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The Role of Endothelinergic and Nitroxidergic Reactions in Predicting the Functional Outcome in Patients with Ischemic Stroke of Different Severity

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Summary

The aim of this study was to assess the value of nitric oxide (NO) and endothelin-1 (ET-1) serum concentrations as potential biomarkers for predicting the functional outcome in patients with acute ischemic stroke.

Material and methods. A total of 37 patients diagnosed with ischemic stroke and admitted to a multidisciplinary vascular center were included in the study. The patients were divided into two groups based on the severity of neurological deficits as determined by the National Institutes of Health Stroke Scale (NIHSS): Group 1 consisted of 20 patients with NIHSS scores <15, and Group 2 consisted of 17 patients with NIHSS scores ⩾15. The functional outcome was assessed using the NIHSS absolute values and the degree of disability measured by the Modified Rankin Scale (mRS) by comparing the values before and after baseline treatment. Lab evaluation included quantitative assessment of stable NO and ET-1 metabolites in patient's serum at admission and on day 10 of hospital stay. The SPSS Statistics V23.0 for Windows software package, Python programming language, and Pandas and SciPy libraries were used for statistical data processing.

Results. Group 1 patients demonstrated a statistically significant decrease in NIHSS (P=0.0013) and mRS (P<0.0001) scores, which was indicative of a favorable functional outcome. Group 2 patients showed some recovery of only neurological deficit measured by NIHSS scale (P=0.0012), changes in degree of disability by mRS were statistically insignificant. On Day10 of hospital stay, both groups showed a clinically significant increase in ET-1 content, and slight change in NO concentration. NIHSS score demonstrated a significant negative correlation with baseline ET-1 concentrations: R=–0.82, P=0.00023 — in Group 1; R=–0.55, P=0.00075 — in Group 2. Modified RS scores showed negative correlation with NO (R=–0.50, P=0.00044) and ET-1 (R=–1.0, P=0.0074) concentrations in Group 1, and positive correlation with NO (R=0.55, P=0.0023) and ET-1 (R=0.33, P=0.04) concentrations in Group 2.

Conclusion. Monitoring of NO and ET-1 serum concentrations provides valuable insights for personalized assessment of the anticipated functional outcome in patients with cerebral ischemia. Further research and the development of prognostic mathematical models are needed to validate the use of endothelial function markers as predictive indicators of patients' recovery potential during the acute phase of ischemic stroke

Keywords: ischemic stroke, nitric oxide, endotelein-1, biomarker

Conflict of interest. The authors declare no conflict of interest.

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Introduction

Cardiovascular disease (CVD) remains the leading cause of death worldwide, accounting for 25% of all deaths [1]. Ischemic stroke (IS) is a major cause of morbidity and mortality as well as a significant socioeconomic problem [2]. The search for predictive biomarkers of stroke progression and functional outcome is ongoing. As such biomarkers, nitric oxide (NO) and endothelin-1 (ET-1) are promising candidates [3]. ET-1 is a multifunctional peptide with cytokine-like activity produced by almost all endothelial cell types [4]. Endothelial dysfunction is characterized by increased ET-1 production in response to a variety of events, including hyperglycemia, hypercholesterolemia, hypertension, estrogen deficiency, and biochemical and mechanical abnormalities [5, 6]. Despite numerous studies in recent years demonstrating the long-term and potent vasoconstrictor effect of ET-1 on cerebral vessels, its role in the pathophysiological mechanisms of cerebral ischemia is still under active investigation [7, 8]. NO is a signaling molecule that is also produced by endothelial cells and has potent vasodilator and anti-inflammatory effects which are necessary to maintain vascular homeostasis [9]. Endothelial dysfunction is directly related to both

a decrease in the production of active NO metabolites and changes in the sensitivity of endothelial cells to NO. Increased NO production occurs in many brain conditions, including acute cerebrovascular accidents and neurodegenerative diseases [10]. The opposing effects of NO and ET-1 on the regulation of local vascular tone are balanced in healthy tissues and dysregulated in cerebral ischemia [11]. Thus, these markers of endothelial dysfunction are of particular interest and can be used as early predictors of blood flow and coagulation disturbances associated with vasoconstriction, leukocyte adhesion, and platelet activation [12]. The role of NO- and ET-driven mechanisms in the regulation of vascular endothelial motor function in acute IS remains poorly understood [13], which provides the rationale for studying changes in the levels of stable NO and ET-1 metabolites in patients with different severity of IS. Mathematical models for prediction and assessment of severity and outcome of stroke are also promising.

The aim of the study was to evaluate the feasibility of using serum levels of nitric oxide and endothelin-1 as prognostic biomarkers of functional outcome in acute ischemic stroke.

Materials and methods

The prospective cohort study was approved by the Independent Ethical Committee of the Center for Clinical Research of the Immanuel Kant Baltic Federal University (protocol 34, dated 29.09.2022). The study included 37 patients admitted to the primary vascular center of an emergency hospital with the diagnosis of ischemic stroke. The sample size of the study was not predetermined. All subjects signed 2 copies of the informed consent prior to all study procedures. To verify the IS subtype according to the TOAST (Trial of Org 10172 in Acute Stroke Treatment) criteria [14], clinical and diagnostic examinations were performed according to the officially approved standards of medical care for stroke patients. At the time of admission, all patients underwent a neurological examination and other routine tests, including transcranial Doppler ultrasound of extra- and intracranial vessels, 12-lead electrocardiography, complete blood count and blood biochemistry, and pulse oximetry. Neuroimaging parameters were assessed by computed tomography (CT) and magnetic resonance imaging (MRI). If necessary, additional tests included MR/CT angiography, echocardiography, ECG Holter monitoring, detailed coagulation study, examination for systemic diseases, and lumbar puncture. All patients were assessed on admission for level of consciousness according to the Glasgow Coma Scale (GCS) and stroke severity according to the National Institutes of Health Stroke Scale (NIHSS). Patients were divided into two groups according to the severity of neurological deficit on the NIHSS scale, which allowed more accurate stratification of the patients studied and use of the clinical data. Group 1 included 20 patients with NIHSS neurological deficit of less than 15 points, of whom 13 (65%) were males and 7 (35%) were females. The mean age of the patients was 68.3±5.6 years. The initial NIHSS score in the group was 6 [2; 9] points, which corresponded to moderate severity.

Group 2 included 17 patients with neurological impairment on the NIHSS scale >15 points, of whom 9 (52.9%) were men and 8 (47.1%) were women. The mean age of the patients was 67.9±4.9 years. The patients' baseline NIHSS score was 18 [15; 28] points, which corresponded to a severe ischemic stroke.

Inclusion criteria were clinical signs and symptoms consistent with the diagnosis of ischemic stroke and age between 60 and 82 years. Exclusion criteria were hemorrhagic stroke and transient ischemic attack.

The functional outcome criteria for acute ischemic stroke were selected from major clinical scales such as the NIHSS Stroke Severity Scale and Modified Rankin Scale (mRS) disability. The change in patient status was expressed in absolute values, and the difference between NIHSS and mRS scores before and after initial treatment was calculated.

The laboratory study included measurement of serum stable metabolites of NO and ET-1. Blood samples were collected on admission and on day 10 of hospitalization.

BD Vacutainer® Plus Serum tubes were used to collect 6 ml of venous blood. CEA482Hu Cloud-Clone Corp. kits, Nitric Oxide Assay Kit A013-2-1 Cloud-Clone Corp. kits were used to determine serum NO and ET-1 levels by enzyme-linked immunoassay.

Statistical data analysis was performed using SPSS Statistics V23.0 for Windows software with Pandas and SciPy libraries, following the appropriate guidelines [15].

The distribution of quantitative variables was evaluated using the Shapiro–Wilk criterion. Quantitative variables with normal distribution were described by arithmetic mean *(M)* and standard deviation *(SD)*. For non-normal distribution, quantitative data were described by median *(Me)*, lower and upper quartiles *(Q1–Q3)*.

Data with normal distribution were compared using ANOVA analysis of variance for dependent and independent samples. For non-normal distribution, the non-parametric Wilcoxon test was used. Differences in the frequencies of parameters in two independent groups were analyzed using Fisher's exact criterion with two-sided confidence limits and the χ^2 criterion with Yates correction. The significance level was set at $P<0.05$. For multiple comparisons of variables, the Bonferroni correction ($P<0.0125$) was used to reject false positives.

The correlation coefficient r was calculated to assess the relationship between functional outcome parameters and laboratory diagnostic parameters. The value of r was [–1; 1], where –1 was complete inverse dependence, 0 was the absence of any dependence, and 1 was complete direct dependence. To evaluate the correlation of continuous values, including parameters measured in points, the Fechner's method for calculating correlation coefficients in small samples was selected:

$$r_f = \frac{n_a - n_b}{n_a + n_b},$$

where n_a is the number of matched signs for differences, n_b is the number of unmatched signs.

The presence of correlation between the values was confirmed if the coefficient exceeded the threshold module value of 0.2. The significance of r was confirmed by calculating the *P*-value [16]:

$$t = \frac{r \times \sqrt{n-2}}{\sqrt{1-r^2}},$$
$$p\text{–}value = 2 \times P(T > t),$$

where *r* is the correlation coefficient, *n* is the sample size, and *P(T>t)* is the probability of obtaining the value of *t* in the distribution *T* with (n-2) degrees of freedom.

The standard value of 0.05 was chosen as the threshold value. If the *P*-value was less than 0.05, the correlation coefficient was considered significant. Correlation coefficients with a *P*-value greater than 0.05 were excluded from consideration.

Results

Patients were treated according to standard stroke care protocols. Thrombolytic therapy was not administered because of contraindications or because patients were hospitalized outside the therapeutic window. Subtypes of ischemic stroke (TOAST criteria), comorbidities, and clinical scores were identified based on clinical and instrumental examinations (Table 1).

Diabetes mellitus was more frequently diagnosed in group 1 than in group 2, whereas the main comorbidity in group 2 was brachiocephalic atherosclerosis according to transcranial Doppler ultrasound. The severity of disability according to mRS was 2 [0; 4] points in group 1 patients, which corresponded to a moderate impairment of performance, and 4 [2; 5] points in group 2 patients, which corresponded to a severe impairment of performance and inability to manage their physical needs without assistance. Patients in group 2 had a significant decrease in mRS and NIHSS scores ($P<0.0001$) compared to group 1. No significant differences were found in basic demographic and laboratory parameters.

Changes in NO and ET-1 concentrations, NIHSS and mRS at the time of admission and on day 10 of hospitalization are shown in Table 2.

Patients in the first group showed a significant decrease in NIHSS score ($P=0.0013$) and mRS disability level ($P<0.0001$), indicating a favorable functional outcome. Patients in the second group showed

Table 1. Main clinical and statistical characteristics of brain ischemic stroke in patients with different severity of disease according to NIHSS.

Parameter	Values in groups		*P*-value
	Group 1, *N*=20	Group 2, *N*=17	
Demographic characteristics			
Men, *N* (%)	13 (65.0)	9 (52.9)	0.455
Women, *N* (%)	7 (35.0)	8 (47.1)	0.455
Mean age, yeas	68.30±5.63	67.90±4.91	0.820
Subtypes of ischemic stroke (TOAST criteria, % of patients)			
IS due to atherosclerosis of large arteries (atherothrombotic)	30.0	29.4	0.968
IS due to cardiogenic embolism (cardioembolic)	40.0	29.4	0.500
IS due to occlusion of small arteries (lacunar)	30.0	35.3	0.731
IS of unknown etiology	0	5.9	0.271
Comorbidity (% of patients)			
Atherosclerosis (>50%)	35.0	64.7	0.0365
Diabetes mellitus	30.0	17.6	0.0228
Atrial fibrillation	25.0	35.3	0.494
Hypertension	75.0	58.8	0.294
Recurrent stroke	25.0	17.6	0.586
Clinical scales (points)			
Glasgow coma scale	15±0.01	14.47±1.2	0.0617
mRS	2 [0; 4]	4 [2; 5]	<0,001*
NIHSS	6 [2; 9]	18 [15; 28]	<0,001*
Laboratory parameters			
NO (mmol/L)	0.001589±0.001	0.0016601±0.002	0.889
ET-1 (pg/mL)	25.02±8.36	24.90±8.62	0.966
ET-1 (pg/mL)	25,02±8,36	24,90±8,62	0,966

Note. * — significant differences between groups.

Table 2. Comparative characteristics of laboratory and clinical parameters at admission and on day 10 of hospital stay.

Parameter	Values in groups				*P*-value
	Group 1, *N*=20		Group 2, *N*=17		
	Day 1	Day 10	Day 1	Day 10	
NIHSS (points)	6 [2; 9]	2.5 [0; 7]	18 [15; 28]	12 [4; 20]	P_1=0.0013* P_2=0.0012* P_3<0.001*
mRS (points)	2 [0; 4]	0.75 [0; 2]	4 [2; 5]	3.5 [2; 5]	P_1⩽0.0001* P_2=0.214 P_3<0.001*
NO (mmol/L)	0.001589±0.001	0.001628±0.001	0.0016601±0.002	0.001330±0.001	P_1=0.875 P_2=0.547 P_3=0.373
ET-1 (pg/mL)	25.02±8.36	31.62±9.14	24.90±8.62	31.24±8.93	P_1=0.018 P_2=0.0431 P_3=0.903

Note. P_1 — difference between the parameters on days 1 and 10 of hospital stay in group1; P_2 — difference between the parameters on days 1 and 10 of hospital stay in group 2; P_3 — difference of parameters between groups on day 10 of hospital stay; * — significant differences.

only an improvement of neurological deficit according to NIHSS (*P*=0.0012). Changes in mRS disability score were insignificant in this group. Analysis of changes in NO and ET-1 showed an increase in ET-1 levels on day 10 of hospitalization in patients in both groups.

No significant changes in NO levels were found in patients of groups 1 and 2 (Fig. 1).

When examining the correlations between baseline levels of NO, ET-1 and functional outcome parameters according to the mRS and NIHSS scales, relationships of different strength and direction were found.

The most significant correlations were found for the NIHSS score with baseline ET-1 levels in group 1 (*r*=–0.82, *P*=0.00023) and group 2 (*r*=–0.55, *P*=0.00075) patients and for NO (*r*=0.50, *P*=0.0036) in group 2 patients (Fig. 2, *a*).

When assessing the association of patient disability at admission based on mRS with NO and ET-1 levels, a negative correlation was found with both NO (*r*=–0.50, *P*=0.00044) and ET-1 (*r*=–1.0, *P*=0.0074) concentrations in group 1 patients. Group 2 patients showed a positive correlation of NO (*r*=0.55, *P*=0.0023) and ET-1 (*r*=0.33, *P*=0.04) with mRS scores (Fig. 2, *b*).

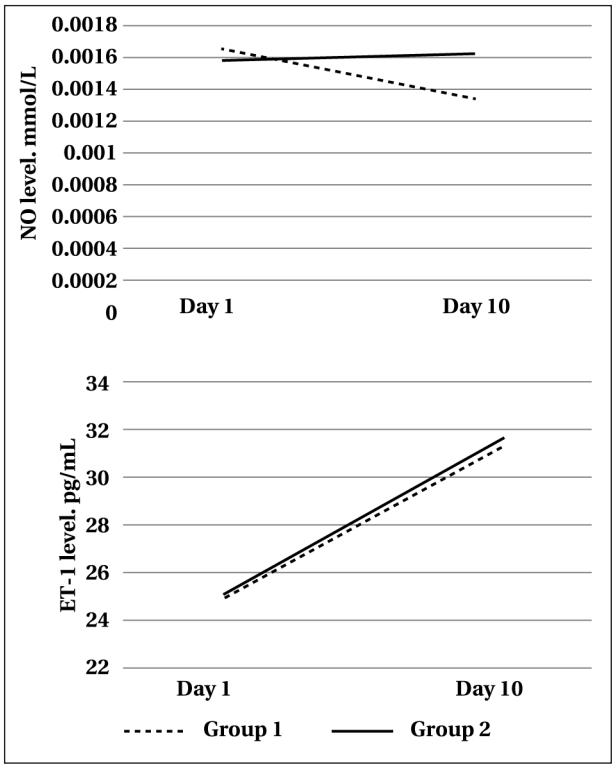

Fig. 1. Changes in serum concentrations of NO and ET-1 in patients with acute ischemic stroke.

Discussion

Prognostication of functional outcome of acute ischemic stroke using various predictors is an essential component of personalized medicine [17]. Evaluation of biochemical markers of endothelial dysfunction together with stroke severity score (NIHSS) and patient independence score (mRS) is a useful clinical decision support tool in the management of acute ischemic stroke [18].

In this study, a greater reduction in mRS independence score was found in patients with severe ischemic stroke compared to patients with moderate neurological deficits according to NIHSS. The results are consistent with other studies reflecting the close relationship between NIHSS and mRS scores and the importance of IS severity in predicting functional disability outcomes after stroke [19–21]. Changes in NIHSS and mRS parameters suggest a favorable functional outcome for patients with moderate IS severity. In patients with severe IS, a significant decrease in NIHSS parameters was also found, but the severity of neurological deficit at day 10 remained high (12 [2; 20] points) and corresponded to a moderate impairment on mRS (3.5 [2; 5] points), which indicates insignificant functional

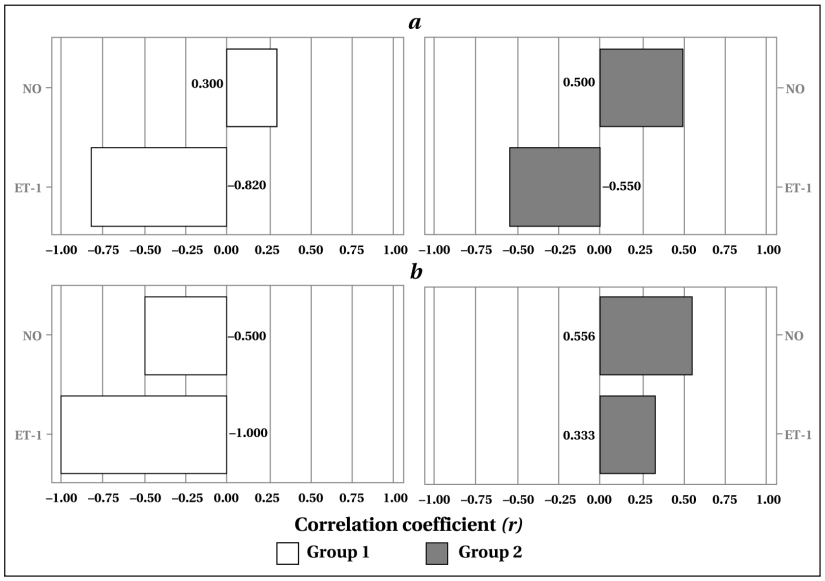

Fig. 2. Correlation between the levels of ET-1 (pg/mL) and NO (mmol/L) and the NIHSS *(a)* and mRS *(b)* scores in patients with acute ischemic stroke.

improvement and cannot be considered as a marker of favorable prognosis.

In patients with IS, circulating endothelin levels are elevated compared to baseline and normal levels [22]. Analysis of changes in laboratory parameters showed an increase in ET-1 levels on day 10 of hospitalization compared to baseline in patients of both groups. This increase may be due to several mechanisms underlying the pathogenesis of IS, such as hypoxia, neuroimmune processes, hypercoagulation, and platelet activation in ischemic areas, which are directly related to impaired vascular endothelial secretion [23]. On the other hand, atherosclerotic vascular changes also predict endothelial dysfunction and, accordingly, increased ET-1 production by damaged endothelial cells [24–26].

In patients with moderate severity of IS, a slight increase in stable NO metabolites has been observed, indicating preserved microcirculatory compensatory mechanisms and a good prognosis in terms of functional outcome [27]. Increased deficiency of endogenous NO in patients with severe neurological deficit may be due to insufficient deactivation of lipid peroxidation and decreased antioxidant defense, which play a critical role in the regulation of cerebral microvascular tone, leading to worsened hypoxia [28, 29]. High ET-1 concentration together with NO deficiency contributes to the persistence and worsening of vasoconstrictor responses, progression of neurological deficits, and predicts an unfavorable functional outcome in patients with a high NIHSS score [30].

Regardless of stroke severity, a negative correlation was found between baseline ET-1 levels and NIHSS neurological deficit scores. Despite the well-established vasoconstrictive effect of ET-1, which negatively affects recovery, the results obtained show an inverse correlation and regression of neurological deficit associated with a significant increase in ET-1.

One of the possible explanations for this is the autocrine-paracrine effect of endotelin-1 at low concentrations, leading to the release of vascular relaxing factors from the endothelium [31–33].

Another neuroprotective mechanism contributing to the regression of neurological signs is also determined by the ET-1-mediated effect and is associated with the inhibition of endothelial cell apoptosis [34]. The positive correlation of NIHSS scores with NO levels has been shown in several studies demonstrating the positive effect of free NO metabolites on the reduction of neurological signs through vasodilation and mediated neuroprotection [35, 36]. The increase in NO and ET-1 levels in patients with moderate ischemic stroke corresponds to the reduction of disability on the mRS scale and indicates a positive functional outcome according to this criterion.

The role of NO and ET-1 expression in the increase in mRS scores in patients with severe ischemic stroke, which reflects a trend toward poor functional recovery and patient disability, is still debated. Limitations of our study include the small number of patients and the lack of a control group.

Conclusion

The study of NO- and ET-1-driven mechanisms of regulation of endothelial vascular function and their role in the pathogenesis of ischemic stroke is a promising direction. Evaluation of NO and ET-1 expression and changes may be used for personalized assessment of predicted functional outcome in patients with acute ischemic stroke.

For a more accurate evaluation of predictors of functional disability in stroke patients, further studies with larger sample size and longer follow-up are warranted, as well as the development of prognostic mathematical models to validate the use of endothelial function markers for predicting patient recovery in acute ischemic stroke.

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