $P = 0.240$, $r = 0.12$, Mann–Whitney test).

NfL supports the diagnostic differentiation of HSP from ALS

To test the hypothesis that serum NfL might support the diagnostic differentiation of HSP from ALS, we compared serum NfL levels between HSP and ALS patients (Fig. 1A, Table 1). NfL levels were significantly higher in ALS patients (158.4 pg/mL (93.2–246.3)) than in HSP patients (24.7 pg/mL (18.1–40.0)) ($U = 275$, $z = 7.07$, $P < 0.001$, $r = 0.62$, two-sided Mann–Whitney test). NfL levels might have been confounded by patients' age, which was significantly higher in the ALS cohort (58.4 years (52.0–62.4)) than in the HSP cohort (51.4 years (41.2–55.0)) ($U = 934$, $z = 3.51$, $P < 0.001$, $r = 0.31$). However, comparison of the ALS cohort with an age-matched subset of the HSP cohort confirmed that serum NfL was significantly higher in ALS (158.4 pg/mL (93.2–246.3)) than in HSP (25.8 pg/mL (19.7–39.8)) ($z = 4.91$, $P < 0.001$, $r = 0.60$, two-sided Wilcoxon test, Appendix S1), also if controlled for age. The receiver operating characteristic indicated that NfL hereby differentiated ALS patients and HSP patients with high accuracy (AUC = 0.91 (0.84–0.99), $P < 0.001$) (Fig. 1C).

Discussion

We here present the first systematic study on NfL levels in HSP, demonstrating that serum NfL is increased in HSPs. A previous study on NfL in motor neuron diseases had already included some HSP patients, yet it included only eight genetically unconfirmed HSP patients, assessed only NfL in CSF, and did not statistically test for differences to healthy controls.[8] Our study recruited a much larger HSP cohort, assessed serum levels, and included a large genetically confirmed HSP cohort. The observed increase in genetically confirmed HSP patients suggests that this NfL increase in the overall HSP cohort was not merely caused by putative patients with HSP phenocopy syndromes. These results support the notion that blood NfL provides a cross-disease biomarker in several neurodegenerative diseases,[4–6] extending this notion by demonstrating that blood NfL increases occur also in degenerative diseases primarily implicating upper motor neurons. Given the established relation between neuronal

damage and blood NfL increases,[4,6,15,16] NfL could serve as an indicator of neuronal damage in HSP. It might hereby be particularly sensitive to axonal destruction of long fiber tracts[11,12] and might reflect particularly upper motor neuron degeneration, as previously suggested in ALS.[11,13] Given that HSP is defined by axonal degeneration of upper motor neurons, NfL is conceptually well suited as a biomarker of neuronal damage in HSP. Quantitatively, the blood NfL increase in HSP was in the same range as measured also by Simoa in other neurological diseases, including inherited peripheral neuropathies[22] and severe parkinsonian disorders like progressive supranuclear palsy (PSP),[23] supporting the relevance of the NfL increase observed in HSP.

The finding of an increase of a biomarker is the necessary prerequisite for a biomarker to serve as disease progression and/or therapy response marker within the intraindividual disease course. Indeed, we here found that the NfL increase in the HSP cohort correlated with patients' cross-sectional disease progression, as defined[20] by the quotient of disease severity (i.e. SPRS score) and disease duration. This provides first indications that serum NfL might also serve as a biomarker of disease progression in HSP. If longitudinal studies confirm our cross-sectional findings, NfL might help to stratify onset and progression of neurodegeneration in natural history studies of HSP, possibly even in the preclinical phase of genetic HSP, and to document the response to therapy in future genotype-specific treatment studies. Hence, further longitudinal and genotype-specific research is warranted and of high interest to investigate NfL as a biomarker of disease severity and progression in HSP, ideally including also investigations of presymptomatic HSP mutation carriers. While this study was not designed to dissociate the effects of disease progression and multisystemic degeneration on NfL levels, future studies may address the differential effects by correlating NfL levels with fine grained clinical parameters and imaging parameters of both disease progression and involvement of multiple neuronal systems.

Moreover, our results suggest that serum NfL might serve as a supportive diagnostic biomarker for differentiating HSP from one specific other motor neuron disease, namely the more rapidly progressive motor neuron disease ALS. Such differential biomarkers within the group of motor neuron diseases would be valuable as these two different types of degenerative motor neuron diseases differ considerably in terms of their prognosis. The diagnostic accuracy of serum NfL for this purpose was high (AUC = 0.91 (0.84–0.99)), and no overlap between the respective 25th and 75th percentile of the two disease groups was observed. To further confirm serum NfL as a biomarker for the differentiation of HSP from ALS, prospective studies are warranted in view of the difference in the underlying dynamics of motor neuron degeneration, particularly in those patients with progressive upper motor neuron involvement who do not yet meet ALS criteria. Quantitatively, NfL levels in HSP were in the intermediate range between the levels of controls and ALS subjects. They thus do not serve as a diagnostic biomarker differentiating HSP in general from controls (Appendix S3). The lesser NfL increase in HSP compared to ALS might reflect the less widespread neurodegeneration in HSP and particularly the slower progression of the disease and its corresponding underlying neurodegeneration. Differences in NfL levels and NfL dynamics might reflect differences in the underlying neurodegenerative process and in the NfL turnover not only between different motor neuron diseases (e.g., between HSP and ALS), but also within one and the same motor neuron disease depending on the disease stage. That is, even if the underlying process in a motor neuron disease is an inherently progressive neurodegenerative process, the associated NfL dynamics might be nonlinear, for example, with a boost of NfL levels around the time of clinical manifestation, and a decrease afterwards, as suggested for ALS.[9]

In sum, our study provides first preliminary proof-of-concept that NfL might serve as a biomarker for several different purposes in HSPs. Particularly, serum NfL might serve as biomarker indicating neuronal damage in HSP and as diagnostic biomarker supporting the differentiation of HSP from ALS. The availability of such a blood-based, easily accessible peripheral biomarker is of considerable practical relevance not only for diagnostic purposes, but also for longitudinal sampling in future registry studies and clinical trials.

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Conflict of Interest

JK's institution (University Hospital Basel) received in the last 3 years and used exclusively for research support: consulting fees from Novartis, Protagen AG; speaker fees from the Swiss MS Society, Biogen, Novartis, Roche, Genzyme; travel expenses from Merck Serono, Novartis; grants from ECTRIMS Research Fellowship Programme, University of Basel, Swiss MS Society, Swiss National Research Foundation (320030_160221), Bayer (Switzerland) AG, Genzyme, Novartis. MS received speaker's honoraria and research support from Actelion Pharmaceuticals, unrelated to the current project and manuscript. The other authors declare no financial disclosures.

Data Availability

The datasets analyzed in this study are available from the corresponding author on reasonable request.

Author Contributions

CW: design and conceptualization of the study, acquisition of data (patient recruitment, patient assessment, blood sampling), analysis of the data, drafting and revision of the manuscript.

TR: acquisition of data (patient recruitment, patient assessment, blood sampling), revision of the manuscript.

MZ: acquisition of data (patient recruitment, patient assessment, blood sampling), revision of the manuscript.

KB: acquisition of data (patient recruitment, patient assessment, blood sampling), revision of the manuscript.

LS: acquisition of data (patient recruitment, patient assessment, blood sampling), revision of the manuscript.

JK: acquisition and analysis of data (blood samples), design and conceptualization of measurements, revision of the manuscript.

RS: design and conceptualization of the study, acquisition of data (patient recruitment, patient assessment, blood sampling), revision of the manuscript.

MS: design and conceptualization of the study, acquisition of data (patient recruitment, patient assessment, blood sampling), drafting and revision of the manuscript.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article:

Appendix S1. Comparison of serum NfL levels between ALS patients and age-matched HSP patients.
Appendix S2. Age-dependency of NfL levels in HSP, ALS and control subjects.
Appendix S3. Receiver operating characteristics of serum NfL for differentiating HSP patients from controls.

