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Predictive Value of Circulating Vascular Endothelial Growth Factor-1 in Arterial Hypertension Patients

Berezin AE[1]* and Lisovaya OA[2]

[1]Internal Medicine Department, State Medical University, Zaporozhye, Ukraine
[2]District Hospital #6, Zaporozhye, Ukraine

Abstract

Aim: The aim of the study was to investigate the predictive value of serial measurements of circulating vascular endothelial growth factor-1 level in hypertensive patients after ischemic stroke.

Methods: 102 patients with mild to moderate arterial hypertension within 3 weeks after ischemic stroke were included in the study. Patients were followed-up for 12 months with 3 month intervals. The circulating of VEGF-1 level was assessed at baseline. Clinical interviews were conducted every 3 months for 1 year after receiving blood samples. As a clinical point we determined following cardiovascular outcomes: recurrent stroke or TIA, ischemic heart disease, sudden death, diabetes mellitus, cardiovascular events, including chronic heart failure and the need for hospitalization for these reasons.

Results: Analysis of obtained outcomes showed that increased VEGF-1 concentration within six months after ischemic stroke has positively associated with incidence of cardiovascular events, when compared with individuals without increased circulating levels of VEGF-1. Adjusted odds ratio for the occurrence of cumulative cardiovascular events in hypertension patients with VEGF-1 concentration at baseline above 403.57 pg/ml was 4.11 (95% CI=2.66-7.28; P=0.001), when compared with lower concentration of VEGF-1.

Conclusion: In conclusion, we found that incremented circulating vascular endothelial growth factor-1 level was an independent predictor of 1 year cumulative cardiovascular events in hypertensive patients after ischemic stroke.

Keywords: Vascular endothelial growth factor-1; Ischemic stroke; Arterial hypertension; Clinical outcomes; Predictive value

Introduction

Circulating inflammatory cytokines may play a pivotal role in manifestation of recurrent cardiovascular events due to atherothrombosis located in any vascular territories [1,2]. However, the role of low intensity proinflammatory activation in turn of modulation of recurrent cardiovascular events is not still understood and controversial [3-5]. It has been postulated that proinflammatory cytokines are able to modulate an activity of endothelial cells via induction of synthesis of vascular endothelial growth factor (VEGF) [6,7]. VEGF-1 belongs to superfamily of endothelial factors and produces pronounced angiopoetic capacity [6]. VEGF-1 realizes its biological effect by cooperation with tirosinkinase receptors located on endothelial cells surface that leads to cells growth, proliferation, and migration, as well as neovascularization and angiogenesis also [8-10]. Paracrine regulation of VEGF-1 activity that is performed by binding with specific solubilized receptor plays a pivotal role in a modulation of processing [11]. Recent studies have revealed that many biological markers of endothelial dysfunction, for example, such as VEGF-1, and some indicators of proinflammatory activation (high sensitive C-reactive protein) have not predicted value for unfavorable clinical outcomes in patients at low and moderate cardiovascular risk [12,13]. In contrast, similar association was found for patients at very high cardiovascular risk [14-16]. However, predicted value of serial measurements of VEGF-1 concentrations for recurrent cardiovascular events among hypertensive patients after ischemic stroke in follow-up is still not understood. The aim of the study was to investigate the predictive value of serial measurements of circulating vascular endothelial growth factor-1 level for recurrent cardiovascular events in hypertensive patients after ischemic stroke.

Methods

Study population

102 patients with mild-to-moderate arterial hypertension in 3 weeks after acute ischemic stroke were enrolled into investigation. Neurological impairment at presentation was assessed by National Institute of Health Stroke Scale (NIHSS) (National Institute of Health Stroke Scale) [17]. The type of acute ischemic stroke was classified according to the TOAST classification: 1) Large Artery Atherosclerosis (LAAS); 2) CardioEmbolic Infarct (CEI); 3) LACunar infarct (LAC); 4) stroke of Other Determined Etiology (ODE); 5) stroke of Undetermined Etiology (UDE) [18]. The Barthel Index [19] and the modified Rankin Scale [20] were used to assess functional disability. The functional outcome using these scales was evaluated at admission and on the 21st day of acute period of stroke before including to the study.

Contrast-enhanced computer spiral tomography

Contrast-enhanced computer spiral tomography (CT) was performed on a "Somatom Spirit" scanner (Siemens, Germany) with 2 rows of detectors. Nonionic contrast "Omnipak" (Amersham Health,

***Corresponding author:** Alexander E Berezin, Internal Medicine Department, State Medical University, Zaporozhye, Ukraine, Tel: +380612729607; Fax: +380612729607; E-mail: dr_berezin@mail.ru

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Ireland) was used. Scanning began at the cranial base and continued cranially for 80 mm. Total acquisition time average was 26 seconds.

Including and excluding criteria

Including criteria are: CEI, LAAS, LAC and types of acute ischemic stroke, mild-to-moderate arterial hypertension, age older than 18 years; sinus rhythm; written informed consent for participation to the study. Excluding criteria are: symptomatic chronic heart failure, left ventricular ejection fraction (LVEF) ≤ 39%, uncontrolled diabetes mellitus, severe kidney and liver diseases that have ability to influence independently on clinical outcomes, malignancy, unstable angina, Q-wave and non-Q-wave MI within 30 days before study entry; creatinin plasma level above 440 μmol/l, GFR index <35 ml/min/m^2, brain injury within 3 months before an enrollment, body mass index above 30 kg/m^2, and less 15 kg/m^2, pulmonary edema, tachyarrhythmia, valvular heart disease, thyrotoxicosis, UDE and ODE types of ischemic stroke, intracranial hemorrhage, acute infections, surgery, trauma, all ischemic events during the previous 3 months, and inflammatory conditions within 1 month, and incident of neoplasm were ruled out by careful medical history and physical examination previous to study entry; pregnancy; an implanted pacemaker, any disorders which according to investigators' opinion can stop the participation of the patients in the study, and patient's refuse to participate and to give consent to this study.

Clinical events determination

Clinical interviews were performed every month during 1 year period after baseline. Clinical events included following: new cases of stroke or TIA; death for any reasons and sudden cardiac death; coronary ischemic events (myocardial infarction, unstable angina, arrhythmia), need for hospitalization for cardiovascular reasons, newly onset of chronic heart failure and diabetes mellitus. Newly diagnosed stroke incidences were obligatory rule in CT. The diagnosis of heart failure was defined as an unplanned hospital admission for which the primary reason was clinical heart failure and it was based on clinical symptoms (limitation of activity, fatigue, and dyspnoea), physical signs (oedema, elevated jugular venous pressure, rales, or third heart sound with gallop), LVEF lowering obtained by Echo-examination, or radiological evidence of pulmonary congestion, and requirement of high dose loop diuretic, intravenous nitrate using or inotropic support. CAD, vascular events, and diabetes mellitus were defined according to contemporary clinical guidelines [18,21,22]. All clinical events were presented as cumulative ones.

Blood samples collection

All samples were collected in cooling vacutaner and after that they were immediately centrifuged (4°C for 6.000×15 min). After centrifugation serum was blind coded and stored at -70° until used. Concentrations of VEGF-1 were measured by ELISA at baseline and in 6 months of observation using laboratory kits produced by Bioscience (USA). All determinations were done by duplicate. The mean intra-assay coefficients of variation were <10% for all cases. High-sensitivity C-RP levels were measured by nephelometric technique and obtained with "AU640 Analyzer" (Olympus Diagnostic Systems Group, Japan). Concentrations of total and HDL cholesterol were determined by a Dimension Clinical Chemistry System (Dade Behring Inc, Newark, NJ). LDL cholesterol was calculated by using the formula of Friedewald W.T., Levy R.I., Fredrickson D.S. (1972).

Statistical analysis

All statistical analyses were performed in SPSS for Windows v. 17.0 (SPSS Inc., Chicago, Il, USA). All values were given as mean and 95% CI or median and percentiles. An independent group t-test was used for comparisons for all interval parameters meeting the criteria of normality and homogeneity of variance. For interval parameters which do not meet these criteria, the non-paramentric Mann-Whitney test was used to make comparisons between groups. Comparisons of categorical variables between groups were performed using the Chi2 test, and the Fisher exact test. The potential factors that may be associated with Cumulative Clinical Events (CCE) were identified first with the univariate analysis (ANOVA), and then Cox proportional hazards multivariate analyses were used to identify predictors of CCE. Receiver operating characteristic (ROC) curves were configured to establish cutoff points of VEGF-1 levels that optimally predicted the occurrence of cumulative clinical events. Kaplan-Meier survival curves were estimated for hypertensive patients depending on VEGF-1 levels. The calculated difference of $P<0.05$ was considered significant.

Results

General characteristics of study patient population

One hundred and two mild-to-moderate hypertensive patients (67 men and 35 women; mean age, 58.38 years [95% CI=54-72 years]) were included in this study in 3 weeks after first clinical signs of ischemic stroke. Baseline characteristics of the study group are presented in Table 1. All included patients were hypertensive at the screening (78 subjects with mild hypertension, and 24 subjects with moderated hypertension). It is noted that all patients were included in the study after achieved goal blood pressure (less than 140/90 mmHg). Besides 45.1% enrolled subjects were dyslipemic ones, 42.2% patients smoked, and 14.7% patients had the history of mild diabetes mellitus. LAAS type of ischemic stroke was defined in 2%, LAC and CEI were observed in 86.3% and 11.7% respectively. We found right-side injury of brain in 63.7% cases; in 34.3% and 2% cases left-side and two-side injuries were defined. NIHSS score of the series at admission and in 21 day after hospitalization date was 10 (interquartile range of 7-18) and 5 (interquartile range of 3-9) respectively. The median Barthel Index score was 65 (interquartile range of 40 to 85) at admission and 75 (interquartile range of 55 to 90) on 21st day of hospitalization; and the median Rankin Scale score was 4 (interquartile range of 2 to 5) at admission and on 21st day before enrollment respectively.

Median of total cholesterol and low-density cholesterol (LDL-C) plasma levels were 5.28 mmol/L (95% CI=3.82-6.74) and 3.26 mmol/L (95% CI=2.14-4.38) respectively. Target levels of LDL-C less 1.8 mmol/L and less 2.5 mmol/L were achieved in 23 (22.5%) and 33 (32.4%) patients at the study entry. Median of hs-CRP concentration was 5.91 mg/L (95% CI=2.90-10.55 mg/L).

No significant differences in hs-CRP levels were observed regarding age, sex, type of acute ischemic stroke occurred, initial BP, vascular risk factors, initial NIHSS score, initial Barthel Index score, initial Rankin Scale score, treatment or time from initial event to blood sampling. There was significantly increased median of hs-CRP concentration in cohort with clinical events (Me=7,24 mg/L, 95% CI=4,43-10,21 mg/L) when compared with free clinical events cohort (Me=4,47 mg/L, 95% CI=3,60-5,80 mg/L; P=0.012). No significant differences between cohorts regarding age, sex, type of acute ischemic stroke occurred, BP at the study entry, cardiovascular risk factors (BMI, dyslipidemia, low-

Variables	All patients (n=102)	Free clinical events cohort (n=55)	Cohort with clinical events (n=47)
Age, years	58.38 (95% CI=54-72)	57.2 (95% CI=56-69)	58.5 (95% CI=55-66)
Male, n (%)	67 (65.7%)	34 (61.8%)	33 (70.2%)
Systolic BP at admission, mm Hg	189.6 ± 2.91	185.2 ± 2.77	190.1 ± 2.33
Diastolic BP at admission, mm Hg	103.2 ± 1.28	103.1 ± 1.25	103.5 ± 1.19
Systolic BP at the study entry, mm Hg	137.9 ± 1.82	137.9 ± 1.82	139.1 ± 1.32
Diastolic BP at the study entry, mm Hg	80.3 ± 1.06	80.1 ± 1.02	81.2 ± 0.47
Mild hypertension, n (%)	78 (76.5%)	44 (80.0%)	34 (72.3%)
Moderate hypertension, n (%)	24 (23.5%)	11 (20.0%)	13 (27.7%)
Left-side localization, n (%)	35 (34.3%)	18 (32.7%)	17 (36.2%)
Right-side localization, n (%)	65 (63.7%)	34 (61.2%)	31 (66.0%)
Two-sides of weakness, n (%)	2 (2%)	1 (1.8%)	1 (2.1%)
LAAS, n (%)	2 (2%)	2 (3.6%)	0 (0%)
LAC, n (%)	88 (86.3%)	46 (83.6%)	42 (89.4%)
CEI, n (%)	12 (11.7%)	5 (9.1%)	7 (14.9%)
Initial NIHSS, mediana	10 (interquartile range of 7-18)	10 (interquartile range of 7-15)	11 (interquartile range of 8-16)
Initial Barthel Index score, mediana	65 (interquartile range of 40-85)	64 (interquartile range of 42-80)	65 (interquartile range of 45-82)
Initial Rankin Scale, mediana	4 (interquartile range of 2 to 5)	4 (interquartile range of 2 to 4)	4 (interquartile range of 2 to 5)
Current Smoking status, n (%)	43 (42.2%)	24 (43.6%)	19 (40.4%)
BMI, kg/m^2	24.8 ± 3.45	24.9 ± 3.12	23.9 ± 2.07
Dyslipidemia, n (%)	46 (45.1%)	22 (40.0%)	24 (51.1%)
T2DM, n (%)	15 (14.7%)	6 (10.9%)	9 (19.1%)*
hs-CPR, mg/L	5,91 (95% CI=2,90-10,55)	4,47 (95% CI =3,60-5,80)	7,24 (95% CI =4,43-10,21)*
Creatinine, μmol/L	96.8 (95% CI=61-138)	87.1 (95% CI =67-100)	99.5 (95% CI =72-122)
Triglycerides, mmol/L	1.57 (95% CI=0.92-2.22)	1.56 (95% CI =0.94-2.16)	1.57 (95% CI =0.92-2.20)
Total cholesterol, mmol/L	5.28 (95% CI=3.82-6.74)	5.02 (95% CI=3.90-5.88)	5.33 (95% CI=4.35-6.23)*
LDL- cholesterol, mmol/L	3.26 (95% CI=2.14-4.38)	3.14 (95% CI=2.19-4.22)	3.42 (95% CI=2.16-4.30)
Fasting glucose, mmol/L	5.61 (95% CI=4.23-6.99)	5.32 (95% CI=4.30-6.10)	5.70 (95% CI=4.72-6.82)
ACE inhibitors at the study entry, n (%)	101 (99%)	54 (98.2%)	47 (100%)
Aspirin before admission, n (%)	87 (85.3%)	48 (87.3%)	39 (83.0%)
Aspirin at the study entry, n (%)	91 (89.2%)	48 (87.3%)	43 (91.5%)
Other antiaggregants at the study entry, n (%)	11 (10.9%)	7 (12.7%)	4 (8.5%)*
Beta-adrenoblockers at the study entry, n (%)	54 (52.9%)	28 (50.9%)	26 (55.3%)
Diuretics at the study entry, n (%)	77 (75.5%)	43 (78.2%)	35 (74.5%)
Statins before admission, n (%)	71 (69.6%)	40 (72.7%)	31 (66.0%)*
Statins at the study entry, n (%)	82 (80.4%)	44 (80.0%)	38 (80.9%)
Calcium channel blockers at the study entry, n (%)	78 (76.5%)	43 (78.2%)	35 (74.5%)

Note: T2DM- Type two Diabetes Mellitus; NIHSS- National Institute of Health Stroke Scale; LAAS- Large Artery AtheroSclerosis; CEI- CardioEmbolic Infarct; BMI- Body Mass Index; LDL- Low Density Lipoprotein; hs-CRP- high-sensitivity C-Reactive Protein.
*- significance differences between cohorts (P<0.05)

Table 1: Baseline characteristics of the study group.

density cholesterol, fasting glucose), initial NIHSS score, initial Barthel Index score, initial Rankin Scale score were found. T2DM was occurred much higher in cohort with clinical events when compared with free clinical events patients. Total cholesterol plasma level was significantly higher in cohort patients with clinical event occurred in comparison to free-event patients. Treatment strategy was similar in both cohorts, but there was a significant increased frequency of antiaggregants distinguished form aspirin in patients with clinical events occurred. Statins before admission were prescribed much often in free-events cohort patients. It was taken into consideration that at the study entry proportions of the patients with prescribed statins were similar. Statins (atorvastatin in 56 cases, and simvastatin in 15 cases) before admission were taken by 71 (69.6%) enrolled subjects. Median of daily doses for atorvastatin and simvastatin were 30 mg orally (interquartile range of 20 mg to 60 mg) and 20 mg (interquartile range of 10 mg to 40 mg) respectively. After admission there was not withdrawing of statins at all, and at the study entry 82 (80.4%) patients have been taking atorvastatin in median daily oral dosage equivalent of 40 mg (interquartile range of 20 mg to 80 mg).

All subjects before study entry were hemodynamically stable and they have controlled arterial hypertension and remained free of any ischemic events during the time elapsed between the first qualifying episode and the inclusion visit date. During observation period 57 cumulative clinical events occurred and they were identified in 48 patients (47.1%). They were distributed in 4 deaths, 6 cardiac arrhythmias, 17 cardiac ischemic events, 9 stroke (5 lacunar infarctions, and 2 cardioembolic strokes), 10 diabetes mellitus, 4 chronic heart failure and 7 hospitalizations for cardiovascular reasons, newly onset chronic heart failure and diabetes mellitus.

Circulating VEGF-1 levels in both cohorts subjects with and without recurrent cardiovascular events

Analysis of obtained results has shown that median of VEGF-1 concentration at baseline in subjects with recurrent cardiovascular events when compared with patients without newly outcomes were similar (Me=344.87 pg/ml, 95% CI=245.67-493.46 pg/ml and Me=352.10 pg/ml, 95% CI=205.31-573.81 pg/ml respectively, P>0.1). The significant difference between VEGF-1 concentrations

in hypertensive patients depended on age, gender, types of ischemic stroke, severity of hypertension, and blood pressure values at baseline as well as in depended on conventional cardiovascular risk factors, NIHSS, Barthel index, and Rankin score index was found.

For further analysis concentration of VEGF-1 was presented depending on numerous of recurrent cardiovascular events in follow-up. We found that circulating of VEGF-1 levels at baseline in patients with one, two, three and more recurrent cardiovascular events were 373.80 pg/ml (95% CI=342.90-479.70 pg/ml), 539.96 pg/ml (95% CI=444.28-865.56 pg/ml) and 724.66 pg/ml (95% CI=558.72-890.66 pg/ml) respectively. Moreover, VEGF-1 levels at baseline for these subjects were significantly higher than free events patients (Me=289.28 pg/ml; 95% CI=279.71-345.88 pg/ml) (P=0.001 for all cases).

Using Receive Operations Curve (ROC) analysis, we found that the most optimal cutoff-point of circulating VEGF-1 in hypertensive patients at baseline was 403.57 pg/ml (sensitivity and specificity were 78.6% and 70.0%, positive and negative likelihood ratio equal 1.12 and 0.305). Area under ROC curve (AUC) was 0.76 (95% CI=0.602-0.917; P=0.001).

Univariant regression analysis has showed that overall one year incidence of cardiovascular events closely and significantly associated with circulating VEGF-1 at baseline more 403.57 pg/ml (R=0.510; P=0.001), circulating hs-CRP (R=0,508; P=0,001), total cholesterol plasma level (R=0.504; P=0.001), T2DM (R=0.468; P=0.001), LDL-C plasma level (R=0.443; P=0.002), age (R=0.431; P=0.001), male sex (R=0.416; P=0.001), current smoking (R=0.402; P=0.001), diastolic blood pressure (R=0.372; P=0.001). Using multivariate analysis we have defined some predictors of cardiovascular events in follow-up. It turned that circulating VEGF-1 at baseline more 403.57 pg/ml (R=0.508; P=0.001), circulating hs-CRP (R=0.498; P=0.001), T2DM (R=0.454; P=0.001), and male sex (R=0.407; P=0.001) were irrespectively associated with incidence of cardiovascular events within one year after the occurrence of ischemic stroke.

The predictive value of OPN concentration in study patient population

Circulating VEGF-1 level at baseline more 403.57 pg/ml (B coefficient=0,002; Wald test=6.515; P=0.011), circulating hs-CRP (B coefficient=0.392; Wald test=5,784; P=0,016), male sex (B coefficient=0.025; Wald test=1.885; P=0.012) had the most significant prognostic potential. It supposed that the explanatory variables were significant for use in the model. In this regard, we have been corrected the data according to gender and level of circulating hs-CRP for subsequent Cox regression analysis. However, it has determined that adjusted odds ratio (OR) in cumulative cardiovascular events in hypertensive patients with VEGF-1 level at baseline more 403.57 pg/ml versus VEGF-1 level less 403.57 pg/ml was 4.11 (95% CI=2.66-7.28; P=0.001).

Kaplan-Meier curves showed (Figure 1) that an accumulation of cardiovascular events was superior in patients with VEGF-1 concentration at baseline more 403.57 pg/ml when compared with subjects with VEGF-1 level less this cutoff point (P=0.001). The curves divergence of events accumulation reached a statistical significance in 14 weeks of observation period.

Thus, these data allowed us to establish the fact that circulating VEGF-1 within 3 weeks after ischemic stroke closely associates with increased risk of recurrent cardiovascular events in a cohort of patients with controlled arterial hypertension.

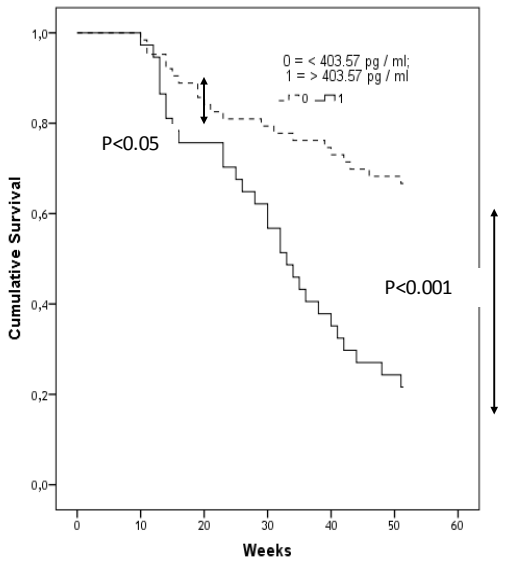

Figure 1: Kaplan-Meier curves indicate differences between groups in cumulative cardiovascular events accumulation depended on circulating VEGF-1.

Discussion

Results obtained by us support the hypothesis that circulating VEGF-1 is an independent one-year predictor of cardiovascular outcomes including atherothrombotic events, in hypertensive patients after serious ischemic brain event. Many recent clinical trials did not indicate predictive value of VEGF-1 peak concentrations among symptomatic atherosclerotic carotid plaque patients after stroke [13], while theoretical backgrounds for such hypotheses are very attractive [23,24]. In particular, it was found that VEGF-1 secretion due to focal brain ischemia mediates to realize a neuroprotection, to improve neoangiogenesis and neurogenesis [25,26]. On the other hand, VEGF-1 is able to induce post-ischemic neurovascular remodeling and apoptosis [27]. Probably, these mechanisms underlie the violation of spatial progressive perivascular citoarchitectonics, expanding penumbra zone and worsening cerebral ischemia [28]. Since an angiopoetic effect of VEGF-1 is systemic, it might be assumed that neovascularization in the vulnerable atheroma cite will promote progressive worsening of mechanical capacity of the atheroma cap, the formation of the phenomenon of "fatigue" cap, appearance of endothelial dysfunction and deregulation of vascular tone, which ultimately leads to a corresponding atherothrombotic events in any vascular territories [29]. Thus, we suggested that in hypertensive patients after ischemic stroke immediate effects of VEGF-1 probably are adaptive in nature, while deferred effects may be associated with recurrent clinical events, in particular, mediated by atherothrombosis [23,30]. This hypothesis was confirmed by the results of our study. It should be noted that all patients included in the trial had controlled blood pressure, and the majority of them continued to receive ACE inhibitors, calcium channel blockers, statins and antiplatelet therapy after stroke. However, despite the use of statins, the target levels of LDL-C were not achieved in most patients. Taken into consideration the fact that statins are able to implement the anti-proliferative and anti-inflammatory effects, our findings can be interpreted as an indirect argument in favor of expanding the use of statins in hypertensive patients directly after stroke. This assumption needs to be confirmed in studies with greater statistical power.

In conclusion, we found that increase of circulating VEGF-1 in hypertensive patients after ischemic stroke had one-year predicted value for cardiovascular recurrent events.

Limitations of the Study

This study has some limitations. We believed that a greater cohort would be desirable to improve the power of the study because low rates of recurrent strokes and deaths were detected. We also relied on clinical data to rule out infection and other inflammatory diseases before sampling, but we cannot exclude that some patients had unrecognized conditions responsible for the elevated hs-CRP and VEGF-1 levels observed. However, additional verification of atherosclerosis as well as intracranial artery occlusive disease can be required. We supposed that these limitations might not have a significant influence to study data interpretation.

Ethical Declaration

The study was approved by the local ethics committee of State Medical University, Zaporozhye, Ukraine. The study was carried out in conformity with the Declaration of Helsinki.

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