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

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ABSTRACT (250 sanaa)

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.

INTRODUCTION

Neurofilaments, major cytoskeletal constituents of neuronal cells, are released into the cerebrospinal fluid (CSF) during neuronal injury or degradation and thus provide a promising tool to evaluate axonal damage in various neurological conditions. Neurofilament light chain (NF-L) is a central nervous system-enriched protein, abundantly expressed in the long myelinated subcortical white matter axons {{327 Zetterberg,H. 2013;}}. Together with the neurofilament medium (NF-M) and heavy (NF-H) subunits, NF-L is one of the scaffolding proteins of the neural cytoskeleton, with important roles in axonal and dendritic branching and growth {{326 Lepinoux-Chambaud,C. 2013; 328 Petzold,A. 2005;}}. Until recently measurement of NF-L was possible only from CSF, where protein concentrations are up to 50 times higher than those measured from blood {{322 Gisslen,M. 2015; 295 Sellner,J. 2011;}}.

In patients with no signs of neurological diseases the upper reference limit and cut-off for NF-L has been set below 100 pg/mL in CSF. Elevated NF-L levels in CSF have been reported in different chronic neurodegenerative disorders and following acute brain injuries including ischemic stroke, subarachnoid hemorrhage (SAH) and traumatic brain injury (TBI) {{309 Van Geel,W.J. 2005; 330 Rose 1996;}}. In axonal damage caused by amyotrophic lateral sclerosis (ALS), NF-L concentrations in CSF may rise over 7 118 pg/mL {{319 Menke,R.A. 2015;}} and in ischemic stroke over 19 800 pg/mL . Measurements of NF-L in CSF have been suggested to have prognostic utility both for transient ischemic attack (TIA) and stroke and seem to correlate with the severity of white matter lesions (WML) {{324 Hjalmarsson,C. 2014;}}.

With the implementation of a new ultra-sensitive Single molecule array (Simoa) assay for NF-L it has become possible to measure the protein reliably in serum and plasma. Plasma

or serum NF-L correlates with CSF NF-L concentration (ref: Gisslén M et al., EBioMedicine. 2015 Nov 22;3:135-40; Kuhle J et al., Clin Chem Lab Med. 2016 Oct 1;54(10):1655-61) and virtually the same information can be derived from these two biofluids. In the future, serum-based NF-L determination (sNF-L) may provide a valuable complement in identifying neuronal degradation or damage and can potentially be utilised clinically for diagnostic and monitoring purposes in traumatic brain injury and neurodegenerative diseases {{335 Shahim,P. 2016; 310 Rohrer,J.D. 2016; 300 Hansson,O. 2017;}} Thus far the literature on sNF-L in patients with acute stroke is scarce and based on previous methods {{295 Sellner,J. 2011; 292 Singh,P. 2011; 290 Traenka,C. 2015;}}.

While careful neurological examination and modern diagnostic imaging are the cornerstones in diagnostics and treatment decisions of acute ischemic stroke, a new blood-based biomarker could improve diagnostics and prediction of outcome of patients with stroke. Therefore we analyzed concentrations of serum NF-L with an ultrasensitive ELISA (Simoa) in 136 patients with acute ischemic stroke or TIA and evaluated the correlations with clinical severity of symptoms, volumes of cerebral infarction, etiology and prognosis of stroke.

MATERIALS AND METHODS

The study was conducted according to the Declaration of Helsinki and the protocol was reviewed and approved by the Kuopio University Hospital Research Ethics Board (N:o

82/2004). Written informed consent was obtained from the patient or the patient's legally authorized representative.

Patients, who were admitted to our university hospital because of acute stroke or TIA with unknown or suspected cardioembolic etiology but without known atrial fibrillation (AF) were evaluated as candidates for this EmbodeteCT study between March 2005 and November 2009 {{13 Sipola 2013; 34 Taina,M. 2013; 75 Muuronen,A.T. 2015;}}. Exclusion criteria included symptoms indicating large-artery atherosclerosis, small-vessel occlusion or a hypercoagulable state. The neurologists involved in this study recruited altogether 162 patients. Three patients refused to participate after giving informed consent and 23 did not give venous blood serum sample.

Diagnostic investigations included combined examination of the brain, heart, aorta, and cervicocranial arteries with computed tomography (CACC-CT) and collection of blood serum samples in the acute phase. Brain CT was controlled in the subacute phase (> two days). Twenty-four (17,6%) patients also underwent MRI of the brain, if CT did not reveal an infarction. The final diagnosis of stroke versus TIA was defined by the neurologist based on all clinical and imaging data. The etiology of stroke/TIA was defined according to the TOAST criteria {{29 Adams,H.P.,Jr 1993;}}, modified by recommendations from the Association of Echocardiography (EAE) for defining the cardiac source of embolism. On the follow-up visit three months after stroke the clinical outcome was evaluated by a neurologist and a blood serum sample was collected.

The two primary outcome measures were 1.) clinical outcome assessed by the modified Rankin Scale (mRS) (with scores ranging from 0 [fully independent] to 6 [dead]) at 90 days and 2.) volume of the cerebral infarction.

Imaging

All patients underwent the contrast-enhanced CACC-CT 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. Volume of the cerebral infarction was measured from 4.5–5 mm transversal slices of the brain CT in the subacute phase and calculated by using Simpson's method.

Measurement of neurofilament in serum

The venipuncture for acute phase blood samples was performed approximately 63.8±50.1 hours after hospital admission. The gel-separator tubes were centrifuged within 20–60 minutes after sampling. Serum was separated, aliquoted and stored at −80 °C pending biochemical analysis. (only reference ?) The antibodies displayed four major epitope regions and different combinations of antibodies for coating and as tracer antibody were evaluated in relation to affinity of the antibodies. The final selection of antibodies was made by combining the two antibodies with the highest affinity with their performance as 'coater' and 'tracer'.

Serum NF-L concentration was measured using the Simoa platform (Quanterix, Lexington, MA, USA), a magnetic bead-based digital ELISA that allows detection of proteins at subfemtomolar concentrations. Magnetic beads (Quanterix, Lexington, MA, USA) were conjugated with capture antibody UD1 (UmanDiagnostics Art No. 37005, Umeå, Sweden) at 0.3 mg/mL according to bead supplier's conjugation protocol. Prior to each run, serum samples were diluted 10 fold, NF-L calibrator (UmanDiagnostics Art No. 27001, Umeå, Sweden) was series diluted and biotin-labeled detection antibody UD2 (UmanDiagnostics Art No. 37017, Umeå, Sweden) was diluted to 0.1 µg/mL in PBS, 0.1 % Tween20, 2 % BSA, 10 µg/mL TRU Block (Meridian Life Science, Inc., Memphis, TN, USA). For each

determination, 400 000 conjugated beads were washed and resuspended in 100 ìL serum sample or calibrator and 20 μL detection antibody was added. After a 30 minute incubation, beads were washed and resuspended in 100 μL streptavidin-conjugated â-galactosidase (Quanterix, Lexington, MA, USA) at 150 pM diluted in SBG Diluent (Quanterix, Lexington, MA, USA). Following five minutes of incubation, the beads were washed and transferred together with resorufin-D-galactopyranoside substrate (Quanterix, Lexington, MA, USA) to an array of wells, each well only big enough to contain one bead. The array was imaged with a charge-coupled device (CCD) camera imaging system and the images were used to differentiate between empty beads and beads with bound analyte, giving a signal expressed as average enzyme per bead (AEB). To extract concentrations from AEBs, each sample AEB was fitted to a four-parameter logistic curve plotted from the known concentrations of the NF-L calibrator run in parallel with the samples. Calibrator points were run in triplicates while samples were run in duplicates. All samples from each individual patient were measured within the same run. Limit of detection (LOD) for the NF-L assay was 0.29 pg/mL and lower limit of quantification (LLOQ) was 2.7 pg/mL when compensated for a four-fold sample dilution. LOD and LLOQ were determined by mean blank signal + 3 SD and + 10 SD, respectively. Average intra-assay duplicate coefficient of variation (CV) for the samples was 6.5 % (SD 8.6 %). All samples were analyzed using the same batch of reagents by board-certified laboratory technicians who were blinded to clinical information.

Statistical analysis

Continuous variables with normal distribution are presented as mean ± SD, and categorical variables as absolute values and percentages. Based on Kolmogorov-Smirnov test, Student's t-test was used to compare normally distributed continuous variables and Mann-Whitney U non-normally distributed continuous variables. With normally distributed

variables two-tailed Pearson correlation and with non-normally distributed variables Spearman correlation was used to investigate the associations between continuous variables. Chi-square test with Pearson or Fisher's correlation was used to compare nominal variables. Receiver operating characteristic (ROC) curves were used to determine the optimal NF-L thresholds for discriminating stroke with ischemic lesion from TIAs without parenchymal loss. Statistical significance was set at $P<0.05$ and high statistical significance at $P<0.01$. Data were analyzed using SPSS for Windows (version 19, 1989–2010 SPSS Inc., Chicago, USA).

RESULTS

The clinical background characteristics and the sNF-L concentrations in relevant subgroups of the patients are presented in Table 1. Most of the study participants were males (68%). The mean sNF-L concentration was 130.0±178.3 pg/mL in acute phase (N=136). Both the acute phase and follow-up sNF-L samples were available in 41 patients. No significant differences were observed between the acute phase sNF-L (117.9±143.1 pg/mL) and three months follow-up sNF-L (160.8±300.0 pg/mL) levels (p=0.24)..sNF-L concentration did not vary between sexes. However age significantly correlated with sNF-L levels the older patients having higher concentrations (r=0.358, P<0.001). Altogether 101 (74 %) of the patients were diagnosed with stroke while 35 (26 %) suffered from TIA and the mean NIHSS was 3.7 on admission. sNF-L was significantly higher (161.4±196.1 pg/mL) in stroke patients compared to those with TIA (39.4±42.2 pg/mL, P<0.001). Eleven patients (8%) were treated with recombinant tissue plasminogen activator. The sNF-L levels of this small group did not differ from the others (P=ns.).

The etiology of stroke, assessed by the five-class TOAST criteria, was associated with sNF-L concentrations in the acute phase. Patients diagnosed with cardioembolism as the stroke etiology had higher sNF-L concentrations compared to other etiological groups (P=0.017). In addition, sNF-L levels in the cryptogenic stroke group significantly differed from those in the small-vessel disease (P=0.042) group. Infarct volumes, mRS points after three months and sNF-L concentrations in different stroke etiologies are shown in Table 2.

Table 1a. Continuous clinical characteristics of the study population and correlation to serum neurofilament concentration.				
Variable	N	Variable (mean±SD)	R	P
Age	136	60.5±10.5years	0.358	<0.001
Body mass index	136	28.1±4.5 kg/m^2	-0.43	ns.
Body surface area	136	2.0±0.2 m^2	-0.004	ns.

r= Spearman correlation coefficient

Table 1b. Categorical variables of the study population.					
Variable	N	Subgroups	N	sNF-L	P
Sex	136	Male	93	120.8±171.7	ns.
		Female	43	150.9±192.4	
Stroke or TIA	136	Stroke	101	161.4±196.1	<0.001
		TIA	35	39.4±42.2	
Hypertension	136	Yes	79	136.6±193.5	ns.
		No	57	120.9±156.1	
Dyslipidemia	136	Yes	54	95.5±116.8	ns.
		No	82	152.7±206.8	
Diabetes	136	Yes	21	117.0±93.8	ns.
		No	115	132.4±190.0	
Smoker	136	Yes	34	78.9±77.7	ns.
		No	102	147.1±198.4	
Atrial fibrillation	136	Yes	17	177.8±223.7	ns.
		No	119	123.2±171.0	

Prior stroke	136	Yes	25	143.0±137.6	ns.
		No	111	127.1±186.7	
Prior myocardial infarction	136	Yes	19	149.0±140.1	ns.
		No	117	127.0±184.1	

Table 2. Infarct volumes (mm³), Modified Ranking Scale (mRS) scores after three months and serum neurofilament light chain (sNF-L) concentrations on addmission according to different stroke etiologies.

Stroke Etiology	Infact volume (mm³)		mRS after 3 months		sNF-L concentration	
	N	Mean±SD	N	Mean±SD	N	Mean±SD
Cryptogenic	73	12 009±32 366	48	0.54±0.85	73	115.3±162.3
Large-artery atherosclerosis	11	3 621±6 463	7	1.86±2.27	11	222.9±311.6
Cardiogenic	22	31 745±53 183	20	0.8±1.28	22	182.8±206.0
Large-artery atherosclerosis and cardiogenic	10	11 300±24 777	8	0.75±1.04	10	79.1±63.1
Small vessel disease	10	354.5±383.6	6	0.33±0.82	10	45.4±30.1

The volume of the cerebral infarction in head CT in the subacute phase significantly correlated with the sNF-L concentration (r=0.413, P<0.001). Patients without an infarction in CT (N=58) had sNF-L concentrations of 82.1±110.7 pg/mL while those with a CT-

detectable infarction (N=78) had concentrations of 165.6±209.0 pg/mL (P<0.001). MRI of the brain was performed in 24 patients out of those 58, who lacked a visible infarction in CT. sNF-L concentration was significantly smaller (75.9±126.2 pg/mL) in the 12 patients without infarction in MRI compared to those suffering from an infarction (80.2±130.8 pg/mL, *P*= ns.).

The diagnostic performance of an elevated sNF-L concentration to separate stroke from TIA was assessed by receiver operating characteristics (ROC) analysis (Figure 1). The highest value of sensitivity and specificity for recognizing stroke from TIA was obtained with the sNF-L cut-off point of 49.35 pg/mL. The area under the ROC curve (AUC) was 81.1%. Sensitivity was 73%, specificity 80%, positive / negative predictive values 91.1% / 49.1% and overall accuracy 73.5%. NIHSS-score at admission significantly correlated to sNF-L concentration (r=0,343, P<0,001).

The clinical outcome measure, three month follow- up mRS score, was available from 89 (65.4%) patients. sNF-L concentrations (r=0.306, P=0.004) and cerebral infarction volumes (r=0.413, P<0.001) were significantly correlated to mRS scores. When the patients were dichotomized to ´good outcome´ (mRs scores 0-2) and ´poor outcome´ (mRs scores 3-6) groups sNF-L concentrations tended to be different (P=0.062).

DISCUSSION

A sensitive and reliable blood biomarker to estimate the amount of tissue damage and to aid in diagnostics of acute stroke patients has eagerly been awaited. In this study we analyzed serum NF-L levels, quantified with a novel ultra-sensitive single molecule array, in a cohort of stroke and TIA patients with different etiologies. This is the first study to analyze blood samples of stroke/TIA patients using the Simoa platform, which is by far the most sensitive method to measure NF-L, allowing measurements also in very low

concentrations in blood samples. A recent study showed that the results obtained by Simoa platform had much stronger correlation between paired CSF and serum samples compared to commercial ELISA {{323 Kuhle,J. 2016;}}. We found strong correlations between sNF-L levels and stroke severity measured with NIHSS, infarct volume in CT and clinical outcome after three months verified with mRS. Importantly, the acute phase sNF-L also provided high accuracy to distinguish TIA from stroke. In addition, the acute phase sNF-L concentrations were significantly higher in patients, who suffered stroke due large-artery atherosclerosis or cardioembolic etiology, leading to larger strokes, compared to patients with cryptogenic or small vessel disease etiologies.

Axonal white matter lesions have been hypothesized to be the major primary determinant of outcome following e.g. traumatic brain injury (TBI) and stroke {{340 Smith,D.H. 2003;}} and recently been shown that sNF-L measurements may be useful also to assess the severity of neuronal damage after severe TBI {{335 Shahim,P. 2016;}}. The aim of the present study was to assess sNF-L in patients with ischemic stroke. Using the blood based method described above, we showed , that sNF-L is increased in patients with stroke compared to those with TIA, in parallel with two previous studies showing that NF-L or NF-M in CSF are elevated in patients with acute ischemic stroke {{324 Hjalmarsson,C. 2014; 339 Martinez-Morillo,E. 2015;}}.

 Three recent studies measuring serum neurofilament subunits (NF-L, NF-H and phosphorylated NF-H (pNF-H)) with another ELISA-method have also reported, that NF-levels are elevated in patients with acute ischemic stroke compared to those with TIA or healthy controls {{295 Sellner,J. 2011; 292 Singh,P. 2011; 290 Traenka,C. 2015;}}. Importantly, in our study sNF-L was able to differentiate between stroke versus TIA patients with high accuracy, in contrast to the study of Gonzales-Garcia et al. where measuring serum neuron specific enolase (NSE) and S100 calcium binding protein B

(S100B) on admission did not improve stroke diagnostics {{342 Gonzalez-Garcia,S. 2012;}}.

One preliminary study of 54 patients analyzing phosphorylated axonal neurofilament subunit H (pNfH) has previously shown correlations with NIHSS and infarct volume measured from MRI with 17 subjects {{292 Singh,P. 2011}}. Our results in a larger patient population are congruent with those preliminary findings and confirm high correlation between sNF-L and NIHSS and infarction volume in CT.

Also, astroglial proteins have been studied to asseess the stroke severity. An earlier study by Herrmann et al. found that serum levels of S-100B and glial fibrillary acidic protein (GFAB) were significantly associations to NIHSS and the volume of infarcted brain area on head CT {{320 Herrmann,M. 2000;}}. On the other hand another neuron specific serum protein, tau ,was found measurable in the blood only in 40 - 50% of stroke patients, thus casting doubt on the utility of the tau as a diagnostic biomarker for stroke {{344 A Lasek-Bal,A. 2016;}} A systematic review of 12 studies including 597 patients showed that measurement of serum NSE correlated with infarct volume and the degree of neurologic deficit, but was of limited value for diagnosis of acute ischemic stroke {{343 Anand,N. 2005;}}. It should be recognized that the dynamics of the astroglial biomarkers described above is different compared to NF-L in stroke patients. S-100 and GFAB have shown to be released to blood quite slowly and raised until fourth day {{320 Herrmann,M.2000;}} whereas NF-L becomes detectable in serum already after 6 h from symptom onset and values have been reported to raise up to three weeks {{ 295 Sellner,J. 2011; 292 Singh,P. 2011;}}.

Evaluating clinical outcome and prognosis is of utmost importance after stroke onset. The main clinical determinants for poor outcome are age, NIHSS on admission, the extent of

damage on imaging and underlining diseases {{350 Counsell, C. 2004;}}. Several different biomarkers and biomarker panels have been studied in the context of stroke outcome {{171 Jickling 2015;169 Whiteley 2008;}}. Whiteley et al. analyzed different serum markers of inflammation, thrombosis, cardiac strain and cerebral damage in a study of 270 patients and poor functional outcome after three months. Higher levels of NT pro-BNP and IL-6 were strongly associated with poor outcome 3 months after stroke or TIA after adjustment for age and NIHSS, however those markers showed limited use to improve classification to ´very high risk´ or ´very low risk´ of poor outcome 3 months after symptom onset i.e. reclassification utility.

We found a significant correlation between sNF-L and mRs after 3 months. In addition, sNF-L concentrations tended to be different, when the patients were dichotomized to ´good outcome´ and ´poor outcome´ groups (P=0.062), however there were only few cases (n=9) in the ´poor outcome´ group. This finding, although not reaching statistical significance, supports the role of sNF-L as a marker of axonal damage in stroke patients and is in line with the observed strong correlations to NIHSS and stroke volume.

Several etiologies of stroke exist and determining the precise underlying cause of stroke is of the essence, when planning for secondary prevention and deciding between anticoagulation versus antiplatelet therapy. There are no earlier studies investigating sNF-L levels in different stroke etiology groups using TOAST criteria. We found highest sNF-L levels in large artery atherosclerosis and cardioembolic groups. Interestingly, there was a significant difference in sNF-L levels between cardioembolic and cryptogenic stroke groups, supporting causes other than emboli behind the cryptogenic strokes. The low sNF-L concentration in the small vessel disease etiological group is in line with the hypothesis, that sNF-L is a marker of axonal damage, because lacunar strokes are the smallest in volume.

Despite the extensive search for stroke etiology in clinical routine, it still remains unknown in up to 35-40% of cases {{352 Petty,G.W. 2000; 354 Yaghi,S. 2017;}}. To find a biomarker-based platform to elucidate the cause of stroke is intriguing , because it might amplify the need of expensive investigations, e.g. prolonged cardiac monitoring, to a limited patient group. In addition to improving stroke diagnostics, this kind of platforms might prove cost-effective. In this context the family of the natriuretic peptides (B-type natriuretic peptide/ N-terminal pro-BNP (BNP/NT- proBNP) and D-dimer have been shown to reliably distinguish cardioembolic from non-cardioembolic strokes {{223 lombart 2015; 353 Isenegger,J. 2010;}}. However none of them is widely used in clinical practice yet. When investigating phosphorylated neurofilament heavy protein (pNfH) levels in serum and using Oxfordshire stroke classification (OSSC) {{355 Bamford,J. 1991;}} Singh et al found that serum pNfH was lowest in lacunar strokes (LACI) and highest in total anterior circulation group (TACI), leading to larger infarcts {{292 Singh,P. 2011;}}.

Limitations

We are aware of the following weaknesses concerning our study. The main limitation is that we were able to measure sNF-L only on two time points; on admission and at three months follow-up visit, thus we could not evaluate the time course of the sNF-L levels in stroke patients. Furthermore we calculated the ischemic infarct volumes from CT imaging instead of diffusion weighted MRI. Most importantly, we could not assess clinical outcome with mRS after three months in all patients because of inexact or missing data.

In conclusion, a recently developed ultrasensitive ELISA allows quantification of NF-L in serum samples. Acute phase sNF-L concentrations proved to distinguish patients with ischemic stroke from those with TIA with high sensitivity and specificity. SNF-L concentrations were elevated in line with stroke severity, assessed either clinically or from

subsequent CT imaging. Furthermore sNF-L concentrations predicted clinical outcome after three months. The independent additional predictive value of sNF-L concentrations must be further investigated in multivariable models.

Disclosures

None.

Figure 1. Receiver operating characteristic (ROC) curve of blood neurofilament light chain (NF-L). ROC curve of blood NF-L to distinguish stroke patients from transient ischemic attack (TIA) patients. Final diagnosis of stroke was rendered by neurologists after reviewing of all clinical, imaging, and conventional laboratory data during admission. The area under the ROC curve (AUC) was 81%. Sensitivity was 73% and specificity 80%.

Figure 2. Comparison of mean serum NF-L (pg/mL) on admission according to the TOAST criteria. There were significant differences found between cryptogenic strokes versus cardioembolic strokes (CE) ($P< 0.05$) and large artery atherosclerosis (LAA) and small vessel disease (SVD) ($P< 0.05$), respectively.

Figure 3. Natural logarithmic correlation of the infarct volume with serum NF-L levels in stroke /TIA patients on admission. A statistically significant positive correlation (Spearman r=0.413, $P<0.001$) was found.

Figure 4. Serum neurofilament light chain (sNF-L, pg/mL) concentrations in patient groups with different modified ranking scale results after three months of stroke/TIA onset. The

mean sNF-L concentration was significantly (P<0.001) higher in those patients, whose stroke symptoms were more severe.