Original article

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The prognostic value of hematological indices in patients with acute ischemic stroke and their correlation with major adipokines

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Abstract

Introduction-Aim: The association between peripheral blood haematological indices and acute ischemic stroke severity, in-hospital and long-term outcome is controversial. We aimed to evaluate this relationship and the potential association of haematological indices with serum adipokines levels and systemic inflammation.

Patients and Methods: We prospectively studied 93 patients consecutively hospitalized for acute ischemic stroke (39.8% males, age 79.7±6.3 years). Peripheral blood haematological indices and serum adiponectin, leptin and resistin levels were determined at admission. Stroke severity at admission was evaluated with the National Institutes of Health Stroke Scale (NIHSS). In-hospital outcome was evaluated by dependency rates at discharge and in-hospital mortality. One year after discharge, functional status, incidence of cardiovascular events and all-cause mortality were

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recorded. Functional status was evaluated with the modified Rankin scale (mRS).
Results: Patients with dependency at discharge had higher total white blood cells (WBC) ($p<0.05$) and absolute neutrophil count (NC) ($p<0.05$). Independent predictors of dependency at discharge were prior history of acute ischemic stroke [Risk Ratio (RR) 7.55, 95% Confidence Interval (CI) 1.69-33.58, $p<0.01$], NIHSS score at admission (RR 1.47, 95% CI 1.17-1.84, $p<0,001$) and serum triglyceride levels (RR 0.98, 95% CI 0.96-0.99, $p<0.05$). Patients with adverse outcome had higher NC ($p<0.05$), lower absolute lymphocyte count (LC) ($p<0.05$) and higher ratios NC/LC (NLR) and platelet count (PC)/LC (PLR) ($p<0.05$). In multivariate analysis, the mRS score at discharge was the only independent predictor of adverse outcome 1 year after discharge (RR 2.78, 95% CI 1.54-5.00, $p<0.001$). Regarding the correlation of haematological indices with major adipokines, there was a positive correlation of adiponectin levels with NLR ($r=0.295$, $p=0.012$) and PLR ($r=0.378$, $p=0.001$), while there was a negative correlation with hemoglobin (Hb) ($r=-0.252$, $p=0.033$). The NLR ($r=0.277$, $p=0.019$) and PLR ($r=0.240$, $p=0.043$) were also positively correlated with high-sensitivity C-reactive protein (hsCRP) levels. Serum resistin levels were positively correlated with NC ($r=0.278$, $p=0.018$). The WBC ($r=0.295$, $p=0.012$) and NC ($r=0.401$, $p<0.001$) showed a positive correlation with hsCRP levels.
Conclusions: WBC, NC, LC, NLR and PLR seem to predict a worse functional outcome of patients with acute ischemic stroke at hospital discharge and one year after hospitalization, due to greater systemic inflammation. Adipokines appear to affect peripheral blood cell counts and are associated with haematological indices. These associations reflect the role of haematological indices as biomarkers of inflammation and possibly their determinant effect, through adipokines and inflammation, on the pathophysiology and prognosis of acute ischemic stroke.

Key words: acute ischemic stroke; haematological indices; adipokines; severity; in- hospital outcome; long-term outcome

Introduction

Ischemic stroke is the second most common cause of mortality and the third most common cause of disability worldwide. [1] In order to predict the outcome of these patients and guide therapeutic decisions, several studies have investigated the predictive value of novel biomarkers, including adipokines and haematological indices. [2-4] The latter ones are consisted of markers which mainly reflect the subclinical systemic inflammation state and subsequent platelet activation in cardiovascular disease (CVD), like the number of total white blood cells count (WBC) and the absolute differential counts, platelet count (PC) and the mean platelet volume (MPV). [5-7] Likewise, ratios such as MPV to PC (MPR), Neutrophil Count (NC) to Lymphocyte Count (LC) (NLR), PC to LC (PLR) seem to more accurately depict the inflammatory and atherosclerotic state in CVD and have also been investigated as potential prognostic markers in CVD and acute ischemic stroke. [8-10]

Adipokines are a group of endogenous protein-derived biomolecules produced mainly by adipose tissue and cells involved in inflammation. [11,12] The most-studied adipokines are adiponectin, leptin and resistin, while a rapidly increasing number of novel adipokines has been described. [13] Adipokines have been associated with established cardiovascular risk factors and the risk of CVD, including acute ischemic stroke, [14,15] which could be attributed to their atherogenic effects, such as insulin resistance, dysregulation of glucose and lipid homeostasis, endothelial dysfunction, subclinical inflammation and stimulation of coagula-

tion. [13,16] However, adipokines seems to exert pleiotropic effects. Adipokines participate in haematopoiesis and affect the proliferation and maturation of haematopoietic cells. [17-19] In addition, they exert parallel effects on the immune system by regulating the immune response and the function of the inflammatory cells. [20-23] Therefore, several research groups have investigated the role of adipokines in haematological diseases, and have associated the corresponding serum adipokines levels with peripheral blood indices. [24-29]

There are limited previous studies regarding the prognostic role of haematological indices on stroke severity and outcome, [8-10] however none of them has ever been conducted in Greek stroke patients. We aimed to evaluate the prognostic value of peripheral blood indices on severity and in-hospital and long-term outcome of patients hospitalized with acute ischemic stroke and their potential association with serum adipokines levels and systemic inflammation.

Patients and methods

Patients

Ninety three consecutive patients (39.8% males, age 79.7±6.3 years) admitted with acute ischemic stroke were prospectively studied. Patients with chronic inflammatory, autoimmune or malignant diseases, as well as with active infectious diseases were excluded from the study.

At admission, demographic data (age, gender), history of cardiovascular risk factors (hypertension, T2DM, atrial fibrillation, smoking, excessive alcohol consumption, family history of CVD), history of concomitant CVD (coronary heart disease, heart failure, previous ischemic stroke) and pharmacological treatment were recorded. Anthropometric parameters (weight, height, waist and hip circumference) and systolic and diastolic blood pressure were also measured. The severity of stroke was assessed at admission with the National Institutes of Health Stroke Scale (NIHSS). Severe stroke was defined as NIHSS at admission ≥ 21.

All patients underwent brain computed tomography at admission and a second brain computed tomography was performed if clinically indicated.

None of the patients underwent thrombolysis. Treatment with an antiplatelet agent (or an anticoagulant agent in case of atrial fibrillation) and a statin were com-

Table 1: Patients' characteristics at admission

Age (years)	79.7±6.3
Males (%)	39.8
Systolic blood pressure (mmHg)	140±21
Diastolic blood pressure (mmHg)	81±14
Heart rate	79±13
Hypertension (%)	76.3
Smoking (current/past, %)	17.2/16.1
Type 2 diabetes mellitus (%)	30.1
Atrial fibrillation (%)	37.6
Weight (kg)	75.4±13.0
Body mass index (kg/m2)	27.7±5.4
Waist (cm)	105±11
Waist/hip	0.98±0.06
Overweight/obese (%)	35.3/27.5
Family history of cardiovascular disease (%)	12.9
Coronary heart disease (%)	37.6
Prior ischemic stroke (%)	47.3
Chronic kidney disease (%)	34.1
Heart failure (%)	28.0
Glucose (mg/dl)	114±48
Low-density lipoprotein cholesterol (mg/dl)	109±38
High-density lipoprotein cholesterol (mg/dl)	45±15
Triglycerides (mg/dl)	117±49
Uric acid (mg/dl)	5.8 (4.6, 7.1)
Estimated glomerular filtration rate (ml/min/1.73m2)	67 (51, 83)
Insulin (μIU/ml)	21.0 (13.8, 31.0)
Homeostasis model assessment of insulin resistance	5.3 (3.3, 8.8)
Quantitative insulin sensitivity check index	0.29±0.03
High-sensitivity C-reactive protein (mg/l)	5.7 (2.2, 7.4)
Leptin (ng/ml)	9.9 (4.0, 24.9)
Adiponectin (μg/ml)	18.1 (11.0, 27.8)
Resistin (ng/ml)	15.8 (11.6, 22.8)
National Institutes of Health Stroke Scale at admission	4 (2, 11)
Modified Rankin scale score at discharge	2 (1, 4)

Table 2: **Haematological indices at admission**	
WBC (x10^3/μl)	9.08±3.37
NC (x10^3/μl)	5.7 (4.3, 7.7)
LC (x10^3/μl)	1.7 (1.1, 2.1)
MC (x10^3/μl)	0.5 (0.4, 0.7)
RBC (x10^6/μl)	4.68±0.69
Hb (gr/dl)	13.2±1.9
Ht (%)	39.9±5.2
PC (x10^3/μl)	223.9±59.1
MPV (fl)	10.54±0.85
MPR (fl/10^3/μl)	0.051±0.016
NLR	3.5 (2.4, 6.1)
PLR	129.2 (98.8, 193.7)

Abbreviations: Hb=Hemoglobin, Ht=Hematocrit, LC=Lymphocyte Count, MC=Monocyte Count, MPR=Mean Platelet Volume to Platelet Ratio, MPV=Mean Platelet Volume, NC=Neutrophil Count, NLR=Neutrophil to Lymphocyte Ratio, PC=Platelet Count, RBC=Red Blood Cells, PLR=Platelet to Lymphocyte Ratio, WBC=White Blood Cells.

menced or continued in all patients, except for patients unable to receive per os medication.

The short-term outcome was assessed by the dependency rates at discharge and the in-hospital mortality. Dependency was defined as a modified Rankin scale (mRS) at discharge between 2 and 5.

Approximately 1 year after discharge, the patients and/or their proxy were contacted by telephone and the functional status was assessed with the mRS. Adverse outcome was defined as mRS between 2 and 6 (i.e. dependency or death). The occurrence of nonfatal ischemic stroke, nonfatal myocardial infarction and death were recorded. In patients who died during follow-up, the cause of death was also recorded.

Biochemical determinations

Routine laboratory investigations were performed after overnight fasting at the first day after admission and included serum levels of glucose, total cholesterol, high- density lipoprotein cholesterol, triglycerides, creatinine and uric acid. Low-density lipoprotein cholesterol levels were calculated using Friedewald's formula. [30] Glomerular filtration rate (GFR) was estimated using the Modification of Diet in Renal Disease equation. [31] Chronic kidney disease was defined as estimated GFR<60 ml/min/1.73m2. Serum insulin and high-sensitivity C-reactive protein (hsCRP) levels as well as serum leptin, adiponectin and resistin levels were measured with enzyme-linked immunosorbent assays (insulin: Insulin ELISA, IBL International GmbH, Hamburg, Germany; hsCRP: CRP high sensitive ELISA, IBL International GmbH, Hamburg, Germany; leptin: Leptin ELISA, IBL Hamburg GmbH, Hamburg, Germany; adiponectin: Human Total Adiponectin Immunoassay, R&D Systems Europe, Abington, UK; resistin: Human Resistin Immunoassay, R&D Systems Europe, Abington, UK) in serum samples which were collected after overnight fasting at the first day after admission and stored at - 80°C until measurement. The homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated according to the following formula: HOMA-IR = fasting insulin (μIU/ml) x fasting glucose (mg/dl) / 405 [HOMA-IR]. [32] The quantitative insulin sensitivity check index (QUICKI) was calculated according to the following formula: QUICKI = 1/[logInsulin (μIU/ml) + logGlucose (mg/dl)][QUICKI]. [33] The haematological indices that were evaluated and extracted from full blood counts were WBC, NC, LC, absolute monocyte count (MC), red blood cells count (RBC), hemoglobin (Hb), hematocrit (Ht), PC, MPV and the ratios MPR, NLR and PLR.

Ethical approval and informed consent

All procedures performed in this study were in accordance with the principles of the Declaration of Helsinki and were approved by the Ethics Committee of the Medical School of the Aristotle University of Thessaloniki. Informed consent was obtained from all individual participants who participated in the study.

Statistical analysis

All data were analyzed with the statistical package SPSS (version 17.0; SPSS, Chicago, IL, USA). Normality of continuous variables' distributions was explored by histograms, Q-Q plots and the Shapiro-Wilk Normality Test. Data are presented as percentages for

categorical variables, as mean and standard deviation (SD) for parametric continuous variables and as median and interquartile range for non-parametric continuous variables. Differences in categorical variables between subgroups were assessed with the chi-square test. Differences in parametric continuous variables and non-parametric continuous variables between subgroups were assessed with the independent samples t- test and Mann-Whitney test, respectively. Binary logistic regression analysis was used to identify independent predictors of severe stroke, in-hospital and long-term outcome. The presence of multicollinearity between independent variables was checked with Pearson Rank Order and Spearman Rank Order Tests before variable input into the regression models. The number of variables included in the regression models was defined by the assumption that the minimum number of cases per independent variable was 10. The potential correlation of different continuous variables with haematological indices was checked with the Pearson Rank Order and Spearman Rank Order Tests. In all cases, a two-tailed $p<0.05$ was considered significant.

Results

Patients' characteristics at admission are shown in **Table 1**, while the haematological indices at admission are depicted in **Table 2**. WBC was positively correlated with body mass index (r=0.33, p=0.025), uric acid (r=0.29, p=0.009) and mRS at discharge (r=0.23, p=0.044), while was negatively correlated with GFR (r=-0.3, p=0.006). NC and NLR were also positively correlated with uric acid (r=0.36, p=0.001 and r=0.33, p=0.003, respectively) and mRS at discharge (r=0.29, p=0.011 and r=0.25, p=0.034, respectively), while were also negatively correlated with GFR (r=-0.32, p=0.003 and r=-0.3, p=0.006, respectively). There was a positive correlation of LC with body mass index (r=0.33, p=0.027) and triglycerides (r=0.23, p=0.042) and of PC and PLR with waist/hip ratio (r=0.44, p=0.019 and r=0.43, p=0.022, respectively).

In total, 14.0% of patients had severe stroke at admission. These patients were older ($p<0.05$) and had higher heart rate ($p<0.05$), higher serum hsCRP levels ($p<0.001$) and lower QUICKI ($p<0.05$) than those with non-severe stroke (**Table 3**). Haematological indices did not differ between patients with severe and non-severe stroke. In binary logistic regression analysis, the only independent predictor of severe stroke was hsCRP levels [risk ratio (RR) 1.43, 95% confidence interval (CI) 1.08-1.91, $p<0.05$]. Moreover, NIHSS score at admission was significantly positively correlated with heart rate (r=0.25, p=0.018), serum hsCRP levels (r=0.55, $p<0.001$), WBC (r=0.31, p=0.004), NC (r=0.33, p=0.002) and NLR (r=0.26, p=0.018). At discharge, 53.0% of patients were dependent. These patients had higher WBC ($p<0.05$) and NC ($p<0.05$). Dependent patients had also more frequently a history of ischemic stroke ($p<0.05$) and had higher serum hsCRP levels ($p<0.005$), lower serum triglyceride levels ($p<0.05$) and higher NIHSS at admission ($p<0.001$). (Table 3). In binary logistic regression analysis, independent predictors of dependency at discharge were the history of ischemic stroke (RR 7.55, 95% CI 1.69- 33.58, $p<0.01$), serum triglyceride levels (RR 0.98, 95% CI 0.96-0.99, $p<0.05$) and NIHSS at admission (RR 1.47, 95% CI 1.17-1.84, $p<0.001$). During hospitalization 10 patients died. Haematological indices did not differ between patients who died during hospitalization and those who were discharged. Patients who died were older ($p<0.001$) and had higher prevalence of atrial fibrillation ($p<0.005$), higher systolic blood pressure ($p<0.01$), higher diastolic blood pressure ($p<0.001$), higher heart rate ($p<0.001$), higher serum hsCRP levels ($p<0.05$) and higher NIHSS ($p<0.001$) (**Table 3**). In binary logistic regression analysis, independent predictors of in-hospital mortality were systolic blood pressure at admission (RR 1.09, 95% CI 1.01-1.19, $p<0.05$) and NIHSS at admission (RR 1.26, 95% CI 1.08-1.48, $p<0.005$).

Regarding the long-term outcome, at 1 year after discharge 57.8% of patients had adverse outcome. These patients had higher NC ($p<0.05$) and lower LC ($p<0.05$), while they had also higher NLR ($p<0.05$) and PLR ($p<0.05$). Moreover, patients with adverse outcome at 1 year were older ($p<0.05$), had higher hsCRP ($p<0.001$) and uric acid levels ($p<0.05$), while they had also higher NIHSS score at admission ($p<0.005$) and higher mRS at discharge ($p<0.001$) (**Table 4**). In binary logistic regression analysis, the only independent predictor of adverse outcome at 1 year was the mRS at discharge (RR 2.78, 95% CI 1.54-5.00, $p<0.001$). During the 1-year follow-up, 28.9% of patients had a cardio-

Table 3: **Patients' significant differences regarding stroke severity, dependency at discharge and in-hospital mortality**

Stroke Severity			
	Patients with severe stroke (n = 13)	**Patients with non-severe stroke (n = 80)**	**p value**
Age (years)	83.1±6.9	79.2±6.1	<0.05
Heart rate	88±15	78±13	<0.05
hsCRP (mg/l)	7.0 (6.6, 9.1)	5.0 (1.8, 7.1)	<0.001
QUICKI	0.28±0.03	0.30±0.03	<0.05
Dependency at Discharge			
	Patients dependent at discharge (n = 44)	**Patients independent at discharge (n = 39)**	**p value**
Prior ischemic stroke (%)	67.6	37.5	<0.05
hsCRP (mg/l)	6.6 (3.0, 8.9)	2.5 (0.9, 5.8)	<0.005
Triglyceride (mg/dl)	106±42	136±50	<0.05
NIHSS score at admission	6.0 (4.0, 11.5)	2.0 (1.0, 3.8)	<0.001
WBC (x103/μl)	9.57±3.11	8.07±2.78	<0.05
NC (x103/μl)	6.9 (4.9, 8.7)	4.7 (4.0, 6.0)	<0.05
In-hospital Mortality			
	Patients who died during hospitalization (n = 10)	**Patients who were discharged (n = 83)**	**p value**
Age (years)	86.2±6.2	78.9±5.9	<0.001
Atrial fibrillation (%)	80.0	32.5	<0.005
Systolic blood pressure (mmHg)	155±22	138±20	<0.01
Diastolic blood pressure (mmHg)	95±19	79±12	<0.001
Heart rate	91±12	78±13	<0.001
hsCRP (mg/l)	7.0 (5.2, 8.5)	5.4 (1.8, 7.9)	<0.05
NIHSS score at admission	30.5 (17.7, 33.5)	4.0 (1.5, 7.5)	<0.001

Abbreviations: hsCRP=high-sensitivity C-reactive Protein, NC=Neutrophil Count, NIHSS=National Institutes of Health Stroke Scale, QUICKI=Quantitative Insulin Sensitivity Check Index, WBC=White Blood Cells.

vascular event. These patients had lower insulin levels (p<0.01) and lower HOMA- IR (p<0.05) at admission, however none of these variables remained significant in multivariate analysis (**Table 4**). Haematological indices did not differ between patients who experienced a cardiovascular event and those who did not. During the 1-year follow- up, 22 patients died. These patients had higher hsCRP levels (p<0.05) at admission, higher NIHSS score at admission (p<0.05) and higher mRS at discharge (p<0.001), while haematological indices did not differ between patients who died during follow-up and those who survived (**Table 4**). In binary logistic regression analysis, the only independent predictor of all-cause mortality during follow-up was the NIHSS score

Table 4: **Patients' significant differences regarding adverse outcome at 1 year after discharge, cardiovascular incidence (nonfatal ischemic stroke, nonfatal myocardial infarction and cardiovascular death) and all-cause mortality during the 1-year follow-up.**

Adverse Outcome			
	Patients with adverse outcome (n = 48)	**Patients independent at 1 year (n = 35)**	**p value**
Age (years)	79.9±5.9	76.4±5.8	<0.05
hsCRP (mg/l)	6.5 (2.8, 9.0)	2.3 (1.0, 5.6)	<0.001
Uric acid (mg/dl)	6.4 (5.0, 7.3)	5.0 (4.0, 6.5)	<0.05
NIHSS score at admission	6 (3, 11)	2 (1, 5)	<0.005
mRS score at discharge	3.0 (1.7, 4.0)	0 (0, 1)	<0.001
NC (x103/μl)	7.1 (5.2, 9.5)	4.7 (3.7, 6.2)	<0.05
LC (x103/μl)	1.4 (1.0, 1.9)	2.2 (1.4, 2.5)	<0.05
NLR	5.0 (3.3, 6.6)	2.3 (1.6, 3.8)	<0.05
PLR	148.9 (102.0, 240.3)	111.9 (73.9, 150.7)	<0.05
Cardiovascular Incidence			
	Patients with a cardiovascular event (n = 24)	**Patients without a cardiovascular event (n = 59)**	**p value**
Insulin (μIU/ml)	16.7 (12.1, 27.1)	23.4 (13.8, 40.2)	<0.01
HOMA-IR	4.8 (3.1, 7.0)	5.6 (3.3, 9.3)	<0.05
All-cause Mortality			
	Patients who died during follow-up (n = 22)	**Patients alive at 1 year (n = 61)**	**p value**
hsCRP (mg/l)	6.7 (4.6, 8.6)	3.3 (1.5, 7.0)	<0.05
NIHSS score at admission	7.0 (3.0, 11.5)	3.0 (1.0, 6.7)	<0.05
mRS score at discharge	4.0 (1.7, 5.0)	1.0 (0, 2.0)	<0.001

Abbreviations: HOMA-IR=Homeostasis Model Assessment of Insulin Resistance, hsCRP=high- sensitivity C-reactive Protein, LC=Lymphocyte Count, mRS=modified Rankin scale, NC=Neutrophil Count, NIHSS=National Institutes of Health Stroke Scale, NLR=Neutrophil to Lymphocyte Ratio, PLR=Platelet to Lymphocyte Ratio.

at admission (RR 1.19, 95% CI 1.04-1.35; p<0.01).

Regarding the potential correlation of haematological indices with major adipokines and inflammation, there was a significant positive correlation of serum adiponectin levels with NLR (r=0.295 and p=0.012) and PLR (r=0.378 and p=0.001), while there was a negative correlation with Hb (r=-0.252 and p=0.033). The NLR (r=0.277 and p=0.019) and PLR (r=0.240 and p=0.043) indices were positively correlated with hsCRP levels, namely with the presence of systemic inflammation. In addition, LC (r=-0.225 and p=0.058) and Ht (r=-0.222 and p=0.060) showed a tendency to negative correlation with adiponectin, while LC had a significant negative correlation with hsCRP (r=-0.292 and p=0.013). The other haematological indices did not show any correlation with adiponectin. Regarding leptin, none of the peripheral blood haematological indices appeared to correlate significantly with its levels. In contrast,

Table 5: **Significant correlations among haematological indices, serum adipokines levels and hsCRP.**

Haematological Indices		Adiponectin	Leptin	Resistin	hsCRP
WBC	r coefficient	-0,036	0,081	0,226	0,295
	p value	0,765	0,517	0,057	0,012
NC	r coefficient	0,033	0,098	0,278	0,401
	p value	0,784	0,434	0,018	<0,001
LC	r coefficient	-0,225	-0,040	-0,137	-0,292
	p value	0,058	0,752	0,251	0,013
Hb	r coefficient	-0,252	0,091	-0,079	-0,123
	p value	0,033	0,468	0,507	0,304
Ht	r coefficient	-0,222	0,158	-0,031	-0,076
	p value	0,060	0,206	0,795	0,523
NLR	r coefficient	0,295	0,009	0,174	0,277
	p value	0,012	0,941	0,143	0,019
PLR	r coefficient	0,378	-0,002	0,056	0,240
	p value	0,001	0,987	0,642	0,043

Abbreviations: HOMA-IR=Homeostasis Model Assessment of Insulin Resistance, hsCRP=high- sensitivity C-reactive Protein, LC=Lymphocyte Count, mRS=modified Rankin scale, NC=Neutrophil Count, NIHSS=National Institutes of Health Stroke Scale, NLR=Neutrophil to Lymphocyte Ratio, PLR=Platelet to Lymphocyte Ratio.

serum resistin levels appeared to correlate positively with NC (r=0.278 and p=0.018), while WBC (r=0.226 and p=0.057) tended to positive correlation with resistin. As expected, the WBC (r=0.295 and p=0.012) and NC (r=0.401 and p<0.001) showed a positive correlation with hsCRP levels. All significant correlations of hematological indices with serum adipokines levels and hsCRP are shown in **Table 5**.

Discussion

The aim of the present study was to evaluate the prognostic value of peripheral blood indices on severity and in-hospital and long-term outcome of patients hospitalized with acute ischemic stroke and their potential association with serum adipokine levels and systemic inflammation. We found that patients with higher WBC and NC at admission had greater disability at discharge. These patients had also higher degree of systemic inflammation, while WBC and NC were positively correlated with hsCRP. However, these associations did not persist in multivariate analysis where stroke severity at admission was the only independent predictor of dependency at discharge. It seems that patients with higher WBC and NC have worse in-hospital outcome due to more severe stroke with greater degree of systemic inflammation. Similarly, patients with adverse outcome at 1 year after discharge had also higher NC at admission, while they had lower LC and higher NLR and PLR. Noteworthy, these patients had also higher degree of systemic inflammation. Apart from WBC and NC, NLR and PLR were also positively correlated with hsCRP, while LC was negatively correlated with inflammation. However, the only independent predictor of adverse outcome at 1 year was dependency at discharge. Taken together, patients with higher WBC and NC have worse in-hospital and long-term outcome due to more severe stroke at admission. Moreover, patients with lower LC and higher NLR and PLR have

more adverse outcome at 1 year due to greater systemic inflammation and higher dependency at discharge. These relations reflect the role of haematological indices as biomarkers of inflammation and the determinant effect of inflammation in stroke pathophysiology and prognosis. However, we cannot exclude a causative effect of haematological indices in stroke outcome. The rest of the haematological indices that were evaluated (MC, RBC, Hb, Ht, PC, MPV, MPR) did not correlate with any of the study end-points.

We found that reduced renal function and uric acid levels correlated with WBC, NC and NLR. Moreover, we also found that stroke patients with higher body mass index and higher waist/hip ratio had higher WBC, LC, PC and PLR. Therefore, it seems that obesity and renal function, which are both characterized by subclinical inflammation, might determine haematological indices in stroke patients and subsequently have an impact on stroke severity and outcome.

Considering the critical role of inflammation in atherosclerotic disease and CVD, previous data show that several inflammatory biomarkers, such as whole blood cell components, have been used to predict the risk of acute ischemic stroke and its prognosis. [34] The effect of high WBC and their subtypes on acute ischemic stroke can be explained by many mechanisms, however inflammation remains the cornerstone of this relationship. Leukocytosis can be considered as an indicator of inflammatory changes in atherosclerotic lesions because leukocytes play a key role in its initiation and progression. [35,36] The number of WBCs and their subtypes, such as NC and LC, are strong and independent predictors of ischemic stroke, and the relative risks are comparable to those of other inflammatory markers, such as CRP. [3,5] Povroznik et al. in a study of 101 newborn children with ischemic encephalopathy and acute ischemic stroke found that in the first few hours after ischemic damage there was an increase in NC and LC, suggesting that activation of the immune system is an important component of ischemic brain injury. [37] Indeed, it appears that after ischemic brain damage, pro-inflammatory signaling molecules mobilize neutrophils and lymphocytes, which then migrate to the injured area of the brain. [38] Experimental studies indicate that neutrophils and lymphocytes are formed in the period just after ischemic damage and that these conformations are often related to stroke severity and long-term neurological outcome. [39,40] A patient-control study in children with ischemic stroke found that patients had higher WBC, NC and lower LC than the control group, however these markers had no prognostic value in overall mortality and further development of children. In acute stress and inflammatory conditions, lymphopenia is a common finding in response to increased corticosteroid excretion and increased lymphocyte apoptosis. [41] However, a retrospective study in 338 Chinese found that low LC was associated with a worse outcome of acute ischemic stroke 3 months after the event, while no relationship was found for NC and total WBC with outcome. [8] Zia et al. in a large prospective study with 28,449 Swedish of the general population also found no significant relationship of WBC and subtypes with mortality. [4] Fang et al. in a retrospective study of 1731 patients with ischemic stroke, found that elevated NC was an independent predictor of severity. [9] Another retrospective study by Fan et al. showed that Chinese patients with ischemic stroke and higher in-hospital mortality had higher WBC and NC, while they had lower LC and MC. In multivariate analysis, the number of WBCs was an independent predictor of in- hospital mortality. [42]

NLR appears to be a reliable indicator of systemic inflammation and in patients with acute ischemic stroke has been associated with CRP, as in the present study. [43] A small patient-control study found that patients had a higher NLR than healthy volunteers, while patients with more severe stroke had also a higher NLR. [44] Fang et al. found that the NLR was an independent prognostic factor of in-hospital mortality, while other smaller studies independently correlated NLR with increased short-term mortality. [9,42,45-47] In addition, several studies in various populations have shown that the highest NLR is independently associated with more severe neurological deficits at hospital admission and discharge, worse functional outcome 3 months after ischemic stroke with or without therapeutic thrombolysis and stroke recurrence. [48-56] A recent meta-analysis of nine studies involving 2947 patients with acute ischemic stroke, concluded that higher NLR rates predict worse outcomes at hospital discharge and 3 months post-stroke, higher trimester mortality and increased risk of symptomatic intracranial hemorrhage.

[57] However, in a study in children with ischemic stroke, although patients had a higher NLR than the control group, the NLR did not appear to predict overall survival and disease free survival. [41]

High PC reflect increased platelet activation, while low LC represents the suppressed immune response associated with undesirable clinical outcomes in CVD. Therefore, both thrombocytosis and lymphopenia are related to the degree of systemic inflammation, and PLR represents a new marker that incorporates both hematological parameters. [58] Only one previous prospective study in patients with acute ischemic stroke who underwent thrombectomy showed that increased PLR is associated with greater cerebral infract and worse functional outcome at 3 months post-stroke. [59]

Considering the pleotropic effects of adipokines, the present study also investigated the possible role of adipokines in hematopoiesis and regulation of the immune system, which interfere with the body inflammatory response and may have an effect on the acute ischemic stroke occurrence, the severity of the event and its outcome. For this reason, peripheral blood indices were used, which are likely to be affected by adipokines, while at the same time serve as biomarkers of systemic inflammation. In particular, there was a significant positive correlation of adiponectin levels with NLR and PLR, while a negative correlation with Hb. Regarding leptin, none of the peripheral blood haematological indices appeared to correlate significantly with its levels. In contrast, serum resistin levels positively correlated with NC, while WBC had a tendency to positive correlation with resistin. Given the positive correlation between hsCRP and WBC, NC, NLR, PLR levels, and negative association with LC, these relationships may be pathogenic to acute ischemic stroke and hematological indices may not be simply biomarkers of inflammation. There are no previous data regarding the correlation of major adipokines with peripheral blood haematological indices in patients with acute ischemic stroke in order to compare the findings of the present study. However, there are limited previous studies for some of the hematologic indices in other populations, which yielded conflicting results. [28,29,60,61]

We acknowledge that this study has some limitations. First, we did not perform a power analysis to determine the required sample size, but we performed appropriate statistical analysis thereby validating our present results. Second, our study might carry random or systemic bias due to its observational design.

In conclusion, in the present study we found that higher WBC and NC at admission predict a worse functional outcome of patients with acute ischemic stroke at hospital discharge and 1 year after discharge due to more severe stroke with greater systemic inflammation. In addition, lower LC and higher NLR and PLR at admission seem to be associated with adverse outcome at 1 year after discharge due to a higher rate of systemic inflammation and greater disability at hospital discharge. Considering adipokines involvement in hematopoiesis and the immune system regulation, adiponectin, leptin and resistin are likely to affect peripheral blood cell counts and may be associated with haematological indices. In particular, adiponectin was positively correlated with NLR and PLR, while negatively with Hb. Also, resistin was positively associated with NC. All of the above associations reflect the role of haematological indices as biomarkers of inflammation and possibly their determinant effect on the pathophysiology and prognosis of acute ischemic stroke, through the effect of adipokines and inflammation. However, larger prospective studies are needed to confirm these results. If proven, peripheral blood haematological indices and adipokines could represent a novel approach for better risk stratification in patients with acute ischemic stroke and better targeting of therapeutic interventions to reduce stroke-related disability and mortality.

Conflicts of interest

We declare no conflicts of interest.

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Περίληψη

Η προγνωστική αξία αιματολογικών δεικτών σε ασθενείς με οξύ ισχαιμικό αγγειακό εγκεφαλικό επεισόδιο και η συσχέτισή τους με τις κυριότερες αδιποκίνες

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Εισαγωγή-Σκοπός: Η σχέση των αιματολογικών δεικτών του περιφερικού αίματος με τη βαρύτητα του οξέος ισχαιμικού αγγειακού εγκεφαλικού επεισοδίου (ΑΕΕ), την ενδονοσοκομειακή και μακροχρόνια έκβαση είναι αμφιλεγόμενη. Σκοπός της παρούσας μελέτης ήταν να διερευνήσουμε αυτές τις σχέσεις και την πιθανή συσχέτιση των αιματολογικών δεικτών με τα επίπεδα των αδιποκινών στον ορό και τη συστηματική φλεγμονή.

Ασθενείς και Μέθοδοι: Μελετήθηκαν προοπτικά 93 ασθενείς που νοσηλεύτηκαν διαδοχικά για οξύ ισχαιμικό ΑΕΕ (39,8% άνδρες, ηλικία 79,7±6,3 έτη). Οι αιματολογικοί δείκτες του περιφερικού αίματος και τα επίπεδα της αδιπονεκτίνης, λεπτίνης, ρεζιστίνης στον ορό προσδιορίστηκαν στην εισαγωγή. Η βαρύτητα του ΑΕΕ κατά την εισαγωγή αξιολογήθηκε με την National Institutes of Health Stroke Scale (NIHSS). Η ενδονοσοκομειακή έκβαση εκτιμήθηκε με την λειτουργική εξάρτηση κατά την έξοδο από το νοσοκομείο και την ενδονοσοκομειακή θνητότητα. Ένα χρόνο μετά την έξοδο, καταγράφηκαν η λειτουργική έκβαση, η συχνότητα εμφάνισης καρδιαγγειακών συμβαμάτων και η ολική θνητότητα. Η λειτουργική εξάρτηση αξιολογήθηκε με την τροποποιημένη κλίμακα Rankin (mRS).

Αποτελέσματα: Οι ασθενείς που ήταν εξαρτημένοι στην έξοδο από το νοσοκομείο είχαν υψηλότερες τιμές συνολικού αριθμού λευκών αιμοσφαιρίων (WBC) ($p<0,05$) και απόλυτου αριθμού ουδετερόφιλων (NC) ($p<0,05$). Ανεξάρτητοι προγνωστικοί παράγοντες της λειτουργική εξάρτησης κατά την έξοδο από το νοσοκομείο ήταν το προηγούμενο ιστορικό οξέος ισχαιμικού ΑΕΕ [σχετικός κίνδυνος (RR, Risk Ratio) 7,55, 95% διάστημα εμπιστοσύνης (CI, Confidence Interval) 1,69-33,58, $p<0,01$), το NIHSS σκορ στην εισαγωγή (RR 1,47, 95% CI 1,17-1,84, $p<0,001$] και τα επίπεδα των τριγλυκεριδίων στον ορό (RR 0.98, 95% CI 0.96-0.99, $p<0,05$). Οι ασθενείς που είχαν δυσμενή έκβαση ένα έτος μετά το εξιτήριο είχαν υψηλότερο NC ($p<0,05$), χαμηλότερο απόλυτο αριθμό λεμφοκυττάρων (LC) ($p<0,05$) και υψηλότερους λόγους NC/LC (NLR) ($p<0,05$) και αριθμού αιμοπεταλίων (PC)/LC (PLR) ($p<0,05$). Στην πολυπαραγοντική ανάλυση, το mRS σκορ εξόδου ήταν ανεξάρτητος παράγοντας κινδύνου δυσμενούς έκβασης στο ένα έτος από το εξιτήριο (RR 2,78, 95% CI 1,54-5,00, $p<0,001$). Σχετικά με τη συσχέτιση των αιματολογικών δεικτών με τις κυριότερες αδιποκίνες, διαπιστώθηκε θετική συσχέτιση των επιπέδων της αδιπονεκτίνης με τον NLR ($r=0,295$, $p=0,012$) και

τον PLR (r=0,378, p=0,001), ενώ αρνητική συσχέτιση με την αιμοσφαιρίνη (Hb) (r=-0,252, p=0,033). Οι δείκτες NLR (r=0,277, p=0,019) και PLR (r=0,240, p=0,043) σχετίστηκαν θετικά και με τα επίπεδα της υψηλής ευαισθησίας C-αντιδρώσας πρωτεΐνης (hsCRP). Τα επίπεδα της ρεζιστίνης στον ορό συσχετίστηκαν θετικά με τον NC (r=0,278, p=0,018). Ο αριθμός των WBC (r=0,295, p=0,012) και ο NC (r=0,401, p<0,001) εμφάνισαν θετική συσχέτιση με τα επίπεδα της hsCRP.
Συμπεράσματα: Οι WBC, NC, LC, NLR και PLR φαίνεται να προβλέπουν χειρότερη λειτουργική έκβαση ασθενών με οξύ ισχαιμικό ΑΕΕ στην έξοδο από το νοσοκομείο και ένα έτος μετά το εξιτήριο, λόγω μεγαλύτερου βαθμού συστηματικής φλεγμονής. Οι αδιποκίνες φαίνεται να επηρεάζουν τα κυτταρικά στοιχεία του περιφερικού αίματος και να συνδέονται με αιματολογικούς δείκτες. Οι παραπάνω σχέσεις αντανακλούν τον ρόλο των αιματολογικών δεικτών ως βιοδείκτες της φλεγμονής και πιθανόν την καθοριστική επίδρασή τους, μέσω της δράσης των αδιποκινών και της φλεγμονής, στην παθοφυσιολογία και την πρόγνωση του οξέος ισχαιμικού ΑΕΕ.

Λέξεις ευρετηρίου: Οξύ ισχαιμικό αγγειακό εγκεφαλικό επεισόδιο, αιματολογικοί δείκτες, αδιποκίνες, ενδονοσοκομειακή έκβαση, μακροχρόνια έκβαση

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