Plasma Neurofilament Light Chain Predicts Large Vessel Occlusions in Patients with Acute Ischemic Stroke

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Research

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Abstract

Background

Plasma neurofilaments light chain (pNfL) is a marker of axonal injury. The aim of this study was to evaluate the role of pNfL as a predictive biomarker for stroke due to large vessel occlusion (LVO).

Methods

This retrospective study was developed from a prospectively collected stroke database, which was conducted at a large academic comprehensive stroke center in western China. Consecutive patients ≥ 18 years with first-ever acute ischemic stroke (AIS) of anterior circulation within 24 hours of symptom onset were included. Stroke severity was analyzed at admission using the NIHSS score. The pNfL drawn within 24 h from symptom onset was analyzed with a novel ultrasensitive single molecule array. The diagnosis of LVO was based on vascular imaging.

Results

A total of 845 patients (male, 480 (56.80%); mean age, 62.67 (± 11.84) years) were included analysis, and 144 (17.00%) were diagnosed with LVO. pNfL was markedly higher in patients with LVO (56.99(± 14.67) versus 37.86(± 13.82) pg/ml; $P < 0.001$) than Non-LVO. pNfL was valuable for the prediction of LVO (OR, 1.099; 95% CI, 1.081-1.118; $P < 0.001$), even adjusted for conventional risk factors (OR, 1.078; 95% CI, 1.058-1.098; $P < 0.001$). The best cut-off value of pNfL to differentiate between patients with LVO and Non-LVO was 43.08 pg/mL, which yielded a sensitivity of 84.70% and specificity of 66.00%, with the area under the curve (AUC) at 0.826 (95% CI, 0.792-0.860; $P < 0.001$). The highest AUC was reached by a combination of pNfL and NIHSS (AUC, 0.876; 95% CI, 0.849-0.902; $P < 0.001$).

Conclusions

Strokes with LVO were distinguishable from those without LVO following the determination of pNfL in the blood samples within 24 hours of onset. The pNfL is a promising biomarker of AIS with LVO.

Clinical trial registration: ChiCTR1800020330.

Introduction

Strokes are among the leading causes of global morbidity and mortality[1]. Strokes due to large vessel occlusion (LVO) are especially dangerous, with mortality or severe morbidity rates of up to 80%[2, 3]. They are also one of the most time-sensitive diagnoses in medicine and require emergent endovascular therapy to reduce morbidity and mortality[4]. The prevalence of LVO among patients with suspected acute ischemic stroke (AIS) ranged from 13% to 52%[5], and the mean prevalence of LVO was 31.1% across all studies[6]. These patients need to be sent directly to the hospital with endovascular treatment

ability[7]. The inability to reliably identify patients with LVO remains an important limitation to optimizing patients and medical resources.

Several clinical grading scales that rely solely on clinical examination and vascular risk factors have been proposed, but they have demonstrated only moderate predictive ability for LVO[8-10], and they are time-consuming, low accuracy and lack of objectivity[9, 11, 12]. It is thus urgent to develop an objective, accurate and pathogenesis based method to predict LVO.

One of the main pathologies of persistent disability after stroke is axonal injury, which is crucial to the functional prognosis of stroke patients[13, 14]. Neurofilament light chain (NfL) might be a suitable candidate for this purpose because it is a part of the neuronal cytoskeleton and exclusively expressed in neurons. Previous studies have reported that NfL is closely related to infarct size[15], symptom severity[16] and prognosis[13, 14, 17, 18]. LVO will cause extensive nerve damage, far more than other types of stroke, so it is speculated that the NfL concentration should be higher than other types of stroke. However, there is no relevant research.

In light of this, this study aimed to investigate the correlation between NfL and the presence of LVO in a cohort of patients with AIS. We hypothesized that plasma NfL (pNfL) measured within 24 h predicts LVO.

Patients And Methods

Participants

Data are available on request from the corresponding author. This study was developed from a prospectively collected stroke database (ChiCTR1800020330). The study was conducted according to the principles expressed in the Declaration of Helsinki. Ethics committee of General Hospital of Western Theater Command approved sample collection and analysis (No. 2018ky06). All patients or their welfare guardians provided written informed consent for the collection of data, blood samples, and subsequent analyses. The database collected consecutive patients ≥ 18 years with first ever AIS within 24 hours of symptom onset between July 1, 2017 and December 31, 2019. AIS was diagnosed according to the World Health Organization criteria and confirmed by brain computed tomography (CT) or magnetic resonance imaging (MRI). Subjects were candidates for inclusion if they met criteria: the lesion of AIS was located in the anterior circulation and underwent vascular imaging using computerized tomography angiography (CTA), Magnetic resonance angiography (MRA) or digital subtraction angiography (DSA) to evaluate for the presence of acute LVO. Patients were excluded if they combined with other non-vascular causes of neural function defects (brain injury, Alzheimer's disease, Parkinson's disease and other neurological diseases); or accompanied by serious medical diseases, tumor, hepatitis or autoimmune disease.

LVO is defined as an occlusion of the M1/M2 segment of the middle cerebral artery (MCA), A1 segment of the anterior cerebral artery (ACA), internal carotid artery (ICA) terminus. Stroke severity was assessed using the National Institutes of Health Stroke Scale Score (NIHSS) and infarct volume (calculated by MRI-DWI). DWI infarct volumes were determined by consensus of two experienced raters unaware of the

clinical and laboratory results. The lesion size was calculated by the commonly used semiquantitative method[19].

Blood sampling and biomarker measurement

Venous blood samples were drawn on admission; the time from stroke onset to blood collected was recorded. After centrifugation plasma (from EDTA tube) was aliquoted. Tubes were frozen locally at -80°C . sNfL was measured by single-molecule assay (SiMoA) platform (Quanterix, Lexington, MA, USA), as described.[20, 21] An in-house pool was used as an internal control and included in each assay for evaluating assay performance. Samples were analyzed in duplicates, and coefficient of variation (CV) was $<11\%$.

Statistical analysis.

Data are presented as mean ($\pm$ SD), median (interquartile range [IQR]), or numbers with percentages. For univariate analysis, the Mann-Whitney U test, Student t test, or χ^2 test were used, as appropriate. Functional outcome was assessed as a binary outcome (mRS score 0–1 vs 2–6), and the NIHSS score was treated as a numeric scale. In order to test for significant correlations between clinical characteristics of patients and the pNfL, Spearman rank correlation was used. The association of pNfL levels with LVO was analyzed by multiple logistic regressions and adjusted for established predictors. Criteria for the entry of variables in the regression analyses was set at $P<0.05$, together with other clinically significant variables. We report odds ratios (OR) along with 95% confidence intervals (CI) as measure of association and uncertainty, respectively. To assess the diagnostic accuracy of sNfL for discriminating LVO and Non-LVO, we calculated the area under the Receiver-Operating-Characteristic (ROC)-curve. The optimal cutoff level for dichotomizing values was selected as the situation maximizing the Youden index. All analyses were performed using SPSS 26 (IBM, Chicago, IL). Two-tailed $P<0.05$ was considered significant.

Results

The study consisted of 845 patients with complete clinical variables, vascular imaging and successful 24-hour blood sample analysis, 144(17.00%) had LVO (ICA 38(26.39%), M1 89(61.81%), M2 9(6.25%), A1 8(5.56%)). Mean age was $62.67(\pm 11.84)$ years; 56.80% were male; mean pNfL was $41.15(\pm 15.70)$; and median clinical severity was 3 points on the NIHSS (IQR, 2–7). The details clinical characteristics of the study cohort are displayed in the Table 1.

After univariate analysis, patients with LVO had significant higher pNfL levels (mean, 56.99 versus 37.86 pg/mL; $P<0.001$, Table 1 and Fig 1), more severe clinical deficits (median NIHSS 2 versus 5; $P<0.001$), and poorer clinical outcome (mRS <2 , 30.56% versus 57.35%; $P<0.001$). With regard to cardiovascular risk factors, patients with LVO suffered more often from atrial fibrillation (22.92% versus 15.12%; $P=0.022$). The blood sampling time did not differ between the groups ($P>0.05$).

The pNfL levels increased with an increasing severity of stroke defined by the NIHSS score (Fig. 2A), the time to blood sampling (Fig. 2B) and slightly correlated with age (Fig. 2C).

After multivariate analysis, pNfL levels (OR, 1.099; 95% CI, 1.081-1.118; $P<0.001$) significant for prediction of LVO after stroke, even after adjusted for age, sex, blood sampling time, baseline NIHSS, history of atrial fibrillation and TOAST classification (OR, 1.078; 95% CI, 1.058-1.098; $P<0.001$; for details, see Table 2).

ROC analysis was drawn for variables remaining significant after multivariate analysis. ROC for sNfL and NIHSS at admission rating with regard to LVO resulted in an area under the curve (AUC) of 0.826 (95% CI, 0.792-0.860; $P<0.001$) and 0.812 (95% CI, 0.851-0.860; $P<0.001$), respectively. The highest AUC was reached by a combination of sNfL and NIHSS (AUC, 0.876; 95% CI, 0.849-0.902; $P<0.001$; Fig. 3).

Discussion

This study showed that the pNfL concentration within 24 hours was an independent risk factor for LVO after an anterior circulation stroke, even after adjusted for potential influencing factors regarded as clinically relevant. In the present study, it was found that LVO patients exhibited higher pNfL and NIHSS scale than Non-LVO patients. Atrial fibrillation and the TOAST classification were also associated with LVO, and the patients with LVO tend to poorer outcome. The pNfL was positively correlated with severity of stroke, slightly correlated with age and the time to blood sampling time. The levels of pNfL showed significant diagnostic accuracy in discriminating patients with LVO from those without LVO, especially when combined with NIHSS. This is the first study that has investigated the pNfL levels in AIS patients with LVO.

NfL, as a protein exclusively expressed in neurons[22], can reflect the severity of neuron injury, and has potential application prospects in patient monitoring, observation and intervention research[21]. Based on the theory that stroke caused by LVO damaged nervous system more serious, so as to express more pNfL. The present study not only confirmed that the expression of pNfL in patients with LVO is higher than that in Non-LVO, but also proved that pNfL has high accuracy in the identification of LVO. Previous studies have shown that NfL expression was associated with dementia[23-26], small vessel disease[27], and other neurodegenerative diseases[28-31]. These results indicate that NfL is a specific marker of nerve injury, which can be highly expressed in a variety of nervous system diseases[32]. Subsequently, an increasing number of studies have demonstrated that NfL levels were associated with clinical characteristics and outcome in stroke patients[13, 17, 18, 21], and the CSF NfL increased months before the first dementia symptoms appeared, suggesting it might serve as a preclinical marker[33]. Interestingly, the higher NfL level was found in a transient ischemic attack (TIA) patient who developed an ischemic stroke 1 day after blood sampling[16]. It might suggest that sNfL releasing ischemic brain injury may have already started before symptoms became clinically apparent. In addition, NfL is related to the clinical severity[34] and can distinguish different nervous system diseases[35], which indicates that the degree of neuronal damage is related to the expression of NfL, and further indicates the feasibility of NfL in differentiating LVO.

The era of endovascular treatment for LVO, the identification and triage of LVO is more complex than ever. With the continuous development of guidelines, tools and systems, an objective and accurate evaluation method is needed to guide the treatment process and the allocation of medical resources. Although previous studies have shown that many LVO screen scales have moderate or even high accuracy[10], the unavoidable defects of LVO screen scales are lack of objectivity and consistency[36, 37]. Especially for inexperienced clinicians, the accuracy of evaluation is significantly lower than that of experienced doctors, and the first medical institution for patients with AIS is often not a comprehensive stroke center. Therefore, the exploration of objective biomarkers has never stopped. Proteins associated with acute LVO pathogenesis and endothelial function may appear in blood samples of AIS patients due to LVO, thus permitting development of blood-based biomarkers for its diagnosis and prognosis. Recent studies identified collateral circulation related biomarker[38] and cardiac biomarkers[39] could provide diagnostic aid to the existing modalities for AIS due to LVO. However, these markers are not neuron specific and may be affected by many factors. Atrial fibrillation, systolic blood pressure and admission NIHSS were also correlated with LVO[40, 41] which is consistent with this study. But the specificity of these indicators is worse. Therefore, NfL is superior to previous biomarkers in the identification of LVO, and the combination of NfL and previous biomarkers may further improve the diagnostic efficiency in the future.

It cannot be denied that the expression of NfL also has some influencing factors. First of all, as mentioned above, NfL may increase on the basis of other nervous system diseases. Therefore, the impact of other nervous system diseases must be excluded in the diagnostic process of using NFL. This study excluded patients with other possible neurological diseases and previous cerebral infarction. Secondly, the expression of NfL changed dynamically with time after stroke[42], so the effect of blood collection time on NfL cannot be ignored. This study showed that there was no significant difference in blood collection time between the two groups. At the same time, NfL was slightly correlated with blood sampling time, which may be related to the relatively concentrated blood collection of patients who are only included within 24 h of onset.

Several limitations to this study should be noted. First, retrospective studies are prone to selection biases. In any case, prospective studies are needed to determine the value of pNfL in making triage decisions for transport of patients with LVO. Future planned studies to expand the cohort, both at our institution and at multicenter, will help to reduce this variance. Second, the small sample size due to strict inclusion criteria raises the risk of chance findings. Third, we were limited to a cross-sectional analysis as longitudinal sNfL measurements were not available. Previous studies showed that sNFL in stroke patients were with a peak at day 7[21], at third week[42], between the acute phase and 3-month post-stroke[43]. More work should be carried out to determine the specific peak value. Finally, single biomarkers may not be sufficient, and multiple biomarkers combined with a machine-learning algorithm should be used to automatically diagnose and predict LVO.

Conclusions

In the era of endovascular therapy for LVO, accurate identification of LVO is critical for clinicians to optimize patients and their medical resources. We conclude that our results support the feasibility of quantifying NfL in acute plasma samples as a potential measure of LVO in AIS. The present study not only confirmed that the expression of pNfL in patients with LVO is higher than that in Non-LVO, but also proved that pNfL has high accuracy in the identification of LVO.

Abbreviations

pNfL, Plasma neurofilaments light chain; LVO, large vessel occlusion; AIS, acute ischemic stroke; NIHSS, National Institutes of Health stroke scale; AUC, area under the curve; CT, computed tomography; MRI, magnetic resonance imaging; CTA, computerized tomography angiography; MRA, Magnetic resonance angiography; DSA, digital subtraction angiography; MCA, middle cerebral artery; ACA, anterior cerebral artery; ICA, internal carotid artery; IQR, interquartile range; CI, confidence intervals; OR, odds ratios; ROC, Receiver-Operating-Characteristic; mRS modified Rankin Scale; TOAST, Trial of ORG 10172 in Acute Stroke Treatment.

Declarations

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

Dongdong Yang: Conceptualization, Methodology, and Software. Qingsong Wang: Conceptualization, Methodology, Supervision and Investigation. Zhiqiang Wang: Data curation, Writing - original draft, review & editing. Rongyu Wang: Data curation, Writing - original draft. Mao Li and Lianyan Jiang: Data curation. Yuxia Li: Data curation, Investigation. Yaodan Zhang: Software, Validation. Jin Fan: Supervision. All authors reviewed the manuscript.

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Data availability

All data are fully available on request from the corresponding author without restriction.

Ethics Statement

The study was conducted according to the principles expressed in the Declaration of Helsinki. Ethics committee of General Hospital of Western Theater Command approved sample collection and analysis (approved No. 2018ky06). All patients or their welfare guardians provided written informed consent for the collection of data, blood samples, and subsequent analyses.

Consent for publication

All authors have read and approved the submitted manuscript. The manuscript has not been submitted elsewhere nor published elsewhere in whole or in part.

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Tables

Table 1 Demographic and clinical characteristics of the patients.			
Factors	LVO (%)	Non-LVO (%)	<i>P</i>
Overall rate, <i>n</i> (%)	144(17.00)	701(83.00)	
Sex (male), <i>n</i> (%)	78(54.17%)	402(57.35%)	0.483
Age (y), mean (±SD)	63.65(±11.30)	62.47(±11.95)	0.276
Vascular risk factors, <i>n</i> (%)			
Hypertension	85(59.03)	411(58.63)	0.381
Diabetes mellitus	49(34.03)	241(34.38)	0.935
Hyperlipidemia	31(21.53)	165(23.54)	0.603
Atrial fibrillation	33(22.92)	106(15.12)	0.022
Smoking	41(28.47)	233(33.24)	0.431
Drinking	29(20.14)	143(20.40)	0.944
NIHSS, median (IQR)	9(5-12)	3(2-4)	<0.001
mRS score<2, <i>n</i> (%)	44(30.56)	402(57.35)	<0.001
Infarct volume (ml), median (IQR)	15.46(10.43-23.32)	9.56(2.29-18.93)	0.058
TOAST classification, <i>n</i> (%)			<0.001
Large-artery atherosclerosis	64(44.44)	314(44.79)	
Cardioembolism	51(35.42)	146(20.83)	
Others	29(20.14)	241(34.38)	
Blood sampling time (h), median (IQR)	10.00(5.50-17.50)	12.00(7.00-18.00)	0.471
Plasma NfL(pg/mL), mean (±SD)	56.99(±14.67)	37.86(±13.82)	<0.001
Abbreviations: LVO, large vessel occlusion ;IQR, interquartile range; NIHSS, NIH stroke scale; mRS modified Rankin Scale; pNfL, plasma neurofilament light chain concentration; and TOAST, Trial of ORG 10172 in Acute Stroke Treatment;			
Bold text indicates a statistically significant difference with a p-value less than 0.05.			

Talbe 2 Multivariate logistic regression analysis for the association of pNfL with LVO			
	OR	95%CI	<i>P</i>
Unadjusted pNfL	1.099	1.081-1.118	<0.001
Model 1 pNfL	1.100	1.081-1.118	<0.001
Model 2 pNfL	1.078	1.059-1.098	<0.001
Model 3 pNfL	1.078	1.058-1.098	<0.001
Abbreviations: pNfL, plasma neurofilament light chain concentration; Model 1 adjusted for age and sex; Model 2 adjusted for Model 1 and blood sampling time and baseline NIHSS; Model 3 adjusted for Model 3 and history of atrial fibrillation and toast classification			

Figures

Figure 1

Plasma neurofilament light chain (pNfL) concentration in the diagnostic groups are shown as scatterplots. The pNfL concentration was higher in the large vessel occlusion (LVO) group compared with the Non-LVO group ($P < 0.001$).

Figure 2

Correlations of the plasma neurofilament light chain (pNfL) concentration with (A) the National Institutes of Health Stroke Scale (NIHSS), (B) blood sampling time (h), (C) age (y).

	Sensitivity	Specificity	AUC	95% CI	<i>P</i>
pNfL	0.847	0.660	0.826	0.792-0.860	<0.001
NIHSS	0.771	0.750	0.812	0.851-0.860	<0.001
pNfL+NIHSS	0.813	0.799	0.876	0.849-0.902	<0.001

Figure 3

Receiver operator characteristic (ROC) curves of variables predicting LVO 24 h after stroke onset. pNFL. Abbreviation: AUC, area under the curve. NIHSS, NIH stroke scale; pNfL, plasma neurofilament light chain concentration.