Serum Neurofilament Light Chain Concentration Correlates with Infarct Volume but Not Prognosis in Acute Ischemic Stroke

Juha Onatsu, MD,* Ritva Vanninen, MD, PhD,†,|| Pekka Jäkälä, MD, PhD,*,‡ Pirjo Mustonen, MD, PhD,§ Kari Pulkki, MD, PhD,¶,# Miika Korhonen, MD,† Marja Hedman, MD, PhD,† Henrik Zetterberg, MD, PhD,**,††,‡‡,§§ Kaj Blennow, MD, PhD,**,†† Kina Höglund, MD, PhD,**,†† Sanna-Kaisa Herukka, MD, PhD,*,‡ and Mikko Taina, MD, PhD†

Background and Purpose: We studied serum neurofilaments diagnostic value in patients with acute ischemic stroke (AIS) or TIA and evaluated any correlation with symptom severity, cerebral infarction volume, aetiology, and clinical outcome. *Methods:* One hundred and thirty-six patients (101 with AIS, and 35 with TIA) were included. Acute-phase serum neurofilament light chain (sNfL) was analyzed with a novel ultrasensitive single molecule array (Simoa). Cerebral infarction volume was measured from brain computed tomography in the subacute phase (>2 days). Stroke aetiology was defined by trial of ORG 10172 in acute stroke treatment classification, severity by National Institute of Health stroke scale (NIHSS) and the degree of disability by the Modified Rankin Scale (mRS) after 90 days. *Results:* sNfL was markedly higher in patients with AIS (89.5 pg/mL [IQR: 44.7-195.3]) than with TIA (25.2 pg/mL [IQR: 14.6-48.0]), $P = <.001$), also after adjusting for age, NIHSS, and stroke volume ($P = .003$). In receiver operating characteristic analysis, sNfL concentration greater than or equal to 49 pg/mL proved to be the best cut-off value to differentiate between patients with stroke and those with TIA (sensitivity of 73% and specificity of 80%). sNfL concentration significantly correlated with cerebral infarction volume ($r = .413$, $P = <.001$), this association remained significant after adjusting for established predictors ($P = .019$). Patients with AIS due to cardioembolism or large artery atherosclerosis had the highest sNfL concentrations. NIHSS on admission ($r = .343$, $P = <.001$) and mRS scores after 3 months ($r = .306$, $P = .004$) correlated with sNfL concentration, however functional outcome 3 months after stroke was not associated with sNfL after adjusting for potential confounders. *Conclusions:* Cases with stroke were distinguishable from those with TIA following the determination of sNfL in the blood samples. The presence and amount of axonal damage estimated by sNfL correlated with the final cerebral infarction volume but was not predictive of degree of disability.

From the *Department of Neurology, NeuroCenter, Kuopio University Hospital, Kuopio, Finland; †Department of Clinical Radiology, Kuopio University Hospital, Kuopio, Finland; ‡Unit of Neurology, Institute of Clinical Medicine, University of Eastern Finland, Kuopio, Finland; §Department of Cardiology, Keski-Suomi Central Hospital, Jyväskylä, Finland; ||Department of Clinical Radiology, University of Eastern Finland, Kuopio Finland; ¶Department of Clinical Radiology and Clinical Chemistry, Kuopio, Finland; #Eastern Finland Laboratory Center and Department of Clinical Chemistry, University of Eastern Finland, Kuopio, Finland; **Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden; ††Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden; ‡‡Department of Molecular Neuroscience, UCL Institute of Neurology, London, United Kingdom; and §§UK Dementia Research Institute, London, United Kingdom.

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Address correspondence to Juha Onatsu MD, Department of Neurology, NeuroCenter, Kuopio University Hospital, P.O.Box 100, Kuopio 70029, Finland. E-mail address: Juha.Onatsu@kuh.fi.

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Introduction

Neurofilaments, major cytoskeletal constituents of neuronal cells, are released into the cerebrospinal fluid (CSF) and blood during neuronal injury or degeneration, and thus constitute a promising tool with which to evaluate axonal damage in various neurological conditions.[1]

Neurofilament light chain (NfL) is a central nervous system-enriched protein, abundantly expressed in the long myelinated subcortical white matter axons.[2] Together with medium and heavy chain neurofilament subunits, NfL is a scaffolding protein of the neural cytoskeleton, with important roles in axonal and dendritic branching, and growth.[3] Until recently, the measurement of NfL was possible only from CSF, where concentrations are up to 50 times higher than those measured in blood.[4,5]

With the implementation of a new ultrasensitive single molecule array (Simoa) assay for NfL, it has become possible to measure the protein reliably in serum and plasma. Plasma or serum NfL correlates with CSF NfL concentration[4,6,7] and virtually the same information can be derived from these 2 biofluids. In the future, serum NfL (sNfL) determination may provide a valuable complement in the identification of neuronal degradation or damage, and could potentially be utilized clinically for diagnostic and monitoring purposes in TBI and neurodegenerative diseases[8-10] Thus far the literature on neurofilaments in patients with AIS is quite scarce and sNfL analysis is based mostly on previous methods.[5,11-13] Although just recently it has been shown that sNfL levels measured with Simoa method are increased in patients with MRI-confirmed recent small subcortical infarcts compared to healthy controls[14] and are related to small vessel disease (SVD) burden.[15] Moreover, a recently published study showed also that sNfL levels 7 days poststroke independently predicted Modified Rankin Scale (mRS) scores 3 months after stroke.[16]

While a careful neurological examination and modern diagnostic imaging are the cornerstones of diagnostic and treatment decisions for acute ischemic stroke, a new blood-based biomarker may improve diagnostics and prediction of outcome of patients with stroke. Therefore, we studied the diagnostic value of acute phase sNfL concentration analyzed with Simoa method to differentiate AIS or TIA and evaluated any correlation with clinical severity of symptoms, cerebral infarction volumes, aetiology, and prognosis of stroke.

Material and Methods

The current study was conducted according to the Declaration of Helsinki and the protocol was reviewed and approved by the Kuopio University Hospital Research Ethics Board (No: 82/2004). Written informed consent was obtained from the patient or the patient's legally authorized representative.

This is a prospective single-center observational cohort study nested within EmbodeteCT Eastern Finland Study. Patients who were admitted to our University Hospital because of AIS or TIA with unknown or suspected cardioembolic aetiology, but without known atrial fibrillation (AF), were evaluated as candidates for this EmbodeteCT Eastern Finland Study (3069 patients during the study period March 2005 to November 2009).[17-19] The inclusion criteria were[1] AIS or TIA with suspicion of cardiogenic source by a neurologist or[2] age less than 50 years and stroke/TIA of undetermined origin, because cardioembolism is the most frequent etiology in these patients.[20] The suspicion of a cardiogenic source was based on the patients' history of prior heart disease or signs and symptoms typical of cardiogenic embolism, such as simultaneous or sequential strokes in different arterial territories, hemorrhagic transformation, simultaneous emboli in other organs, decreased consciousness at stroke onset, isolated aphasia, or a visual-field defect.[21] Exclusion criteria were: AF on electrocardiogram on admittance, signs, and symptoms indicating large artery atherosclerosis, small vessel occlusion, or a hypercoagulable state. Altogether 162 patients were recruited. Three of them refused to participate after giving informed consent and 23 did not provide a venous blood serum sample for sNfL analyses.

Diagnostic investigations included a combined examination of the brain, heart, aorta, and cervicocranial arteries with computed tomography and the collection of blood serum samples within 1-12 days after symptom onset during randomization. Brain CT was controlled in the subacute phase (>2 days). Twenty-four patients also underwent magnetic resonance imaging (MRI) of the brain, because infarction was not found on repeated CT imaging. AIS was defined as neurological dysfunction lasting greater than 24 hours with an acute lesion on CT or MRI and no intracranial bleeding. TIA was defined as neurological dysfunction lasting less than 24 hours and caused by focal brain ischemia without an acute lesion on CT or MRI (a modified tissue-based definition).[22] Stroke/TIA aetiology was determined according to the trial of ORG 10172 in acute stroke treatment (TOAST) classification,[23] modified by recommendations from the Association of Echocardiography for defining the cardiac source of embolism. The clinical outcome was evaluated by a trained stroke neurologist at the follow-up visit at 3 months after stroke in outpatient clinic and a blood serum sample was collected again. The control group was not available.

The 2 primary outcome measures were an assessment of the clinical outcome using the mRS, with scores ranging from 0 (fully independent) to 6 (dead)

at 90 days; and determination of the volume of cerebral infarction.

Imaging

The patients underwent contrast-enhanced cervicocranial arteries with computed tomography scan (Somatom Sensation 16 or Somatom Definition AS; Siemens Medical Solutions, Forchheim, Germany) of the aortic arch, cervical arteries, and intracranial arteries, immediately followed by scanning of the ascending aorta and heart. The volume of the cerebral infarction was measured from 4.5 to 5 mm transversal slices of the brain CT in the subacute phase and calculated by using Simpson's rule.[24] If infarction was not found on repeated CT imaging, MRI of the brain was performed (1.5T, Siemens Avanto, Erlanger, Germany).

Measurement of Neurofilament in Serum

Venipuncture for the acute phase blood samples was performed approximately 63.8 ± 50.1 hours after hospital admission; range 1-12 days. The gel separator tubes were centrifuged 20-60 minutes after sampling. The serum was separated, aliquoted, and stored at −80 °C pending biochemical analysis.

The serum NF-L concentration was measured using the Simoa platform (Quanterix, Lexington), a magnetic bead-based digital ELISA that allows the detection of proteins at subfemtomolar concentrations. Magnetic beads (Quanterix) were conjugated with capture antibody (UD1 (UmanDiagnostics, Umeå, Sweden) at .3 mg/mL, according to the bead supplier's conjugation protocol. Prior to each run, serum samples were diluted 10 fold. An NfL calibrator (UmanDiagnostics) was series diluted and biotin-labeled detection antibody UD2 (UmanDiagnostics) was diluted to .1 μg/mL in phosphate-buffered saline, .1% Tween 20, 2% of bovine serum albumin, and 10 μg/mL TRU Block (Meridian Life Science, Inc., Memphis). For each determination, 400,000 conjugated beads were washed and resuspended in 100 μL of the serum sample or calibrator, and 20 μL detection antibody was added. After a 30-minute incubation, the beads were washed and resuspended in 100 μL streptavidin-conjugated â-galactosidase (Quanterix) at 150 pM, diluted in SBG Diluent (Quanterix). After 5 minutes of incubation, the beads were washed and transferred together with resorufin-D-galactopyranoside substrate (Quanterix) to an array of wells, each only large enough to contain 1 bead. The array was imaged with a charge-coupled device camera imaging system and the images were used to differentiate between empty beads and analyte-bound beads, providing a signal expressed as average enzyme per bead (AEB). To extract concentrations from AEBs, each sample AEB was fitted to a 4-parameter logistic curve, plotted from the known concentrations of the NfL calibrator, run in parallel with the samples. The calibrator points were run in triplicates and the samples were run in duplicates. The samples from each patient were measured within the same run. The limit of detection for the NfL assay was .29 pg/mL and the lower limit of quantification was 2.7 pg/mL, when compensated for a 4-fold sample dilution. Limit of detection and lower limit of quantification were determined by a mean blank signal +3 standard deviation (SD) and +10 SD, respectively. The average intra-assay duplicate coefficient of variation for the samples was 7% (SD 9%). The samples were analyzed using the same batch of reagents by board-certified laboratory technicians who were blinded to clinical information.

Statistical Analysis

Continuous variables are presented as means or medians (interquartile range [IQR]), and categorical variables as absolute counts and percentages. sNfL values were $\log_{10}$ transformed in order to achieve a normal distribution and these were used for all analyses. Student's *t* test was used to compare normally distributed continuous variables and the Mann-Whitney *U* test to analyze the non-normally distributed continuous variables. Two-tailed Pearson correlation coefficient and Spearman`s rank correlation coefficient were used to investigate the association between continuous normally distributed and non-normally distributed variables, respectively.

The chi-square test with Pearson's or Fisher's correlation was used to compare nominal variables. Receiver operating characteristic (ROC) curves were used to determine the optimal sNfL thresholds for discriminating between AIS and TIA. To assess the independent association of sNfL with dichotomous endpoint (ie, AIS versus TIA), we computed multivariate logistic regression models adjusted for established predictors. Ordinal regression models were used to test the association between sNfL and 3-month outcome (ie, increased mRS category). Analysis of variance and linear regression models were used to compare sNfL levels in different etiological groups. All regression models were adjusted by age, NIHSS, and brain infarct volume. Testing was 2-sided and *P* values less than .05 were considered to be statistical significant. The data were analyzed using IBB SPSS for Windows, version 19.0 (SPSS Inc., Chicago).

Results

The clinical background characteristics and the sNfL concentrations for the relevant patient subgroups are presented in Table 1. Most of the study participants were males (68%). The median sNfL concentration was (65.7 pg/mL [IQR: 25.8-162.2]) in the acute phase (n = 136). Both the acute phase and follow-up sNfL samples were available for 41 patients. Significant differences were not observed between the acute phase sNfL (36.0 pg/mL [IQR: 22.6-165.4]) and 3-month follow-up sNfL (54.1 pg/mL [IQR: 28.2-106.7]) levels (*P*= .170). sNfL

Table 1. *Clinical characteristics of the study population and correlation with serum neurofilament light chain concentration*

Variable	Unit	Spearman's correlation coefficient or serum NF-L in pg/mL in acute phase (median ± IQR)	*P*
Age (N = 136)	Years	.358	<.001
Sex (N = 136)	Males (N = 93, 68%)	60.5 (32.6-153)	.89
	Females (N = 43, 32%)	72.4 (24.2-165.9)	
Stroke or TIA (N = 136)	Stroke (N = 101, 74%)	89.5 (44.7-195.3)	<.001
	TIA (N = 35, 26%)	25.2 (14.6-48.0)	
Hypertension (N = 136)	Yes (N = 79, 58%)	68.0 (31.4-142.1)	.43
	No (N = 57, 42%)	59.4 (24.6-171.1)	
Body mass index (N = 136)	kg/m^2	−.43	.62
Body surface area (N = 136)	m^2	−0.004	.96
Dyslipidemia (N = 136)	Yes (N = 54, 40%)	50.2 (24.4-104.0)	.26
	No (N = 82, 60%)	68.4 (28.7-187.2)	
Diabetes (N = 136)	Yes (N = 21, 15%)	82.4 (63.7-142.8)	.07
	No (N = 115, 85%)	53.5 (23.6-163.8)	
Smoker (N = 136)	Yes (N = 34, 25%)	44.8 (22.8-109.1)	.09
	No (N = 102, 75%)	72.7 (27.1-177.4)	
Prior stroke (N = 136)	Yes (N = 25, 18%)	85.0 (42.7-202.2)	.10
	No (N = 111, 82%)	54.5 (24.6-155.8)	
Prior myocardial infarction (N = 136)	Yes (N = 19, 14%)	112.5 (41.6-205.2)	.11
	No (N = 117, 86%)	60.5 (24.4-156.7)	

Abbreviations: IQR, interquartile range; SD, standard deviation; sNfL, serum neurofilament light chain concentration; TIA, transient ischemic attack.

concentration did not vary between the sexes. However, age significantly correlated with the sNfL levels with higher concentrations in the older patients (r = .358, *P*= <.001). Altogether, 101 (74%) patients were diagnosed with stroke and 35 (26%) were found to have TIA. The mean NIHSS was 3.7 on admission. sNfL was significantly higher (89.5 pg/mL [IQR: 44.7-195.3]) in stroke patients, compared to that with TIA (25.2 pg/mL [IQR: 14.6-48.0]), *P*= <.001). Eleven patients (8%) were treated with recombinant tissue plasminogen activator. The sNfL levels in this small group did not differ from those in the other groups (*P*= .35).

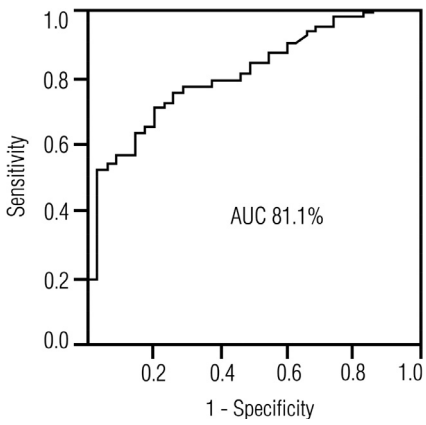

Figure 1. *Receiver operating characteristic curve for the neurofilament light chain in blood.*

Abbreviation: AUC, area under curve.

Stroke aetiology, assessed using the 5-class TOAST classification, was associated with sNfL concentrations in the acute phase in univariate analysis. Patients diagnosed with cardioembolism had higher sNfL concentrations than the other etiological groups (*P*= .017). In addition, sNfL levels in the cryptogenic stroke group differed significantly from those in the SVD (*P*= .042) group, (Fig 2), however there were not statistical significance after adjustment with age, NIHSS, and stroke volume. Infarct volumes, mRS scores after 3 months, and sNfL concentrations in relation to the different stroke aetiology are shown in Table 2.

The volume of cerebral infarction in head CT in the subacute phase significantly correlated with the sNfL concentration (r = .413, *P* = <.001), (Fig 3). Patients without infarction on CT imaging (n = 58) had sNfL concentrations of (40.9 pg/mL [IQR: 18.3-83.9]) while those with a detectable infarction on CT (n = 78) had concentrations of (87.3 pg/mL [IQR: 46.1-187.2]) (*P*= <.001). MRI of the brain was performed for 24 patients of the 58 patients who lacked visible infarction on CT imaging. There was no difference between the 12 patients without infarction on MRI (25.1pg/mL [IQR:13.3-49.1]) versus the 12 patients with infarction (24.8 1pg/mL [IQR:16.7-81.5]; *P*= .60). When a comparison was made of the sNfL consentration levels in patient with lesions on MRI or CT (n = 124) and that for the

Table 2. *Infarction volumes, Modified Rankin Scale scores after 3 months and serum neurofilament light chain concentrations on admission, according to different stroke aetiology*

	Infaction volume (mm^3)		mRS score after 3 months		sNfL concentration	
Stroke aetiology	n	Median(IQR)	n	Mean ± SD	n	Median(IQR)
Cryptogenic stroke	73	906.7(0-9349.5)	48	.54 ± .85	73	47.5(21.7-160.2)
Large artery atherosclerosis	11	915.8(-2846.7)	7	1.86 ± 2.27	11	76.9(48.7-230.4)
Cardiogenic stroke	22	17924.4(0-28018.9)	20	.80 ± 1.28	22	113.5(63.3-201.1)
Large artery atherosclerosis and cardiogenic stroke	10	0(0-10946.9)	8	.75 ± 1.04	10	53.7(29.3-125.3)
Small vessel disease	10	298.0(0-650.4)	6	.33 ± .82	10	37.1(15.5-73.4)

Abbreviations: IQR, interquartile range; mRS, Modified Rankin Scale; SD, standard deviation, sNfL; serum neurofilament light chain concentration.

12 patients without infarction, a statistically significant difference was found (*P*= <.010).

An evaluation of elevated sNfL levels was performed to separate stroke from TIA using ROC analysis, (Fig 1). The highest sensitivity and specificity value required to make a distinction between stroke and TIA was obtained using a sNfL cut-off point 49.35 pg/mL. The area under the ROC curve was 81%. Sensitivity was 73%, specificity 80%, and the positive/negative predictive values 91% and 49%, respectively, with overall accuracy of 74%. NIHSS score at admission correlated significantly with sNfL concentration (r = .343, *P*= <.001).

The clinical outcome measure, 3-month follow-up mRS score, was available from only 89 (65%) patients. The sNfL concentration (r = .306, *P*= .004) and cerebral infarction volumes (r = .413, *P*= <.001) were correlated significantly with the mRS scores. However sNfL was not alone predictive for poor outcome after adjustment for confounding factors.

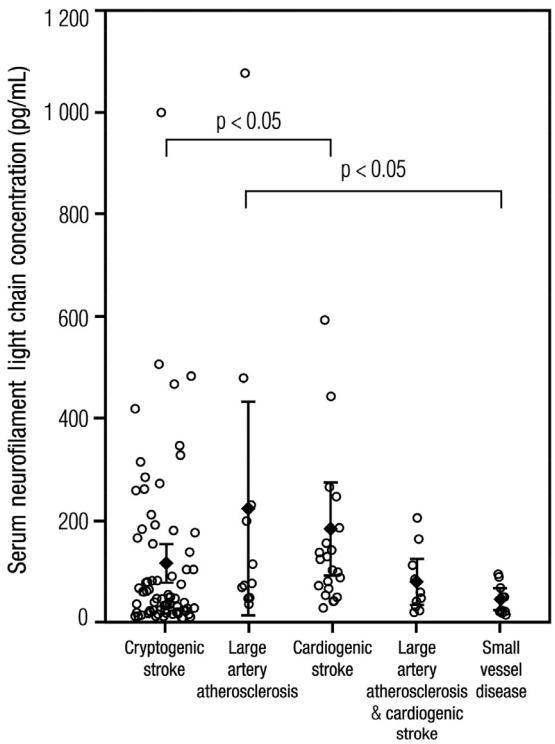

Figure 2. *Comparison of the mean serum neurofilament light chain on admission, according to the trial of ORG 10172 classification.*

Significant differences were found between cryptogenic strokes and cardioembolic strokes (P= <.05) *and large artery atherosclerosis and small vessel disease* (P= <.05), *respectively. However statistical significance was lost in different etiological groups after adjustment for confounding factors.*

Discussion

In the current study, sNfL levels were analyzed and quantified using a novel ultrasensitive technique in a cohort of AIS and TIA patients with different aetiologies. This is the first study in which blood samples of stroke and TIA patients were assessed using the Simoa platform, which is by far the most sensitive method to measure NfL.[25] It also facilitates the measurements of very low concentrations in blood samples. It was shown in a recent study that the results obtained by Simoa platform were more strongly correlated between the paired CSF and

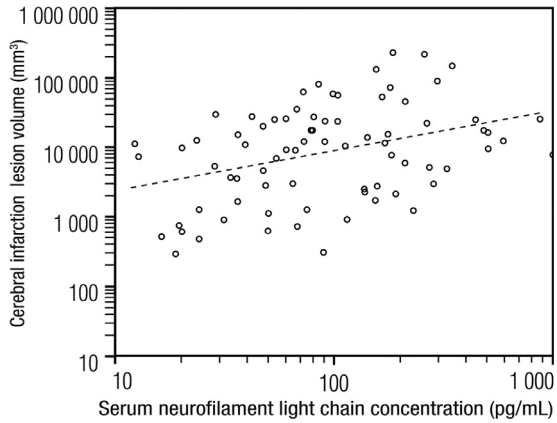

Figure 3. *Natural logarithmic correlation of infarction volume with serum neurofilament light chain concentration levels in stroke and transient ischemic attack patients on admission.*

A statistically significant positive correlation (Spearman r = .413, P= *<.001) was found and it remained after adjusting for age, NIHSS, and sex.*

serum samples compared to commercial ELISA.[6] The objective of the current study was to assess sNfL in patients with ischemic stroke. Using the blood-based method described herein, it was demonstrated that sNfL increased in patients with stroke compared to that in patients with TIA, in parallel with the findings of a previous study in which it was shown that NfL in CSF was elevated in patients with acute ischemic stroke.[26,7] We found a clear correlations between sNfL levels and stroke severity measured with NIHSS, infarct volume in CT, and clinical outcome after 3 months verified with mRS, although the 3-month outcome was not associated, after adjusting for established predictors, with sNfL levels on admission. Acute phase sNfL was capable in distinguishing between TIA from stroke. In addition, sNfL concentrations were significantly higher in patients, who had had a stroke due to large artery atherosclerosis or cardioembolic aetiology, compared to those in patients with cryptogenic stroke or SVD aetiology.

NfL levels were shown to be elevated in patients with AIS compared to those with TIA or in healthy controls in 4 recent studies in which serum neurofilament subunits [NfL, neurofilament heavy, and phosphorylated neurofilament heavy subunits] were measured using ELISA and mass selective detection.[5,11-13] Importantly, in our study, sNfL could be used to differentiate between stroke versus TIA patients with higher accuracy compared to recently published study, in which an area under the curve of sNfL was .64.[13] This finding strengthens the conception, that Simoa platform is the most sensitive method to measure sNfL also in the blood samples and sNfL levels may be considered a measure of structural brain lesion, in addition to CT or MRI. Accordingly, in a study analyzing sNfL levels with Simoa method showed higher sNfL levels in patients with MRI-confirmed recent small subcortical infarcts compared to healthy controls.[14]

We found, that patients with AIS had 3.5-fold higher sNfL levels compared with patients with TIA, whereas 2 other studies reported 1.7 and 6.6-fold difference, respectively.[12,13] However sNfL was assessed in different time points in those studies and with a different methodology. In the study of De Marchis et al the median time interval between AIS onset and the first blood sample was much shorter (2.8 hours) compared to that of ours (63.8 hours) or up to 30 days in a study on patients with nontraumatic cervical artery dissection and AIS.[12] Further, sNfL levels in our cohort tended to be higher at 3-month follow-up visit compared to those assessed on acute phase. Likewise, a recent study showed that sNfL levels remained increased at the 3-month follow-up but returned to normal at 15 months after active SVD.[14] These results might reflect a sustained release of NfL molecules first to CSF and then into the blood, where they can be quantified. Alternatively, the late rise of sNfL might reflect ongoing axonal degeneration due to poststroke immune and inflammatory processes.[27,28]

A significant correlation between NIHSS and sNfL levels has been showed in several studies[12,13,15,16] and in a study in which pNf-H was analyzed in 54 patients, a correlation was shown also between NIHSS and infarction volume measured using MRI in 17 subjects.[11] Our results in a larger patient population were congruent with these preliminary findings and is a first study, which confirmed a clear correlation between sNfL, NIHSS, and infarction volume in CT imaging and this association remained also after adjustment for confounders. This finding contrast with a previous cohort study, which lacked association between infarct size on MR-DWI and sNfL levels on admission.[13] However, measurement of infarct size was semiquantitative and sNfL assessed with a different method in blood drawn as early as 24 hours after symptom onset

Similarly, a recent study with larger patient population,[13] we found a significant correlation between sNfL and mRS after 3 months, but this association was not significant after correction for age, NIHSS, and infarct size. However, a newly published study showed also that sNfL levels 7 days poststroke independently predicted mRS scores 3 months after stroke.[16] sNfL levels in different stroke etiology groups, using TOAST criteria, have not been investigated in earlier studies. In univariate analysis we found the highest sNfL levels in the large artery atherosclerosis and cardioembolic groups. In addition sNfL levels varied between cardioembolic and cryptogenic stroke groups, supporting causes other than emboli in our cryptogenic stroke cohort. Again, the associations were not significant after adjustment for confounders. The low sNfL concentration in the SVD etiological group supports the hypothesis, that sNfL reflects the damage in white matter, because lacunar strokes are smallest in volume. Accordingly, when investigating pNF-H levels in serum and using Oxfordshire stroke classification Singh et al found that serum pNF-H was lowest in lacunar strokes and highest in the total anterior circulation group, leading to larger infarcts.[11]

Limitations

The main limitation to the current study is that primarily the EmbodeteCT study focused on patients with suspected cardioembolic AIS/TIA and excluded patients with known AF. Exclusion criteria were also symptoms indicating large-artery atherosclerosis, small-vessel occlusion, and hypercoagulable states; hence the study included only a small subpopulation of all patients with stroke/TIA. This reduces generalizability of these results to all patients with stroke.

We were able to measure sNfL at 2 time points only; on admission and at 3-months follow-up visit. Thus, we could not evaluate the time course of the sNfL level changes in our stroke patients. Furthermore, we calculated the ischemic infarction volumes from CT imaging

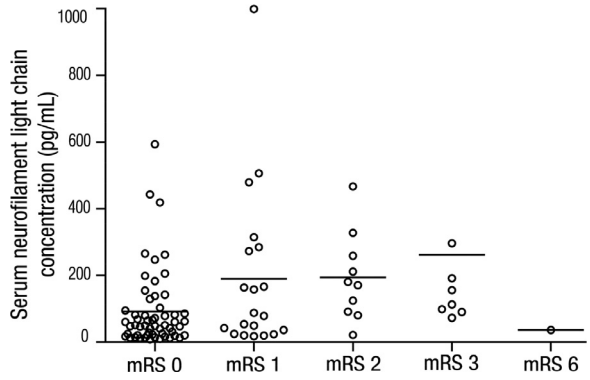

Figure 4. *Serum neurofilament light chain concentrations in patient groups with different Modified Ranking Scale results after 3 months of stroke or transient ischemic attack onset.*

Abbreviation: mRS, Modified Rankin Scale.

The mean serum neurofilament light chain concentration was significantly higher in those patients whose stroke symptoms were more severe after 3 months (P= <.001). Statistical significance was lost after adjusting for potential confounders.

instead of from diffusion- weighted MRI. Finally, we could not assess clinical outcome with mRS after 3 months in all patients owing to inexact or missing data.

Conclusion

A recently developed ultrasensitive ELISA allows the quantification of NfL in serum samples. sNfL concentrations drawn approximately two and half days after stroke onset were capable in distinguishing between AIS and TIA patients, demonstrating reasonably high sensitivity and specificity. The sNfL concentrations were elevated in line with stroke severity, assessed either clinically or from subsequent CT imaging. Furthermore, they were predictive of clinical outcome after 3 months, although statistical significance was lost after adjustment for confounding predictors. Thus, the independent additional predictive value of sNfL concentrations should be investigated further in multivariable models (Fig 4).

Authors' Contributions

J.O., P.J., M.H., and RV recruited patients and analyzed clinical, radiological, and cardiological data to the Embodect Eastern Finland study.

H.Z., K.B., K.H., P.M., K.P., and S.H. were involved in assay development, sample processing, and analysis.

J.O., M.T., and R.V. drafted the initial version and figures and M.T. and J.O. performed the statistical analysis.

All authors contributed to reviewing and editing the manuscript.

Disclosures

H.Z. and K.B. are cofounders of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg. H.Z. has served on the advisory boards of Roche Diagnostics, Eli Lilly, and Pharmasum Therapeutics. K.B. has served on the advisory boards or as a consultant for Alzheon, Eli Lilly, Fujirebio Europe, IBL International, Pfizer, and Roche Diagnostics. J.O., R.V., P.J., P.M., K.P., M.K., M.H., K.H., S.H., and M.T. have nothing to declare in regard to this study.

References

1. Khalil M, Teunissen CE, Otto M, et al. Neurofilaments as biomarkers in neurological disorders. Nat Rev Neurol 2018;14:577-589.
2. Zetterberg H, Smith DH, Blennow K. Biomarkers of mild traumatic brain injury in cerebrospinal fluid and blood. Nat Rev Neurol. 2013;9:201-210.
3. Lepinoux-Chambaud C, Eyer J. Review on intermediate filaments of the nervous system and their pathological alterations. Histochem Cell Biol 2013;140:13-22.
4. Gisslen M, Price RW, Andreasson U, et al. Plasma concentration of the neurofilament light protein (NFL) is a biomarker of CNS injury in HIV infection: a cross-sectional study. EBioMedicine 2015;3:135-140.
5. Sellner J, Patel A, Dassan P, et al. Hyperacute detection of neurofilament heavy chain in serum following stroke: a transient sign. Neurochem Res 2011;36:2287-2291.
6. Kuhle J, Barro C, Andreasson U, et al. Comparison of three analytical platforms for quantification of the neurofilament light chain in blood samples: ELISA, electrochemiluminescence immunoassay and Simoa. Clin Chem Lab Med 2016;54:1655-1661.
7. Pujol-Calderón F, Portelius E, Zetterberg H, et al. Neurofilament changes in serum and cerebrospinal fluid after acute ischemic stroke. Neurosci Lett 2018;698:58-63.
8. Shahim P, Gren M, Liman V, et al. Serum neurofilament light protein predicts clinical outcome in traumatic brain injury. Sci Rep 2016;6:36791.
9. Rohrer JD, Woollacott IO, Dick KM, et al. Serum neurofilament light chain protein is a measure of disease intensity in frontotemporal dementia. Neurology 2016;87:1329-1336.
10. Hansson O, Janelidze S, Hall S, et al. Blood-based NfL: a biomarker for differential diagnosis of parkinsonian disorder. Neurology 2017;88:930-937.
11. Singh P, Yan J, Hull R, et al. Levels of phosphorylated axonal neurofilament subunit H (pNfH) are increased in acute ischemic stroke. J Neurol Sci 2011;304:117-121.
12. Traenka C, Disanto G, Seiffge DJ, et al. Serum neurofilament light chain levels are associated with clinical characteristics and outcome in patients with cervical artery dissection. Cerebrovasc Dis 2015;40:222-227.
13. De Marchis GM, Katan M, Barro C, et al. Serum neurofilament light chain in patients with acute cerebrovascular events. Eur J Neurol 2017.
14. Gattringer T, Pinter D, Enzinger C, et al. Serum neurofilament light is sensitive to active cerebral small vessel disease. Neurology 2017;89:2108-2114.
15. Duering M, Konieczny MJ, Tiedt S, et al. Serum neurofilament light chain levels are related to small vessel disease burden. J Stroke 2018;20:228-238.
16. Tiedt S, Duering M, Barro C, et al. Serum neurofilament light: a biomarker of neuroaxonal injury after ischemic stroke. Neurology 2018;91:e1338-e1347.
17. Sipola P, Hedman M, Onatsu J, et al. Computed tomography and echocardiography together reveal more high-risk

findings than echocardiography alone in the diagnostics of stroke etiology. Cerebrovasc Dis 2013;35:521-530.
18. Taina M, Vanninen R, Hedman M, et al. Left atrial appendage volume increased in more than half of patients with cryptogenic stroke. PLoS One 2013;8:e79519.
19. Onatsu J, Taina M, Mustonen P, et al. Soluble urokinase-type plasminogen activator receptor predicts all-cause 5-year mortality in Ischemic stroke and TIA. In Vivo 2017;31:381-386.
20. Putaala J, Metso AJ, Metso TM, et al. Analysis of 1008 consecutive patients aged 15 to 49 with first-ever ischemic stroke: the Helsinki young stroke registry. Stroke 2009;40:1195-1203.
21. Ferro JM. Cardioembolic stroke: an update. Lancet Neurol 2003;2:177-188.
22. Sacco RL, Kasner SE, Broderick JP, et al. An updated definition of stroke for the 21st century: a statement for healthcare professionals from the American Heart Association/American Stroke Association. Stroke 2013;44:2064-2089.
23. Adams Jr. HP, Bendixen BH, Kappelle LJ, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke 1993;24:35-41.
24. Graham Jr. TP, Jarmakani JM, Atwood GF, Canent Jr. RV. Right ventricular volume determinations in children. Normal values and observations with volume or pressure overload. Circulation 1973;47:144-153.
25. Kuhle J, Barro C, Disanto G, et al. Serum neurofilament light chain in early relapsing remitting MS is increased and correlates with CSF levels and with MRI measures of disease severity. Mult Scler 2016;22:1550-1559.
26. Hjalmarsson C, Bjerke M, Andersson B, et al. Neuronal and glia-related biomarkers in cerebrospinal fluid of patients with acute ischemic stroke. J Cent Nerv Syst Dis 2014;6:51-58.
27. Shichita T, Sugiyama Y, Ooboshi H, et al. Pivotal role of cerebral interleukin-17-producing gammadeltaT cells in the delayed phase of ischemic brain injury. Nat Med 2009;15:946-950.
28. Yan J, Greer JM, Etherington K, et al. Immune activation in the peripheral blood of patients with acute ischemic stroke. J Neuroimmunol 2009;206:112-117.