UDC: 616.127-005.8:616.124-007.61-07:577.112

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Predictive value of circulating inflammatory biomarkers in hypertensive patients after acute ischemic stroke

Objective. The aim of the study was to investigate the predictive value of inflammatory biomarkers in hypertensive patients after acute ischemic stroke. Methods. 102 patients with mild to moderate arterial hypertension within 3 weeks after ischemic stroke were included in the study with follow-up observation during 12 months with 3 month intervals. The circulating level of vascular endothelial growth factor-1 (VEGF-1), metalloproteinase-9 (MMP-9), and high sensitive C-reactive protein (hs-CRP) was assessed at baseline. Clinical interviews had been conducted every 3 months for 1 year after receiving blood samples. All recurrent cardiovascular outcomes were determined as end points. Results. Analysis of obtained outcomes have been shown that circulating levels of VEGF-1 over 403.57 pg/mL (Odds Ratio [OR]=2.78, 95% confidence interval [CI]=2.4–2.95; Wald test=6.515; P=0.011) appears to be the most significant prognostic value for cumulative clinical events (CCE). However, circulating levels of MMP-9 (OR=2.61; 95% CI=2.37–3.16; Wald test=7.32; P=0.001), diabetes mellitus, type 2 (OR=2.11; 95% CI=1.47–2.59; Wald test=3.52; P=0.001), hs-CRP (OR=1.62; Wald test=5,784; P=0,003), and male sex (OR=1.25; Wald test=1.885; P=0.012) were also determined as clinically significant predictors for CCE after acute ischemic stroke. Conclusions. Using reclassification methods, however, we found that addition of both biomarkers combination (hs-CRP + MMP-9) to the ABC model (VEGF-1) improved the relative IDI for CCE by 9.3%. In conclusion, we found that combination of three inflammatory circulating biomarkers (VEGF-1, hs-CRP, MMP-9) in hypertensive patients after ischemic stroke represent the best one-year predictive values for cardiovascular recurrent events risk, when compared to either of biomarker alone.

Key words: *vascular endothelial growth factor-1, metalloproteinase-9, high sensitive C-reactive protein, ischemic stroke, hypertension, clinical outcomes, predicted value recurrent cardiovascular outcomes.*

Introduction

Ischemic stroke is one of the leading causes of disability and death in industrialized countries (Go A.S. et al., 2013). Recent studies indicate that low intensity inflammatory activation might play a pivotal role in modulation of recurrent cardiovascular events (Luo Y. et al., 2012; Tuttolomondo A. et al., 2012). Ischemia may activate the brain proteases such as metalloproteinases (MMP), endogenous tissue plasminogen activator, and other types of inflammatory cytokines, and factors that promote vascular remodeling (vascular endothelial growth factor and high-moblity-group-box-1). Therefore, proinflammatory cytokines, such as C-reactive protein (CRP) are able to modulate an activity of endothelial cells via induction of synthesis of vascular endothelial growth factor (VEGF) (Ferrara N. et al., 2003; Takahashi H., Shibuya M., 2007) and extracellular remodeling through MMP-9 over expression (Blankenberg S. et al., 2003).

VEGF-1 belongs to superfamily of endothelial factors and has pronounced angiopoetic capacity (Orecchia A. et al., 2003). VEGF-1 realizes their biological effect thereby cooperation with tirosinkinase receptors located on endothelial cells surface that leads to cells growth, proliferation, and migration, as well as neovascularization and angiogenesis (Shen F. et al., 2011; Luque A. et al., 2003; Siow R.C.M., Churchman A.T., 2007). Paracrine regulation of VEGF-1 activity thereby binding with specific solubilized receptor plays a pivotal role in processing modulation (Ridker P.M. et al., 2000).

Proinflammatory cytokines facilitate leukocytes infiltration into brain tissue by activating and inducting molecules' adhesion on vascular endothelial cells. As a result, leukocyte-derived MMP-9 is secreted by activated macrophages and may damage the neurovascular unit and promote blood-brain barrier (BBB) disruption (Amantea D. et al., 2009). This process associates with reactive oxygen species overproduction and may lead to hemorrhagic transformation that is a common complication of ischemic stroke with poor clinical outcomes (Jickling G.C. et al., 2014).

Recent studies revealed that number of biological markers of endothelial dysfunction, such as VEGF-1, MMP-9, and some indicators of proinflammatory activation (high sensitive-CRP) have not predicted value for unfavorable clinical outcomes in patients at low and moderate cardiovascular risk (Khurana D. et al., 2013; Adams H.P. et al, 1993). In contrast, the association between high sensitive CRP and VEGF-1 was found to be of high prognostic value for patients at high cardiovascular risk (Ridker P.M. et al., 2001; 2008; Lyden P.D. et al., 2001). While inflammatory biomarkers predict incident and recurrent cardiac events, their relationship to stroke prognosis is still uncertain and poorly understood (Elkind M.S. et al., 2014).

The aim of the study was to investigate the predictive value of inflammatory biomarkers in hypertensive patients after acute ischemic stroke.

Methods

102 patients with mild-to-moderate arterial hypertension in 3 weeks after documented acute ischemic stroke and achieved clinical stabilization period have been enrolled in the study. The sample size was computed on sample size calculators (*http://www.sealedenvelope.com/power/binary-superiority/*) taken into consideration that the level of significance is 5%, Zα/2 is 1.96. The result indicates that the total sample size required is 93 patients. With adjustment for non-compliance/cross-over and lost for follow-up patients (10%) the total sample size was calculated for 102 subjects.

Neurological impairment at presentation was assessed by National Institute of Health Stroke Scale (NIHSS) (American College of Cardiology Foundation/American Heart Association Task Force1; American Stroke Association; American Association of Neuroscience Nurses et al., 2011). The type of acute ischemic stroke was classified according to the TOAST (Trial of Org 10172 in Acute Stroke Treatment) classification for follow major categories (Adams H.PJr. et al., 1993):

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) (Collin C. et al., 1988)].

The Barthel Index (Hacke W. et al., 1998) and the modified Rankin Scale (Williams S.V. et al., 2001) were used to assess functional

disability. The functional outcome of the patents was evaluated at admission and after achieving clinical stabilization period (in average on the 21st day of acute period of stroke) before including in the study.

Contrast-enhanced computer spiral tomography (CT) scans were performed with using «Somatom Spirit» scanner (Siemens, Germany) at admission with control scanning at 24 to 48 hours, or after neurological worsening occurred. Nonionic contrast «Omnipak» (Amersham Health, Ireland) was used. Scanning began at the cranial base and continued cranially for 80 mm. Total acquisition time average was 26 seconds. Symptomatic intracranial hemorrhage was defined as absolute exclusion criteria and was determined as bleeding at any site in the brain associated with neurological deterioration. CEI, LAAS, LAC and other types of acute ischemic stroke, mild-to-moderate arterial hypertension, age >18 years; sinus rhythm; obtained informed consent for participation to the study were determined as including criteria. Excluding criteria were defined as symptomatic chronic heart failure, left ventricular ejection fraction (LVEF) ⩽ 39%, uncontrolled diabetes mellitus, severe kidney and liver diseases that have ability to independently influence to clinical outcomes, malignancy, unstable angina, Q-wave and non-Q-wave MI within 30 days before study entry; creatinin plasma level >440 μmol/L, globular filtration rate (GFR) index < 35 ml/min/м^2, pulmonary edema, tachyarrhythmia, valvular heart disease, thyrotoxicosis, intracranial hemorrhage, acute infections, surgery, trauma, all ischemic events during the previous 3 months, inflammatory conditions within 1 month, and incident of neoplasm were ruled out by thorough evaluation of medical history and physical examination before entering the study; pregnancy; an implanted pacemaker, any disorders that according to investigators' opinion could be the cause either of withdrawing, or participation refusal or obtaining informed consent.

Blood samples

All samples were collected in cooling vacutaner at baseline and with 6 months interval of follow-up period, and immediately centrifuged (at t =4 °C for 6,000 rpm × 15 min). After centrifugation serum was blind coded and stored at t=–70 °C until used.

Levels of VEGF-1 and MMP-9 were measured by ELISA using commercial laboratory kits («Bioscience», USA; «R&D System Europe, Ltd.», United Kingdom) respectively. All assays were performed in duplicate for each biomarker. The mean intra-assay coefficients of variation were <10% for all cases.

Hs-CRP levels were measured by nephelometric technique and obtained by using «AU640 Analyzer» (Olympus Diagnostic Systems Group, Japan). Concentrations of total and HDL cholesterol were evaluated with the usage of Dimension Clinical Chemistry System («Dade Behring Inc.», Newark, NJ).

Low-density cholesterol (LDL-C) was calculated by using the formula of W.T. Friedewald et al. (1972).

Clinical event determination

Clinical interviews were performed every month during 1 year period since baseline. Clinical events included following: new cases of stroke or transient ischemic attack (TIA); death for any reasons and sudden cardiac death; coronary ischemic events (myocardial infarction, unstable angina, arrhythmia), need of hospitalization for cardiovascular reasons, newly onset chronic heart failure and diabetes mellitus. Newly diagnostic 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 was based on clinical symptoms (limitation of activity, fatigue, and dyspnea), physical signs (edema, 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. Coronary artery disease, vascular events, and diabetes mellitus were defined according to the current clinical guidelines (Collin C. et al., 1988; Williams S.V. et al., 2001; Sacks D.B. et al., 2011). All clinical events have been represented as cumulative.

Ethical Declaration

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

Statistical Analysis

All statistical analyses were performed in SPSS (Statistical Package for the Social Sciences) for Windows v. 20.0 («SPSS Inc.», Chicago, Il, USA). All values were given as mean (M) and standard deviation (±SD) or median (Me) and 95% confidence interval (CI). For scaled values interquartile range was calculated. An independent group *t*-test was used for comparisons all the interval parameters meeting the criteria of normality and homogeneity of variance. For interval parameters not meeting 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 likely associated with Cumulative Clinical Events (CCE) were identified first with the univariate analysis (ANOVA). Receiver operating characteristic (ROC) curves were configured to establish cutoff points of biological markers that optimally predicted the occurrence of CCE. Cox proportional odds multivariate analyses were used to identify predictors of CCE. Reclassification methods with IDI (integrated discrimination index) and NRI (net reclassification improvement) calculation were used for comparison of predictive models. A calculated difference of P<0.05 was considered significant.

Results

One hundred two mild-to-moderate arterial hypertension patients (67 men and 35 women; mean age, 58.38 years [95% CI=54–72 years]) were included in the 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, and 24 subjects with moderated hypertension). All patients were included in the study after achieved goal blood pressure levels (⩽140/90 mm Hg). Besides 45.1% enrolled subjects were dyslipidemic, 42.2% patients were smoked, and 14.7% patients had a history of mild diabetes mellitus. LAAS type of ischemic stroke was defined in 2% of cases, LAC and CEI were observed in 86.3% and 11.7% of cases, respectively. We found right-side injury of brain in 63.7% of cases; in 34.3% and 2% — left-side and two-side injuries, respectively, were defined. NIHSS score of the series at admission and in 21 days after hospitalization was 10 (interquartile range 7–18) and 5 (interquartile range of 3-9), respectively. The median Barthel Index score was 65 (interquartile range 40–85) at admission and 75 (interquartile range 55–90) on the 21st day of hospitalization; and the median Rankin Scale score was 4 (interquartile range 2–5) at admission and on the 21st day before enrollment, respectively.

Median of total cholesterol and 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. Median of hs-CRP concentration was 5.91 mg/L (95% CI=2.90–10.55 mg/L).

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 20–60 mg) and 20 (interquartile range 10–40 mg), respectively. After admission there was no withdrawing of statins at all, and at the study entry to 82 (80.4%) patients atorvastatin had been administered in median daily orally dosage equivalent 40 mg (interquartile range 20–80 mg). Target levels of LDL-C — <1.8 mmol/L and <2.5 mmol/L — had been achieved in 23 (22.5%) and 33 (32.4%) patients at the study entry.

No significant differences between cohorts regarding age, sex, type of acute ischemic stroke occurred, BP at the study entry, cardiovascular risk factors (body mass index (BMI), dyslipidemia, low-density cholesterol, and fasting glucose), initial NIHSS score, initial Barthel Index score, and initial Rankin Scale score. The type two diabetes mellitus (T2DM) was occurred a much higher in cohort with clinical events when compared with free clinical events patients. Total cholesterol plasma level was a significant 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 agntiaggregants distinguished form aspirin in patients with clinical events occurred. Statins before admission were prescribed much often in free-event cohort patients. It has taken into consideration that at the study entry proportions of the patients with statins prescribing was similar.

Table 1 Baseline characteristics of the study group

	All patients (n=102)	Free clinical events cohort (n=55)	Clinical events cohort (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, median	10 (interquartile range 7–18)	10 (interquartile range 7–15)	11 (interquartile range 8–16)
Initial Barthel Index score, median	65 (interquartile range 40–85)	64 (interquartile range 42–80)	65 (interquartile range 45–82)
Initial Rankin Scale, median	4 (interquartile range 2–5)	4 (interquartile range 2–4)	4 (interquartile range 2–5)
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)
Acetylsalicylic acid before admission, n (%)	87 (85.3)	48 (87.3)	39 (83.0)
Acetylsalicylic acid 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)

Abbreviations: ACE – angiotensin converting enzyme; *significance differences between cohorts (P<0.05).

Clinical event determination within the study

All subjects before entering the study were hemodynamically stable, with 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 CCE occurred and were identified in 48 patients (47.1%). The events distributed as follows: 4 — deaths, 6 — cardiac arrhythmias, 17 — cardiac ischemic events, 9 — LAAS stroke, 5 — LAC and 2 — CEI, 10 — diabetes mellitus, 4 — chronic heart failure, and 7 — hospitalizations for cardiovascular reasons, new-onset chronic heart failure and diabetes mellitus.

Inflammatory biomarkers in two patients cohorts

Analysis of obtained results has been shown that median of VEGF-1 concentration at baseline in subjects with recurrent cardiovascular events when compared with patients without clinical outcomes were similar (Me=344.87 pg/mL, 95% CI=245.67–493.46 pg/mL, and Me=352.10 pg/mL, 95% CI=205.3–573.81 pg/mL, respectively, P=0.42). However, when we presented VEGF-1 in relation with numerous of recurrent cardiovascular events in follow-up, we found that circulating VEGF-1 levels in subjects 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 (P=0.001 for all cases).

Median of circulating MMP-9 in patients with recurrent cardiovascular events compared to those without them were 1059.50 ng/mL (95% CI=801.1–1514.51 ng/mL) and 679.77 ng/mL (95% CI=590.11–769.43 ng/mL), respectively (P<0.001). No significant changes of MMP-9 depending on numerous recurrent cardiovascular events in follow-up.

There was a significantly increased median of hs-CRP concentration in clinical events cohort (Me=7.24 mg/L, 95% CI=4.43–10.21 mg/L) when compared to free clinical event cohort (Me=4.47 mg/L, 95% CI=3.60–5.80 mg/L; P=0.012). We did not found significant changes of MMP-9 level depending on numerous of recurrent cardiovascular events.

The significant difference between concentrations of inflammatory biomarkers in hypertensive patients depended on age, gender, types of ischemic stroke, severity of hypertension, distribution of conventional cardiovascular risk factors, NIHSS, Barthel index, and Rankin score index was not found.

Predictive value of inflammatory biomarkers

Using ROC-analysis, we found that the most optimal decremented cut-off point of irculating VEGF-1 in hypertensive patients was 403.57 pg/mL (sensitivity and specificity were 78.6% and 70.0%, respectively). Area under ROC curve (AUC) was 0.76 (95% CI=0.602–0.917; P=0.001). The most optimal cut-off point of circulating MMP-9 was 1001.0 ng/mL (sensitivity =85.7%, specificity =72.1%), AUC was 0.814 (95% CI=0.671–0.958; P=0.001). The cut-off point with the best decremented value of hs-CRP was 5.58 mg/L (AUC=0.814; 95% CI=0.702–0.925; sensitivity =76.7%, specificity =80.3%).

Univariant regression analysis has shown that overall one year incidence of cardiovascular events closely and significantly associated with circulating VEGF-1 more 403.57 pg/ml (r=0.510; P=0.001), circulating hs-CRP (r=0,508; P=0,001), MMP-9 over 1001.0 ng/ml (r=0.68; 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 identified some predictors of cardiovascular events. However, circulating VEGF-1 >403.57 pg/mL (r=0.508; P=0.001), MMP-9 >1001.0 ng/mL (r=0.622; 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) independently predicted incidence of cardiovascular events within one year after acute ischemic stroke.

Circulating VEGF-1 over 403.57 pg/mL (odds ratio [OR]=2.78, 95% CI=2.41–2.95; Wald test=6.515; P=0.011) demonstrated the most significant prognostic value for CCE. However, circulating MMP-9 (OR=2.61; 95% CI=2.37–3.16; Wald test=7.32; P=0.001),

T2DM (OR=2.11; 95% CI=1.47–2.59; Wald test=3.52; P=0.001), hs-CRP (OR=1.62; Wald test=5,784; P=0,003), and male sex (OR=1.25; Wald test=1.885; P=0.012) were also determined as clinically significant predictors for CCE after acute ischemic stroke.

Using reclassification methods, however, we found that the including both biomarkers (hs-CRP + MMP-9) into the ABC model (VEGF-1) improved the relative IDI by 9.3% for CCE (Table 2).

For category-free NRI, 11% of events (p=0.001) and 26% of non-events (p=0.0001) were correctly reclassified by the addition of hs-CRP+MMP-9 to the ABC model for CCE (Table 3). Thus, we suggested that VEGF-1+hs-CRP+MMP-9 remained statistically significant predictors for CCE in hypertensive patients after acute ischemic stroke.

Discussion

Results of the study support the hypothesis that inflammatory biomarkers are independent one-year predictors of cardiovascular outcomes in hypertensive patients after acute ischemic stroke. Many of recent clinical trials did not indicated a predicted value of VEGF-1 peak concentrations among symptomatic atherosclerotic carotid plaque patients after stroke (Adams H.P. et al., 1993), while theoretical backgrounds for such hypotheses are promising (Sun Y. et al., 2003; Zhao H. et al., 2011). On the one hand, VEGF-1 secretion due to focal brain ischemia mediates to provide neuroprotection, to improve neoangiogenesis and neurogenesis (Hayashi T. et al., 1998; Hermann D.M., Zechariah A., 2009). On the other hand, VEGF-1 is able to induce post-ischemic neurovascular remodeling and apoptosis (Lo E.H., 2008). Probably these mechanisms underlie the violation of spatial progressive perivascular cytoarchitectonics, expanding penumbra zone and worsening post-acute cerebral ischemia (Testa U. et al., 2008).

Since an angiopoetic effect of VEGF-1 consider 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 (Zachary I. et al., 2000). Therefore, biomarkers of brain injury, such as MMP-9 and hs-CRP, were identified as powerful predictors of clinical outcomes, including intracerebral hemorrhage, in acute stroke, while their role in postacute period is still not understood (Luo Y. et al., 2012; Sapojnikova N. et al., 2014; Wen D. et al., 2014).

Thus, we suggested that in hypertensive patients after ischemic stroke immediated effects of VEGF-1 probably are adaptive in nature, while deferred effects may be associated with recurrent clinical events, in particular, mediated by atherothrombosis (Elkind M.S. et al., 2014). With regard to this, combination of three inflammatory biomarkers, characterized both sides of pathogenesis of postacute stroke — injury and repair — represent the best detrimental predictive value for clinical outcomes. This hypothesis was confirmed by the results of the study. It should be noted that all patients included in the trial had controlled blood pressure, and the majority of them continued to receive treatment with ACE inhibitors, calcium channel blockers, statins and antiplatelet therapy during after-stroke period. However, despite the use of statins, most patients failed to achieve targeted levels of LDL-C. Taken into consideration the fact that statins are able to provide anti-proliferative and anti-inflammatory effects, our findings could 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 combination of three inflammatory circulating biomarkers (VEGF-1, hs-CRP, MMP-9) in hypertensive patients after ischemic stroke represent the best one-year predictive value for cardiovascular recurrent event when compared with each biomarker alone.

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, MMP-9 and VEGF-1 levels observed. However, additional verification of atherosclerosis as well as intracranial artery occlusive disease could be required. We supposed these limitations might not have a significant influence to study data interpretation.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interest

The authors declare no conflict of interest.

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Table 2 C-statistics for Models with circulating VEGF-1, MMP-9, hs-CRP, T2DM, and male sex as continuous variables

Models	AUC (95% CI)	ΔAUC	IDI (±SE)	Relative IDI (%)
	Cumulative Clinical Events			
Model 1 (VEGF-1)	0.760	–	–	–
Model 1 + hs-CRP	0.794	–	–	–
Model 1 + hs-CRP *vs* Model 1	–	0.034; P=0.01	0.02±0.006	4.9
Model 1 + MMP-9	0.826	–	–	–
Model 1 + MMP-9 *vs* Model 1	–	0.066; P=0.01	0.03±0.01	6.7
Model 1 + T2DM	0.813	–	–	–
Model 1 + T2DM *vs* Model 1	–	0.053; P=0.01	0.04±0.012	5.2
Model 1 + male sex	0.788	–	–	–
Model 1 + male sex *vs* Model 1	–	0.028; P=0.01	0.01±0.004	4.2
Model 1 + hs-CRP + MMP-9	0.834	–	–	–
Model 1 + hs-CRP + MMP-9 vs Model 1	–	0.074; P=0.001	0.03±0.011	9.3
Model 1 + MMP-9 + T2DM	0.814	–	–	–
Model 1 + MMP-9 + T2DM *vs* Model 1	–	0.054; P=0.01	0.04±0.014	5.2
Model 1 + hs-CRP + T2DM	0.812	–	–	–
Model 1 + hs-CRP + T2DM *vs* Model 1	–	0.051; P=0.01	0.02±0.008	3.6

Relative IDI – calculated as the ratio of IDI over the discrimination slope of the model without VEGF-1.
Abbreviations: AUC – area under curve, SE – standard error.

Table 3 Prediction performance analyses for Models with VEGF-1, MMP-9, hs-CRP, T2DM, and male sex as continuous variables

	Model 1 + hs-CRP + MMP-9 *vs* Model 1
	Cumulative Clinical Events
Categorical NRI	0.19 (95% CI=0.11–0.25)
Percentage of events correctly reclassified	5 (p=0.14)
Percentage of non-events correctly reclassified	10 (p=0.011)
Categorical free NRI	0.63 (95% CI=0.48–0.81)
Percentage of events correctly reclassified	11% (p=0.001)
Percentage of non-events correctly reclassified	26% (p=0.0001)

Model 1– circulating VEGF-1.

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Прогностична цінність циркулюючих прозапальних біомаркерів у пацієнтів із гіпертонічною хворобою III стадії після перенесеного мозкового ішемічного інсульту

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Резюме. *Мета дослідження — вивчення прогностичної цінності прозапальних біомаркерів у пацієнтів із гіпертонічною хворобою після перенесеного мозкового ішемічного інсульту. Об'єкт і методи дослідження. У дослідження включено 102 пацієнти із артеріальною гіпертензією м'якого та помірного ступеня через 3 тиж після розвитку ішемічного інсульту. Повторні дослідження в період спостереження проводили із 3-місячним інтервалом протягом 1 року. Циркулюючі рівні васкулярного ендотеліального фактора росту-1 (ендотеліального фактора росту-1 — VEGF-1), металопротеїнази-9 (matrix metalloproteinase-9 — ММП-9), високочутливого (high sensitivity) С-реактивного протеїну (hs-СРП) оцінювали на початку дослідження. Клінічні огляди проводили кожні 3 міс протягом 1 року після отримання зразків крові. Кінцевими точками визначено всі рекурентні серцево-судинні події. Результати та їх обговорення. Аналіз отриманих даних продемонстрував, що концентрації у плазмі крові VEGF-1 >403,57 пг/мл (відношення шансів [ВШ]=2,78; 95% довірчий інтервал [ДІ] 2,41–2,95; тест Вальда=6,515; p=0,011) мають найвище прогностичне значення для кардіоваскулярних подій при одноріч ному спостереженні. Окрім того, такі фактори, як ММП-9 (ВШ=2,61; 95% ДІ 2,37–3,16; тест Вальда=7,32; p=0,001), наявність цукрового діабету 2-го типу (ВШ=2,11, 95% ДІ 1,47–2,59; тест Вальда=3,52; p=0,001), hs-СРП (ВШ=1,62; тест Вальда=5,784; p=0,003), чоловіча стать (ВШ=1,25; тест Вальда=1,885; p=0,012) також визначені як клінічно значущі предиктори кумулятивних серцево-судинних подій у період після перенесеного ішемічного інсульту. Застосування методів реклассифікації показало, що включення комбінації двох біомаркерів (hs-СРП+ММР-9) до моделі ABC (VEGF-1) сприяло підвищенню декримінаційного індексу для кардіоваскулярних подій на 9,3%. Висновки. Поєднання трьох прозапальних біомаркерів (VEGF-1, hs-СРП, ММР-9) у пацієнтів із гіпертонічною хворобою з ішемічним інсультом в анамнезі мають найкращу прогностичну цінність щодо ризику розвитку рекурентних кардіоваскулярних подій протягом 1 року спостереження порівняно з ізольованим застосуванням будь-якого з біомаркерів.*

Ключові слова: *васкулярний ендотеліальний фактор росту-1, металопротеїназа-9, високочутливий С-реактивний протеїн, ішемічний інсульт, артеріальна гіпертензія, клінічні наслідки, прогностичне значення, рекурентні кардіоваскулярні події.*

Прогностическая ценность циркулирующих провоспалительных биомаркеров у пациентов с гипертонической болезнью III стадии после перенесенного мозгового ишемического инсульта

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Резюме. *Цель исследования — изучение прогностической ценности провоспалительных биомаркеров у пациентов с гипертонической болезнью после перенесенного ишемического инсульта. Объект и мето-*

ды исследования. В исследование включены 102 пациента с артериальной гипертензией мягкой и умеренной степенями через 3 нед после развития ишемического инсульта. Повторные исследования в период наблюдения проводили с 3-месячным интервалом в течение 1 года. Циркулирующие уровни в плазме крови (vascular endothelial growth factor-1 — VEGF-1), металлопротеиназы-9 (matrix metalloproteinase-9 — ММП-9), высокочувствительного (high sensitivity) С-реактивного протеина (hs-СРП) оценивали в начале исследования. Клинические осмотры проводили каждые 3 мес в течение 1 года после получения образцов крови. В качестве конечных точек определены все рекуррентные сердечно-сосудистые события. Результаты и их обсуждение. Анализ полученных данных продемонстрировал, что концентрации в плазме крови циркулирующего VEGF-1>403,57 пг/мл (отношение шансов [ОШ]=2,78; 95% доверительный интервал (ДИ) 2,41–2,95; тест Вальда=6,515; p=0,011) обладают наиболее высоким прогностическим значением для кардиоваскулярных событий при одногодичном наблюдении. При этом ММП-9 (ОШ=2,61, 95% ДИ 2,37–3,16; тест Вальда=7,32; p=0,001), наличие сахарного диабета 2-го типа (ОШ=2,11, 95% ДИ 1,47–2,59; тест Вальда=3,52; p=0,001), hs-СРП (ОШ=1,62; тест Вальда=5,784; p=0,003), мужской пол (ОШ=1,25; тест Вальда=1,885; p=0,012) также определены в качестве клинически значимых предикторов развития кумулятивных сердечно-сосудистых событий в период после перенесенного ишемического инсульта. Применение методов реклассификации показало, что включение комбинации двух биомаркеров (hs-СРП и ММР-9) в модель ABC (VEGF-1) способствует повышению дискриминационного индекса для кардиоваскулярных событий на 9,3%. Выводы. Сочетание трех провоспалительных биомаркеров (VEGF-1, hs-СРП, ММР-9) у пациентов с гипертонической болезнью с ишемическим инсультом в анамнезе демонстрируют наилучшую прогностическую ценность в отношении риска развития рекуррентных кардиоваскулярных событий на протяжении 1 года наблюдения в сравнении с изолированным применением какого-либо из биомаркеров.

Ключевые слова: *васкулярный эндотелиальный фактор роста-1, металлопротеиназы-9, высокочувствительный С-реактивный протеин, ишемический инсульт, гипертония, клинические исходы, прогностическое значение, рекуррентные кардиоваскулярные события.*

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Received 23.05.2014

Реферативна інформація

Насколько безопасна вакцинация детей?

Согласно результатам нового систематического обзора, преимущества вакцинации значительно перевешивают возможные риски. Исследователи во главе с Маргаритой Маглионе (Margaret Maglione) провели обзор литературы по безопасности вакцин, рекомендуемых детям в возрасте <6 лет: комбинированной вакцины против кори, эпидемического паротита и краснухи (КПК), вакцины против коклюша с ацеллюлярным компонентом, дифтерии и столбняка (АаКДС), вакцины против гемофильной инфекции типа b, вирусного гепатита (ВГ) А, ВГ В, полиомиелита, гриппа и др. В обзор включили 67 исследований, в которых наблюдали группу вакцинированных участников с предусмотренным механизмом контроля. Результаты также оценивали по силе доказательств: высокая (ВСД) (маловероятно, что дальнейшие исследования изменят результат), средняя (ССД) (дальнейшие исследования могут изменить выводы), низкая (НСД) (высокая вероятность, что дальнейшие исследования повлияют на выводы) и недостаточная (доказательств недостаточно для формирования выводов). Также использованы данные доклада Института медицины по безопасности вакцин за 2011 г. (Institute of Medicine consensus report — IOM) для сравнения результатов.

Краткий обзор выводов представлен ниже.

АаКДС

Согласно докладу IOM, прямых доказательств каузальной взаимосвязи между этой вакциной и каким-либо патологическим состоянием нет. Имеющиеся доказательства свидетельствуют в пользу того, что эта вакцина каузально не связана с развитием сахарного диабета 1-го типа.

М. Маглионе и соавторы не выявили каких-либо дополнительных доказательств, опровергающих вывод IOM (ВСД).

ВГ В

IOM сообщает о наличии доказательств в пользу того, что у определенных индивидов вакцина против ВГ В может вызвать анафилактические реакции. Однако эти данные получены не в рамках эпидемиологических исследований.

В одной из новых работ вакцинация против ВГ В в первые 6 мес жизни была ассоциирована с повышением иммуноглобулина Е у детей без клинических проявлений аллергии, но с положительным семейным анамнезом. М. Маглионе и соавторы заключили, что этих данных недостаточно для формирования каких-либо выводов.

В научных работах сообщалось о возможной каузальной взаимосвязи между вакцинацией и демиелинизирующими неврологическими заболеваниями, однако специалисты IOM заключили, что этих доказательств недостаточно. В данном обзоре ученые также пришли к выводу со ССД, что каузальной взаимосвязи нет.

Еще в одном из исследований выявлено, что вакцинация в первые месяцы жизни связана с 3-кратным повышением риска аутизма в сравнении с вакцинацией в более поздние сроки и теми, кому эту вакцинацию не проводили (в этом исследовании НСД и высокий риск системных ошибок).

КПК

По данным IOM, существует взаимосвязь между этой вакциной и проходящей артралгией у детей. Что касается аутизма, подобной ассоциации не выявили.

Обзор исследований М. Маглионе и соавторов показал отсутствие каузальной взаимосвязи между вакцинацией и аутизмом со ССД. Однако вакцина КПК с ВСД связана с анафилактическими реакциями у детей с аллергическими заболеваниями, со ССД — преходящей артралгией и тромбоцитопенической пурпурой.

Полиомиелит

Сделан вывод об отсутствии взаимосвязи между вацинацией против полиомиелита и развитием любых патологических состояний.

Грипп

По данным IOM, отсутствуют какие-либо доказательства в пользу того, что вакцина против гриппа связана с патологическими реакциями. По данным ряда исследований эта вакцина ассоциирована с легкими гастроинтестинальными нарушениями и фибрильными судорогами (ССД) и гриппоподобными симптомами (НСД).

Другие вакцины

С ВСД вакцина против ветряной оспы связана с анафилактическими реакциями и диссеминацией/реактивацией вируса в организме. Также со ССД ротавирусная вакцина ассоциирована с инвагинацией кишечника.

Несмотря на то что для части вакцин все же нашли доказательства связи со специфическими побочными эффектами, они возникали невероятно редко.

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