



Leucocyte count indicates carotid plaque instability in stroke patients

Broj leukocita pokazuje nestabilnost karotidnog plaka kod bolesnika sa akutnim infarktom mozga

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Abstract

Background/Aim. Increasing evidence points to the inflammatory character of atherosclerosis and several parameters of inflammation have been proposed as cerebrovascular risk markers. The objective of the research was to examine the connection of serum inflammatory parameters and ultrasound (US) characteristics of the structure and size of carotid plaque. We assumed that the number of leukocytes (Le) was an indicator of carotid plaque instability and an increased risk of stroke. **Methods.** Serum inflammatory parameters: erythrocyte sedimentation rate in the first (ESR I) and second hour (ESR II), the number of Le, high sensitivity C-reactive protein (hsCRP) and fibrinogen were measured by standard methods. All the subjects ($n = 75$) were divided into 3 groups (symptomatic, asymptomatic and control). US evaluation of extracranial carotid arteries was performed in a duplex system. Plaques were classified into categories according to stenosis percentage ($\geq 50\%$, $< 50\%$) and pursuant to echomorphological characteristics (Gray-Weale classification). In the subjects with stroke an ischemic lesion was confirmed by computed tomography. **Results.** The average values of biochemical parameters in the symptomatic group were: ESR I 29.57 ± 29.87 cm, ESR II 51.60 ± 36.87 cm, the number of Le $10.10 \pm 3.20 \times 10^9$ U/L, hs-

CRP 8.15 ± 5.50 mg/L and fibrinogen 4.03 ± 0.70 g/L. The average values of all testing biochemical parameters in symptomatic patients were significantly higher than in the asymptomatic ones and the control group: for ESR I ($p < 0.05$) and ESR II ($p < 0.05$); for the number of Le ($p < 0.001$); for hsCRP ($p < 0.001$) and fibrinogen ($p < 0.001$). Category I of echomorphological characteristics in the symptomatic group was present in 66.7% of the cases and it was significantly higher than in the asymptomatic (40.0%; $p < 0.05$) and the control group (20.0%; $p < 0.01$). Univariate logistic regression analysis confirmed that all testing biochemical parameters are indicators of stroke risk. Multivariate logistic regression analysis confirmed a statistically significant correlation of the number of Le and stroke risk, while the increase in the value by a unit of measurement was associated with the growth of risk by 3.22 times (from 1.67 to 6.22). **Conclusion.** The number of Le is associated with the phenomenon of carotid plaque instability and may be a useful additional marker of increased risk for developing acute cerebral infarction.

Key words:

stroke; carotid stenosis; plaque, atherosclerotic; leukocyte count.

Apstrakt

Uvod/Cilj. Porast dokaza o inflamatornom karakteru ateroskleroze istakao je više parametara zapaljenja kao pokazatelje cerebrovaskularnog rizika. Cilj istraživanja bio je da se ispita povezanost parametara zapaljenja u serumu i ultrazvučnih (UZ) karakteristika strukture i veličine karotidnog plaka. Pretpostavili smo da je broj leukocita (Le) pokazatelj nestabilnosti karotidnog plaka i povećanog rizika od razvoja akutnog infarkta mozga. **Metode.** Broj Le i ostali serumski parametri zapaljenja [sedimentacija eritrocita u prvom (Se Er I) i drugom satu (Se Er II), visokosenzitivni C-reaktivni protein (hs-CRP) i fibrinogen] mereni su standardnim metodama. Svi ispitanici ($n = 75$) imali su kompletan klinički pregled i bili podeljeni u tri grupe (simptomatska, asimptomatska i kontrolna). Ultrazvučni (UZ) pregled ekstrakranijalnih karotidnih arterija rađen

je u dupleks sistemu. Plakovi su klasifikovani u kategorije prema procentu stenoze ($\geq 50\%$, $< 50\%$) i prema ehomorfološkim karakteristikama (Gray-Weale klasifikacija). Kod ispitanika sa akutnim infarktom mozga ishemijska lezija je potvrđena kompjuterizovanom tomografijom. **Rezultati.** Prosečne vrednosti ispitivanih biohemijskih parametara u simptomatskoj grupi bile su: Se Er I $29,57 \pm 29,87$ cm, Se Er II $51,60 \pm 36,87$ cm, broj Le $10,10 \pm 3,20 \times 10^9$ U/L, hs-CRP $8,15 \pm 5,50$ mg/L i fibrinogen $4,03 \pm 0,70$ g/L. Prosečne vrednosti svih ispitivanih biohemijskih parametara u simptomatskoj bile su značajno više nego u asimptomatskoj i kontrolnoj grupi: za Se Er I ($p < 0,05$) i Se Er II ($p < 0,05$); za broj Le ($p < 0,001$) za hsCRP ($p < 0,001$) i fibrinogen ($p < 0,001$). Zastupljenost I kategorije ehomorfoloških karakteristika u simptomatskoj grupi (66,7% ispitanika) bila je značajno viša nego u asimptomatskoj (40,0%; $p < 0,05$) i kontrolnoj (20,0%; $p < 0,01$)

grupi. Univarijantna logistička regresiona analiza potvrdila je da su svi ispitivani parametri indikatori rizika od akutnog infarkta mozga. Multivarijantna logistička regresiona analiza potvrdila je statistički značajnu povezanost broja Le i rizika od akutnog infarkta mozga. Povećanje vrednosti za jednu mernu jedinicu povezano je sa porastom rizika za 3,22 puta (1,67–6,22). **Zaključak.** Broj Le povezan je sa fenomenom nestabilnosti karotidnog plaka i može biti koristan dodatni pokazatelj povećanog rizika od razvoja akutnog infarkta mozga.

bilnosti karotidnog plaka i može biti koristan dodatni pokazatelj povećanog rizika od razvoja akutnog infarkta mozga.

Ključne reči:
mozak, infarkt; aa. carotis, stenoza; aterosklerotički plak; leukociti, broj.

Introduction

Ischemic brain disease as the final stage of cerebral atherosclerosis includes the pathological processes on extracranial carotid arteries. Clinical experience of neurologists indicate that acute cerebral infarction is often the first manifestation of the progression of carotid atherosclerosis. Carotid plaque is not a stable lesion in spite of prophylactic drug therapy and carries the risk of definite or transient ischemic complications¹⁻³.

The scientific research dealing with the vulnerability of atherosclerotic lesions on experimental and human models provided theoretical knowledge that numerous biochemical markers in peripheral blood and modulators and mediators of inflammation in vulnerable atherosclerotic lesions represent a part of complex pathophysiological destabilization mechanisms^{2,3}.

The objective of the research was to examine the connection of serum inflammatory parameters, applicable in everyday clinical work, and ultrasound (US) characteristics of the structure and size of carotid plaque. We assumed that the number of leukocytes (Le) could be an indicator of carotid plaque instability and an increased risk of developing acute cerebral infarction.

Methods

The study included 75 subjects of both sexes, 50–70 years of age, processed in hospital and treated in the Intensive Care Unit of the Department of Neurology, Military Hospital in the town of Niš and on outpatient treatment, neurologically monitored in the Section for Neurology of the Military Hospital.

The basic criteria for the inclusion of subjects in this study were the sample structure according to age and sex, with defined traditional risk factors of atherosclerosis⁴, ultrasound (US) diagnosed carotid atherosclerosis^{5,6} and multislice scanner (MSCT) diagnosed acute cerebral infarction^{4,7}.

The exclusion criteria were: subjects with clinical symptoms and signs of current or recent infection (< 4 weeks), verified by a physical examination, diagnostic assessment by indications (by organ systems) and laboratory confirmation of clinically important infections; patients with diagnosed chronic or specific infections by organ systems; subjects with potentially cardioembolic etiology of acute cerebral infarction after clinical and electrocardiographic/echocardiographic evaluation (intermittent/continuous atrial fibrillation/flutter, recent myocardial infarction < 6 weeks, mitral/aortic stenosis, prolapse, calcification, vegetation or prosthetic valve replacement, aneurysm or left atrial myxoma, thrombosis in the left ventricle, persistent fo-

ramen ovale, right-to-left shunt, congestive heart failure, congenital heart disease, endocarditis ...) ⁸; subjects diagnosed with kidney failure, based on the clinical stage of disease and laboratory confirmation ⁹; patients with diagnosed immunological, malignant diseases and disorders of hemostasis; subjects with trauma or surgery in the past 12 months; subjects on corticosteroids, antioxidant or hormone therapy; subjects with MSCT confirmed alterations of the brain parenchyma not corresponding to acute cerebral infarction by the clinical categorization and pathogenesis of atherothrombosis^{7,8}.

Each patient was taken a detailed medical history and subjected to a neurological examination. Additional tests included: laboratory blood test, ultrasound of extracranial carotid arteries and MSCT of endocranium in patients with acute cerebral infarction.

The sample size was calculated on the basis of the results of clinical studies with similar objectives¹⁰ and preliminary results taking into account that $\alpha = 0.05$, and the study power 0.8 according to a flexible statistical power analysis program G* Power 3¹¹.

The subjects were divided into three groups: the symptomatic group (30 subjects) comprised subjects with acute cerebral infarction, or with focal or global disturbance of cerebral function, which occurred rapidly and lasted longer than 60 minutes and their clinical categorization and pathogenesis which corresponded to atherothrombotic cerebral infarction^{4,7}.

The diagnosis of acute cerebral infarction was confirmed by MSCT of endocranium⁴. Carotid atherosclerosis was diagnosed by the ultrasound examination of extracranial carotid arteries and confirmation of the localized atherosclerosis lesion^{5,6}.

The asymptomatic group (30 subjects) involved subjects with carotid atherosclerosis and verified hemodynamically significant carotid burification plaque ($\geq 50\%$)⁵.

The control group (15 subjects) consisted of subjects with carotid atherosclerosis and verified hemodynamically insignificant carotid burification plaque (< 50%)⁵. The subjects of the asymptomatic and control group, in their medical history, had no anamnestic data on current/prior episodes of rapidly developing focal/global disturbances of cerebral function which lasted longer than 60 minutes, without MSCT confirmed densimetric alterations of the brain parenchyma⁷.

In this research we valued and analyzed biochemical, ultrasound, clinical and neuroradiological parameters.

Biochemical parameters. Blood for the required analyses was taken from the medial cubital vein into tubes with a vacuum system. The number of Le was determined on an automated hematology analyzer from a tube with ethylenediamineacetic acid (EDTA) as an anticoagulant; high sensitivity C-reactive protein

(hs-CRP) was determined on a biochemical analyzer from a tube without anticoagulants; erythrocyte sedimentation rate (ESR) and fibrinogen were determined from a tube with sodium citrate as an anticoagulant. Ready-made commercial blood tests were used for the analysis. Blood for analyses in the subjects of the symptomatic group was taken on admission in the time frame of "therapeutic window"¹². Blood for analyses in the subjects of the asymptomatic and control group was taken from 7.30 AM to 8.00 AM, on an empty stomach and before morning administration of the therapy. The listed biochemical analyses were performed in the Clinical Biochemical Laboratory, of the Military Hospital, Niš.

Ultrasound parameters. By the anatomical and morphological depiction of the carotid arteries (B-mode) we analyzed the characteristics of blood vessels: the degree of stenosis expressed by the ratio of the diameter in the stenotic area and the residual lumen diameter according to the following formula – $d2-d1/d2 \times 100$ ¹³; echomorphological plaque characteristics; using a grey-scale median, (GSM) for visual assessment, carotid plaques were divided into 4 types according to the standard classification (Gray-Weale): I – predominantly echolucent plaque with a thin echogenic cap; II – substantially echolucent plaque with small areas of echogenicity, < 25%; III – predominantly echogenic plaque with small areas of echolucency, < 25%; IV – uniformly echogenic plaque, equivalent to homogenous one¹⁴. The tested blood vessels were observed in the longitudinal and transverse projection and adequate positions. The ultrasound examination was performed on the appliance HITACHI EUB 5500, Ultrasound Scanner, Japan with a 10 MHz probe. The measurements were taken before the ostium of the internal carotid artery (ICA) and the distal part of the common carotid artery (CCA) in five consecutive sections, and the medium values were used for the statistical analysis of data. Ultrasound examination of the subjects of the symptomatic group was performed on the day of admission, of the subjects of the asymptomatic and the control group after obtaining blood samples for analysis.

Clinical parameters. The degree of neurological deficit in symptomatic patients was assessed by a standardized scale, National Institute of Health Stroke Scale (NIHSS) – admission NIHSS score⁴.

Neuroradiological parameters. MSCT of endocranium was performed in symptomatic patients natively or following a contrast agent application. The MSCT was performed on the appliance TOSHIBA AQUILION, 16 multisliced scanner, Japan. The largest diameter (mm) was used in the assessment of the ischemic lesion size. Densimetric alterations which by their clinical categorization and pathogenesis did not correspond to atherothrombotic cerebral infarction were not included in the study⁷.

All the subjects gave a written consent to be included in the study after an insight into the written information of the planned research. Those with acute cerebral infarction and altered consciousness were required the consent of the closest family members.

The preliminary design had the authorization of the Ethic Committee of the home institution that it met the profes-

sional and ethic criteria, that there was no deviation from the principles stated in the Declaration of Helsinki and that the planned research could be conducted in the home institution.

The assessment of the correlation between the values of different characteristics was performed by correlation analysis. The Friedman's test with (post-hoc) χ^2 to follow or Fisher's test were used for the comparison of the frequency of certain modalities of attributive characteristics. The analysis of variance (ANOVA) with Dunnett's test to follow were used for the comparison of numerical values among the three groups of subjects. The assessment of the influence of certain factors on the degree of stenosis was performed by univariate or multivariate linear regression analysis. The assessment of the significance of certain factors in the prediction of cerebral infarction was done by the application of univariate or multivariate logistic regression analysis, as well as Receiver Operating Characteristic (ROC) analysis.

Results

The average age of all the subjects was 66.21 ± 4.19 years. The average age of the subjects in the symptomatic group was 66.27 ± 4.27 years, and the differences compared to the subjects in the asymptomatic (66.93 ± 4.72 years) and the control group (64.67 ± 2.29 years) were not statistically significant (ANOVA and post-hoc Tukey's test: $p > 0.05$). The symptomatic group included 17 (56.7%) men and 13 (43.3%) women, and the asymptomatic involved 11 (36.7%) men and 19 (63.3%) women, the control group comprised 8 (53.3%) men and 7 (46.7%) women. The distribution according to sex was not homogeneous, but the differences among the compared groups in the structure by sex were not statistically significant ($\chi^2 = 2,62$; $p = 0,27$).

The average value of the erythrocyte sedimentation rate in the first hour (ESR I) in the subjects of the symptomatic group was 29.57 ± 29.87 cm, and in the second hour (ESR II) it was 51.60 ± 36.87 cm (Table 1). The number of leukocytes in the subjects of the symptomatic group was approximately $10.10 \pm 3.20 \times 10^9/L$, the average value of hsCRP was 8.15 ± 5.50 mg/L and fibrinogen was 4.03 ± 0.70 g/L (Table 1). The average value of ESR I in the subjects of the asymptomatic group was 15.73 ± 10.82 cm and ESR II was 32.87 ± 19.61 cm (Table 1). The number of leukocytes in the subjects of the asymptomatic group was $6.59 \pm 1.33 \times 10^9/L$, the average value of hsCRP was 4.12 ± 1.60 mg/L and fibrinogen was 3.43 ± 0.65 g/L (Table 1). The average value of ESR I in the subjects of the control group was 13.60 ± 9.33 cm and ESR II was 28.53 ± 19.09 cm (Table 1). The number of leukocytes in the subjects of the control group was $6.14 \pm 1.25 \times 10^9/L$; the average value of hs-CRP was 3.33 ± 1.25 mg/L and fibrinogen was 3.05 ± 0.80 g/L (Table 1).

The average value of ESR I in the subjects of the symptomatic group was significantly higher than in those of the asymptomatic (ANOVA and post-hoc Dunnett's test: $p = 0,030$) and the control group ($p = 0,043$). The average value of ESR II in the subjects of the symptomatic group was significantly higher than in the subjects of the asymptomatic ($p = 0,029$) and the control group ($p = 0,028$). The number of leukocytes in the subjects of the symptomatic group was

Table 1

Characteristics	Group			Comparison
	Symptomatic (n = 30)	Asymptomatic (n = 30)	Control (n = 15)	
Erythrocyte sedimentation rate I (cm)	29.57 ± 29.87	15.73 ± 10.82	13.60 ± 9.33	A*, B*
Erythrocyte sedimentation rate II (cm)	51.60 ± 36.87	32.87 ± 19.61	28.53 ± 19.09	A*, B*
Leukocyte count (×10 ⁹ /L)	10.10 ± 3.20	6.59 ± 1.33	6.14 ± 1.25	A‡, B‡
hs CRP (mg/L)	8.15 ± 5.50	4.12 ± 1.60	3.33 ± 1.25	A‡, B‡
hs CRP range, n (%)				
< 3.30 mg/L	0 (0.0)	7 (23.3)	6 (40.0)	A†, B*
> 3.30 mg/L	30 (100.0)	23 (76.7)	9 (60.0)	
Fibrinogen (g/L)	4.03 ± 0.70	3.43 ± 0.65	3.05 ± 0.80	A†, B‡

hsCRP – high sensitivity C-reactive protein; A – symptomatic vs asymptomatic; B – symptomatic vs control; **p* < 0.05; †*p* < 0.01; ‡*p* < 0.001.

significantly higher than in the subjects of the asymptomatic ($p < 0.001$) and the control group ($p < 0.001$); the average value of hsCRP in the subjects of the symptomatic group was significantly higher than in the subjects of the asymptomatic ($p < 0.001$) and the control group ($p < 0.001$) and the average value of fibrinogen was notably higher in the subjects of the symptomatic than in the subjects of the asymptomatic ($p = 0.005$) and the control group ($p < 0.001$).

All the subjects of the symptomatic group (100.0%) had the measured level of hsCRP higher than 3.3 mg/L, which was a significantly higher incidence than in the subjects of the asymptomatic (76.7%; $\chi^2 = 7.79$; $p = 0.005$) and the control group (60.0%; $\chi^2 = 13.54$; $p < 0.01$) (Table 1).

The representation of the category I of echomorphological characteristics (Figure 1) in the subjects of the symptomatic group was present in 20 (66.7%) of the cases and it was significantly higher than in the asymptomatic group where this category was found in 12 (40.0%) of the subjects ($\chi^2 = 4.21$; $p = 0.040$), as well as in the control group where category I of findings was confirmed in 3 (20.0%) of the subjects ($\chi^2 = 8.52$; $p = 0.003$). The representation of the category III of echomorphological characteristics in the subjects of the symptomatic group was present in 2 (6.7%) of the cases and it was significantly lower than in the control group where this category was found in 7 (46.7%) of the subjects ($\chi^2 = 9.78$; $p = 0.002$).

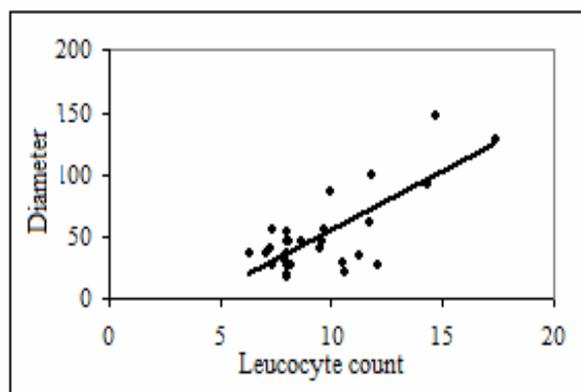


Fig. 1 – Correlation between ischemic cerebral lesion diameter and leukocyte count.

In the echomorphological category I there were 20 (57.1%) of the subjects of the symptomatic group, which was significantly higher than in the category II (8 subjects, 29.6%; $\chi^2 = 4.58$; $p = 0.032$) and the category III (2 subjects, 15.4%; $\chi^2 = 6.52$; $p = 0.011$). The echomorphological category III contained 7 (53.8%) of the subjects of the control group, which was a significantly higher incidence than in the category I (3 subjects, 8.6%; $\chi^2 = 12.72$; $p < 0.01$) and the category II (5 subjects, 18.5%; $\chi^2 = 5.08$; $p = 0.024$). $p < 0.05$). The average age of the subjects, as well as the structure by sex, were not statistically different in the compared categories of echomorphological characteristics.

The average value of the number of leukocytes in the subjects with the category I of echo findings was $8.82 \pm 3.62 \times 10^9/L$, the average value of fibrinogen was 3.87 ± 0.75 g/L (Table 2). In subjects with the category II of echo findings the average value of the number of leukocytes was $7.27 \pm 1.73 \times 10^9/L$, the average value of fibrinogen was 3.35 ± 0.71 g/L (Table 2). The average value of the number of leukocytes in subjects with the category III of echo findings was $6.76 \pm 1.59 \times 10^9/L$, the average value of fibrinogen was 3.37 ± 0.86 g/L (Table 2).

The average value of the number of leukocytes in subjects with the category I was notably higher than in those with the category II (ANOVA and post-hoc Dunnett's test: $p = 0.043$) and the category III ($p = 0.024$); the average value of fibrinogen in the subjects with the category I was notably higher than in those with the category II ($p = 0.022$). There was no significant difference in the values of other biochemical markers in the subjects with the three compared categories.

The percentage of stenosis in the subjects of the asymptomatic group was $57.17 \pm 5.47\%$ and it was notably higher than in the subjects of the symptomatic group ($32.20 \pm 10.69\%$; $\chi^2 = 51.63$; $p < 0.001$), as well as in the control group ($34.73 \pm 6.46\%$; $\chi^2 = 44.00$; $p < 0.001$). In all the 30 (100.0%) subjects of the asymptomatic group the percentage of stenosis was higher than 50%, which was a significantly higher incidence than in those of the symptomatic group (χ^2 -test: $p < 0.001$) where such findings were confirmed in 2 (6.7%) of the cases, as well as in the subjects of the control group ($p < 0.001$) where such findings were not confirmed in any of the cases. The subjects with 50% stenosis (28–65.1%) belonged to the symptomatic group, and 15 (34.9%)

Table 2

Characteristics	Morphological characteristics			Comparison
	Type I (n = 35)	Type II (n = 27)	Type III (n = 13)	
Erythrocyte sedimentation rate I (cm)	25.97 ± 28.63	17.22 ± 11.37	14.54 ± 10.42	n.s.
Erythrocyte sedimentation rate II (cm)	44.29 ± 35.43	37.41 ± 22.50	30.92 ± 21.33	n.s.
Leukocyte count ($\times 10^9$ /L)	8.82 ± 3.62	7.27 ± 1.73	6.76 ± 1.59	A*, B [†]
hs CRP (mg/L)	6.92 ± 5.60	4.47 ± 2.05	4.21 ± 1.11	n.s.
hs CRP range, n (%)				
< 3.30 mg/L	7 (20.0)	5 (18.5)	1 (7.7)	n.s.
> 3.30 mg/L	28 (80.0)	22 (81.5)	12 (92.3)	
Fibrinogen (g/L)	3.87 ± 0.75	3.35 ± 0.71	3.37 ± 0.86	A*

hsCRP – high sensitivity C-reactive protein; A – symptomatic vs asymptomatic; B – symptomatic vs control; ns – no significance; * $p < 0.05$; [†] $p < 0.01$; [‡] $p < 0.001$.

to the control group. The subjects with over 50% stenosis (2–6.3%) belonged to the symptomatic group, and 30 (93.8%) to the asymptomatic group. The difference in the structure of belonging to certain groups among subjects with varying degrees of stenosis was statistically significant ($\chi^2 = 67,37$; $p < 0.001$). The results showed no statistically significant sex and age distribution of the percentage of stenosis. The average number of leukocytes was significantly higher in the subjects with 50% stenosis (8.83 ± 3.35 : $6.66 \pm 1.32 \times 10^9$ /L, ANOVA and post-hoc Dunnett's test: $p = 0.001$) (Table 3). The values of other biochemical parameters were not notably different among groups formed according to the percentage of stenosis (Table 3).

The average value of NIHSS score in the subjects of the symptomatic group was 5.10 ± 2.86 , and the ischemic lesion diameter was 52.00 ± 30.83 mm (Figure 2). The correlation analysis showed a very high level of interdependence between the values of NIHSS score and diameter ($r = 0.949$, $p < 0.001$). These two characteristics also show the significant correlation with ESR I (NIHSS score: $r = 0.445$ and $p = 0.014$; diameter: $r = 0.537$ and $p = 0.002$), the number of leukocytes (NIHSS score: $r = 0.822$ and $p < 0.001$; diameter: $r = 0.824$ i $p < 0.001$) (Table 4, Figure 3).

The univariate logistic regression analysis as significant predictors of CVI confirmed ESR I, ESR II, the number of leukocytes, hs-CRP, and fibrinogen. The increase in the

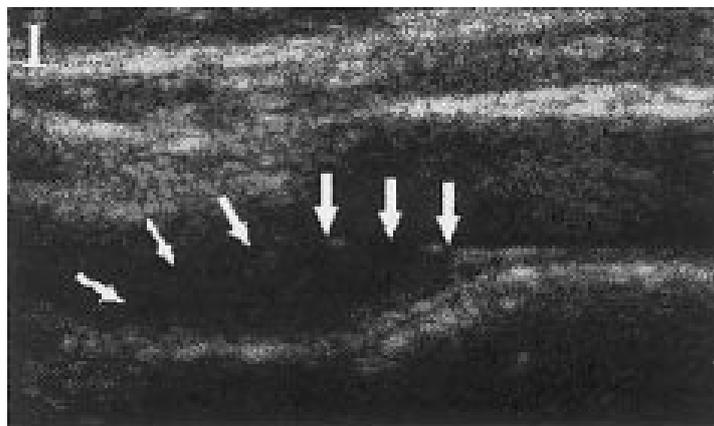


Fig. 2 – Predominantly echolucent plaque with a thin echogenic cap (category I, Gray-Weale classification).

Table 3

Parameters	Stenosis		Comparison
	≤ 50% (n = 43)	≥ 51% (n = 32)	
Erythrocyte sedimentation rate I (cm), $\bar{x} \pm SD$	23.98 ± 26.33	16.63 ± 11.68	n.s.
Erythrocyte sedimentation rate II (cm), $\bar{x} \pm SD$	43.47 ± 33.45	34.16 ± 21.71	n.s.
Leukocyte count ($\times 10^9$ /L), $\bar{x} \pm SD$	8.83 ± 3.35	6.66 ± 1.32	0.001
hs CRP (mg/L), $\bar{x} \pm SD$	6.22 ± 4.89	4.70 ± 2.92	n.s.
hs CRP range, n (%)			
< 3.30 mg/L	6 (14.0)	7 (21.9)	n.s.
> 3.30 mg/L	37 (86.0)	25 (78.1)	
Fibrinogen (g/L), $\bar{x} \pm SD$	3.69 ± 0.89	3.47 ± 0.64	n.s.

n.s. – no significance; hsCRP – high sensitivity C-reactive protein.

Table 4
Correlation between NIHSS score and serum levels of inflammatory parameters in the symptomatic group

Characteristics	NIHSS scor		Diameter	
	r	p	r	p
Erythrocyte sedimentation rate I (cm)	0.445	0.014	0.537	0.002
Erythrocyte sedimentation rate II (cm)	0.254	0.176	0.338	0.068
Leukocyte count ($\times 10^9$ U/L)	0.822	0.000	0.824	0.000
hs CRP (mg/L)	-0.256	0.172	-0.228	0.225
Fibrinogen (g/L)	0.141	0.458	0.178	0.346

hsCRP – high sensitivity C-reactive protein; NIHSS – National Institute of Health Stroke Scale.



Fig. 3 – Ischemic cerebral lesion.

value of each of the following factors by a unit of measurement was associated with a significant increase in the risk of CVI as follows: ESR I by 5% (95% CI; 1 to 9%; $p = 0.029$),

ESR II by 3% (95% CI; 1 to 5%; $p = 0.007$), the number of leukocytes by 3.17 times (95% CI; 1.80–5.56 times; $p = 0.001$), hsCRP by 91% (95% CI; 33–173%; $p = 0.001$), fibrinogen by 3.86 times (95% CI; 1.83–8.16 times; $p < 0.001$) (Table 5). On the contrary, the increase in stenosis percentage value by a unit of measurement was associated with a significant decrease in the risk of CVI by 10% (95% CI; 6–15%) (Table 5). In the subjects with category I of echomorphological characteristics the risk of CVI was notably higher than in the subjects with categories II and III by 4 times (95% CI; 1.50–10.66 times) (Table 5).

The multivariate logistic regression analysis as the most important predictor of CVI singled out the number of leukocytes. Each increase in the value of this characteristic by an unit of measurement was associated with a significant increase in the risk of CVI by 3.22 times (1.67– 6.22 times) (Table 6).

The regression model containing these two factors and the regression constant as independent variables, explained even 66% of the variability of the risk of CVI in subjects of the sample (determination coefficient - $R^2 = 0.66$).

Table 5
Stroke risk factors stratified by inflammatory parameters (univariate analysis)

Factors	OR	95% CI bounds		p
		Lower	Upper	
Erythrocyte sedimentation rate I (cm)	1.05	1.01	1.09	0.020
Erythrocyte sedimentation rate II (cm)	1.03	1.01	1.05	0.007
Leukocyte count ($\times 10^9$ /L)	3.17	1.80	5.56	< 0.001
hs CRP (mg/L)	1.91	1.33	2.73	< 0.001
Fibrinogen (g/L)	3.86	1.83	8.16	< 0.001
Stenosis (%)	0.89	0.84	0.94	< 0.001
I category Eho morph	0.82	0.31	2.17	0.695
II category	0.79	0.31	2.05	0.631
III category	1.71	0.59	4.99	0.323

OR – odds ratio; CI – confidence interval; NIHSS – National Institute of Health Stroke Scale. hsCRP –high sensitivity C-reactive protein.

Table 6
Stroke risk factors stratified by inflammatory parameters (multivariate analysis)

Risk factor	OR	95% CI bounds		p
		lower	upper	
Leukocyte count ($\times 10^9$ /L)	3.22	1.67	6.22	< 0.001

OR – odds ratio; CI – confidence interval.

Discussion

Contemporary knowledge about the potential reversibility of ischemic cerebral damage¹² influences the formation of a therapeutic approach in the prevention and treatment of acute cerebral infarction. The results of studies dealing with the morphology of atherosclerotic lesions have provided epidemiological support of the hypothesis that the vulnerability of carotid plaque is an etiopathogenic factor of acute cerebral infarction¹⁵. Studies on the therapeutic effect of endarterectomy in symptomatic/asymptomatic patients emphasize stenosis as risk factor for cerebrovascular complications¹⁶ in clarifying the mechanisms which associate atherosclerosis with ischemic cerebral damage. Ultrasound evaluation of the morphological and hemodynamic status of carotid plaque^{17, 18} influence the formation of the attitude that the size and structure of atheroma should be considered as separate risk factors of acute cerebral infarction in the assessment of embolic potential of atherosclerotic lesions.

Epidemiological support of the hypothesis that echolucency carries an increased risk of cerebrovascular complications¹⁷⁻¹⁹, the definition of stable/unstable plaque and classification on the basis of echo-morphological characteristics¹⁴ confirm that echomorphological characteristics are an indicator of the vulnerability and risk of future neurological symptomatology^{17,18}.

The traditional opinion that the size of atherosclerotic lesion is the criterium in the assessment of high-risk changes²⁰ does not encompass the concept of inflammation as a basic mechanism of atherogenesis²¹. Scientific research on experimental and human models provide theoretical knowledge and assumptions that inflammation can predispose distal embolization^{22, 23}. Even though we emphasize the importance of chronic subclinical inflammation in blood vessel wall, precise opinions on the proinflammatory response and the role of inflammatory mechanisms in destabilization of carotid plaque have not been formed. The connection of inflammatory, rheological and coagulation/fibrinolytic processes in the network of complex interactions^{21, 22} gives the possibility of considering numerous parameters altered by the biochemical conditions of vulnerable carotid plaque.

By statistical analysis of biochemical markers characteristics it has been determined that the average values of the examined parameters are significantly higher in the subjects of the symptomatic group than in the subjects of the asymptomatic and control group.

The independent connection of Er dysfunction and carotid plaque incidence in the results of clinical studies represents a rational basis of the assumption that inflammation and oxidative stress (OS) are the factors which change Er homeostasis through the alteration of the morphology and activity^{22, 24}. The influence of plasma proteins fibrinogen, immunoglobulin, lipoprotein, α_2 macroglobulin on the ESR and increased aggregation potential under the influence of cytokines^{22, 24} confirm that the altered activity of Er is a part of the chronic inflammatory processes of atherogenesis. In estimating the role of the ESR in the progression and stability of carotid plaque there were no statistically signifi-

cant differences in the values of the ESR I and ESR II in the subjects of the three compared categories of echomorphological characteristics. The average value of fibrinogen was notably higher in the subjects with category I than in those with category II of echomorphological characteristics ($p < 0.05$). The values of the ESR I, ESR II and fibrinogen were not significantly different among the two groups formed according to the hemodynamic significance of stenosis percentage ($\geq 50\%$, $< 50\%$). The obtained results were in accordance with the opinion formed after the publication of the Norwegian study TROMSO, that the size and structure of carotid plaque should be considered as separate factors in assessing the risk of the development of acute cerebral infarction^{17,18}.

Fibrinogen as a reactant of the acute phase is an important determinant of the ESR^{22, 24-26} which indicates that within a framework of the systemic response, the ESR can be the measure of response of brain alteration at an early stage of ischemia²⁷.

Significantly higher values of the ESR I and ESR II in the subjects of the symptomatic group compared to the subjects of the asymptomatic and control group represent a rational basis of the conclusion that the ESR can be an acceptable test in the monitoring of chronic inflammatory processes related to atherosclerosis. In assessing the influence of the ESR as risk factor for cerebrovascular ischemic complications, its predictive significance was confirmed by the univariate logistic regression analysis, as well as that the increase in the value of this parameter by a unit of measurement was associated with a significant risk growth. Even though the prognostic significance of appearance of the clinical manifestations of the progression and complications of carotid disease was not confirmed in multivariate logistic regression analysis, in the statistical processing of the interdependence between the values of NIHSS score (ischemic lesion diameter) and examined biochemical markers, a significant correlation during the first hour indicates that the ESR can carry important information for the early prognosis of acute cerebral infarction.

No interdependence has been found between the concentration of fibrinogen, markers of the severity of neurological deficit and the size of ischemic brain lesion, even though there has been confirmation of the association of the average values of fibrinogen with echomorphological characteristics. That is in accordance with the results of immunohistochemical tests that hyperfibrinogenemia is associated with the specific composition of carotid plaque predisposing it to rupture and thrombosis^{28, 29}. Fibrinogen is a part of coagulation/fibrinolytic, rheological and inflammatory process and a marker of the progression of atherosclerosis through the mechanisms of the increase in platelet aggregability and blood viscosity, increased generation of fibrinous formations and decrease in fibrinolysis²², which can explain that the calculated average value of fibrinogen in the conducted research was significantly higher in the subjects of the symptomatic group than of the asymptomatic and control group. A connection of fibrinogen concentration and the risk of developing acute cerebral infarction in the this research was confirmed by the univariate logistic regression analysis. Each

increase in the value for an unit of measurement is associated with the risk increase. Similar results have been published by Atherosclerosis Risk in Communities Study (ARIC) and Tromsø studies emphasizing the significance of pharmacological control of carotid atherosclerosis progression^{15, 30}. The mechanisms of the pleiotropic effect of statin on multi-metabolic disorders encompass, within antiinflammatory effects, also the reduction of the concentration of fibrinogen in the primary and secondary prevention of acute cerebral infarction³¹. As a reactant of the acute phase, a part of the systemic response within the framework of cerebral ischemia can be a purposeful parameter in the assessment of carotid atherosclerosis progression and prediction at early stage of acute cerebral infarction.

Statistical analysis of biochemical parameters shows that the number of Le in the subjects of the symptomatic group is notably higher than in the subjects of the asymptomatic ($p < 0.001$) and the control group ($p < 0.001$). A connection of the number of Le and clinical progression of carotid disease and the influence of the increased number of Le on the risk of developing acute cerebral infarction is reflected in the phenomena of inflammation and infection in the process of atherogenesis^{32, 33}. As the study on Risk Factors in Impaired Glucose Tolerance for Atherosclerosis and Diabetes (RAID) points out, the number of Le is an independent determinant of the initiation and progression of atherosclerotic vascular disease³⁴. Low grade inflammation and subclinical infection, expressed through the number of Le, are a more informative indicator of the focal than general endothelial damage³⁵. The mechanisms of aggregation, adhesion and migration of Le³², connection of the number of Le, thickness of intima-media complexes and atherosclerotic plaque³¹ indicate that Le are a part of chronic subclinical inflammation. The rheological significance of Le stems from their size and deformability characteristics and the ability to release biologically active substances such as prostaglandins, leukotrienes, cytokines make them a part of the inflammatory response of the arterial wall²².

In statistical analysis of the characteristics of biochemical markers, it was determined no statistically significant difference in the average values of Le among the asymptomatic and the control group. The connection of the number of Le and reduction in the blood vessel diameter, an insignificant difference of the number of Le in the subjects with hemodynamically significant carotid plaque ($\geq 50\%$, asymptomatic group) and the subjects with hemodynamically insignificant carotid plaque ($< 50\%$, control group) confirm that the clinical neurologist should include both the size and structure of the atherosclerotic lesion when considering the appropriate treatment. In the conducted research the average value of Le in the subjects with category I of echomorphological characteristics was notably higher than in the subjects with the categories II and III.

The results of an experimental research suggest the mediating and modulatory role of Le in the acute inflammation inside the fibrous cap³⁴. The presence of inflammatory and immunocompetent cells in the human atheroma, synthesis and release of the numerous molecules with proinflammatory effects³⁶ confirm that inflammation is a basic determinant of the

vulnerability of atherosclerotic lesions. In defining the histological criteria of destabilization, we emphasized the ability of Le to dilute tissue by the secretion of proteolytic enzymes and that they are rarely present in intact plaques³. Starting from the premise that the presence of carotid plaque is the risk factor for cerebrovascular ischemic disorders, the results of the Northern Manhattan Stroke Study (NOMASS) give an epidemiological confirmation of the association of the number of Le with the incidence of carotid plaque in persons who did not suffer from acute cerebral infarction³³.

The majority of studies on the connection of carotid disease and acute cerebral ischemia^{15, 37, 38} stemmed from the results of the study Aortic Plaque and Risk of Ischemic Stroke (APRIS) suggesting no connection of the increased number of Le, appearance and size of atherosclerotic plaque and risk of acute cerebral infarction³³. The importance of clinical studies lies in the additional information which influences the formation of attitudes that there is a connection of the number of Le and subclinical atherosclerosis, independent on traditional risk factors³² and that the connection of the number of Le, carotid atherosclerosis and acute cerebral infarction confirm that the number of Le can be a significant predictor of cerebrovascular ischemic complications^{17, 30}. The altered permeability of the blood-brain barrier, accumulation of Le in the zone of acute ischemia, secretion of the proinflammatory cytokines, increased endothelial permeability and production of reactive oxygen metabolites (ROM), increased expression of the potentially neurotoxic enzymes are a part of physiological changes within a framework of the inflammatory reaction which affects ischemic brain tissue³⁹. The reduced flexibility of Le under a reduced pressure in the zone of acute cerebral infarction, adhesion to endothelium, impaired hemodynamics, occlusion of capillaries and altered blood viscosity lead to an increase of the zone of ischemic brain damage³⁹. In the conducted research the average number of Le was statistically significantly higher in the group of the subjects with acute cerebral infarction compared to the asymptomatic ($p < 0.001$) and the control group ($p < 0.001$). Correlation analysis established the values of the numerical characteristics of NIHSS score (ischemic lesion diameter) in a significant connection with the number of Le. The obtained results are in accordance with the attitude that the increased number of Le can be related to the risk of developing acute cerebral infarction¹⁵ and that the degree of Le infiltration in the ischemic zone is in a positive correlation with the size of tissue damage and the disease outcome³⁹.

Assessing the tested factors influence on the occurrence of ischemic cerebral complications by the univariate logistic regression analysis showed that the values of the number of Le in the tested sample represented significant predictors of acute cerebral infarction. The multivariate logistic regression analysis singled out the number of Le as the most significant predictor of acute cerebral infarction.

The increased number of Le in the initial phases of atherogenesis and the increase with the progression of the disease indicate that the number of Le can be an indicator of subclinical inflammation of the arterial walls. Starting from the premise that significantly higher values of the number of Le in subjects with

carotid plaques of lower echogenicity are a part of pathophysiological mechanisms of the change of atherosclerosis lesion phenotype from a structurally vulnerable to functionally unstable form and that there is an increase of the number of Le as a reactant of the acute phase, as well as a positive correlation with the lesion size⁴⁰ in the neuroinflammatory response and considering the results of the conducted research, it can be concluded that the number of Le is an informative parameter in the clinical assessment of the stability of carotid plaque and risk of developing acute cerebral infarction.

In the pathophysiological mechanisms of atherogenesis the penetration of hsCRP into the arterial wall at the sites of endothelial dysfunction and the presence of a deposit in the early atherosclerotic lesion, binding to Le, synthesis by monocytes and macrophages, increased platelet aggregation, proliferation of smooth muscle cells and reduced expression of endothelial nitric oxide synthase (e-NOS)^{1, 38, 41} confirm the proinflammatory and proatherogenic effects of hsCRP in the initiation and progression of atherosclerotic vascular disease. By measuring numerous mediators of the inflammatory process, scientific studies on inflammation and atherosclerotic vascular disease emphasized that hsCRP as indicator of the risk of cerebrovascular ischemic complications was in correlation with the incidence of acute cerebral infarction^{35, 38, 39, 42, 43}.

The association of the concentrations of hsCRP with histological determinants of atherosclerotic lesion vulnerability in symptomatic and asymptomatic patients^{23, 44} indicates that hsCRP can be indicator of the change in atheroma phenotype from a stable to unstable form and a significant indicator of the risk of developing acute cerebral infarction⁴⁵.

Analysis of the connection of biochemical and ultrasound parameters showed the average values of hsCRP were not significantly different among the groups formed according to the hemodynamic significance and structure of carotid plaque. In the atherogenic profile of subjects the concentration of hsCRP was considered a reactant of the acute phase, from the perspective of neurological practice as a part of the systemic response in acute cerebral ischemia without the presence of the extrahepatic synthesis factor of hsCRP²².

The average value of hsCRP was notably higher in the symptomatic group compared to the asymptomatic and control

ones. All the subjects of the symptomatic group had the level of hsCRP above the reference values. Even though there was no correlation between the concentrations of hsCRP and severity of neurological deficit expressed through NIHSS score, in assessing the influence of the concentrations of hsCRP on the development of acute cerebral infarction by univariate logistic regression analysis it was established that hsCRP was a significant indicator of the possible cerebrovascular ischemic complications. Multivariate logistic regression analysis confirmed no predictive significance of hsCRP. The results of the conducted research and the results of other studies^{44, 45} confirm a connection of the increased concentrations of hsCRP, carotid atherosclerosis progression and the incidence of acute cerebral infarction and that hsCRP can be an indicator of the presence of unstable carotid plaque and the risk of ischemic cerebral complications. If we accept the attitude that hsCRP is a part of the process of inflammation in the pathophysiology of cerebral ischemia^{18, 46-48} and that the increased concentration is related to the severity of neurological deficit¹⁰, the fact that there are patients with normal values of hsCRP after acute cerebral infarction leads to the conclusion that the relation between hsCRP and brain damage is much more complex within a framework of the acute phase^{10, 45, 46}. Clinical trials in which the increase of the concentration of hsCRP in defined time intervals after acute cerebral infarction was compared to the values prior to the disease, confirmed that the significance of hsCRP in the pathogenesis of acute cerebral ischemia is the expression of inflammatory system individual response^{10, 44, 45}.

Conclusion

The predictive significance of the number of Le confirms that the number of Le is associated with the phenomenon of atherosclerotic plaque vulnerability and may be a useful, additional marker in the clinical practice of neurologists in discovering new pharmacological approaches in the prevention of cerebrovascular complications. It is possible that the risk of developing acute cerebral infarction could be reduced by controlling the carotid plaque stability through the continuous therapeutic influence on the pathogenic mechanisms of destabilization.

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