IMAGE AND LABORATORY ASPECTS OF CAROTID ATHEROSCLEROSIS

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ABSTRACT

Carotid atherosclerosis is a main risk factor for ischemic stroke. Plaque instability is determined by the morphological characteristics of the plaque and can be characterized by immunological biomarkers. The study aimed to examine the connection between serum levels of hs-CRP, fibrinogen, ICAM-1, VCAM-1 and carotid atherosclerosis and the different types of atherosclerotic plaques imaged by ultrasound and magnetic resonance. The study involved 120 patients with carotid atherosclerosis and 33 patients without carotid atherosclerosis. Blood samples were collected to analyze the serum level of hs-CRP, fibrinogen, ICAM-1 and VCAM-1. The ultrasound analysis included detection of atherosclerotic plaques in the internal carotid arteries, measurement of artery stenosis in percentage and determination of plaque types by the classification of Gray-Weales/Gerolacus. A small subset of 30 patients with carotid atherosclerosis performed 3T magnetic resonance imaging. Atherosclerotic plaques were classified into 8 types based on the modified MR classification of the American Heart Association. Significantly higher serum levels of hs-CRP ($p < 0.001$) and fibrinogen ($p = 0.018$) were observed in patients with carotid atherosclerosis compared to patients without atherosclerosis. Criterion values for hs-CRP > 4.13mg/l and for fibrinogen > 3.6 g/l were associated with the presence of carotid plaques with accuracy of 70%. No relation was observed between the investigated biomarkers, the artery stenosis and the types of atherosclerotic plaques determined by ultrasound and magnetic resonance diagnostic methods. Hs-CRP and fibrinogen are reliable serum markers whose increased serum concentrations are connected with the presence of carotid atherosclerosis.

Keywords: *Carotid atherosclerosis, biomarkers, image diagnostics, ultrasound, MRI.*

INTRODUCTION

Atherosclerosis of the internal carotid artery is a main risk factor for ischemic stroke (1). Atherogenesis is modified by immunological processes and specific biomarkers can characterize them (2). A histological classification of atherosclerotic plaque was proposed by American Heart Association (3). Morphological structures like large lipid-rich necrotic nucleus, plaque hemorrhage, thin fibrous capsule with surface irregularities determine the instability of the plaques (4). Non-invasive diagnostic methods such as ultrasound and magnetic resonance imaging can show plaque morphology and distinguish vulnerable plaques.

The study aimed to examine the connection between serum levels of hs-CRP, fibrinogen, ICAM-1 and VCAM-1 and carotid atherosclerosis and the different types of atherosclerotic plaques imaged by ultrasound and magnetic resonance.

MATERIAL AND METHODS

An observational, cross-sectional study has been conducted for 6 months (from November 2017 till May 2018) in the Clinic of Neurology, UMHAT "St.George"– Plovdiv, Bulgaria. It enrolled consecutively 153 patients that were sent for ultrasound examination of the carotid arteries in the Ultrasound Laboratory of the Clinic. The inclusion criteria were all indications for ultrasound investigations of the carotid artery such as acute ischemic stroke or primary stroke prevention because of risks factors such as history of hypertension, carotid atherosclerosis, atrial fibrillation, diabetes mellitus and cardiovascular diseases. The exclusion criteria were autoimmune diseases, cancer and inflammatory diseases that could influence the biomarkers. Two main groups were formed: patients with carotid atherosclerosis (120 patients) and patients without carotid atherosclerosis (33 patients). The study protocol was approved by the Local Ethics Committee (31.03.2016/protocol №2). All subjects provided written informed consent prior to their participation in the study.

Laboratory investigations

Blood samples from all patients were collected to analyze the serum level of hs-CRP, fibrinogen, ICAM-1 and VCAM-1. The sample were taken in the morning between 6-8 a.m. as atraumatically as possible on the next day after the ultrasound investigation. For the patient with an acute stroke, blood samples were taken during the first 24 hours after the onset of the stroke. The high sensitive C-reactive protein was determined immunoturbimetrically using an automated OLIMPUS AU400 clinical chemistry analyzer, Beckman Coulter USA. The measurement of hs-CRP is in mg/L. Serum fibrinogen was measured coagulometrically by an automated Sysmex Cs 2000 analyzer. The fibrinogen test is in g/l with a reference range of 2-4g/l. Serum concentrations of VCAM-1 and ICAM-1 were measured by ELISA analysis using commercially available CE brand kits (Bender Med Systems,GmbH). There are no definite data in the literature on the reference serum values of VCAM-1 and ICAM-1.

Image investigations

The ultrasound examinations were performed with the ultrasound machine Philips ClearVue 500. The analysis included the detection of atherosclerotic plaques in the internal carotid arteries, the measurement of artery stenosis in percentages using the local method and the determination of plaque types according the classification of Gray-Weales / Gerolacus (5). A small subset of 30 patients with ultrasound proven atherosclerosis in the internal carotid artery performed 3T magnetic resonance imaging. MR scans were done on a 3T MR imaging system (Discovery MR750; GE Healthcare) with a 24-channel head and neck coil. A standardized protocol was used, which includes 4 different contrast-weighted images in the axial plane - 3D time of flight magnetic resonance angiography with high resolution (HR art 3D TOF MRA), T1-weighted (T1W), proton density weighted (PDW), T2 -weighted (T2W) images and a sagittal modality T1 3D Cube. Both carotid bifurcations were identified by 2D MR PC (Phase contrast) coronary angiography. Using 2D MR PC-MRA as a localizer, the four impulse sequences (HR art 3D TOF, T1-W, T2-W, PD) were performed on bilateral carotid arteries in a transverse plane 2 cm proximal and 2 cm distal to the carotid bifurcation. T1W, PDW, T2W and T1 3D Cube were FSE (Fast spin echo) sequences in which was used the Black Blood (BB) plaque imaging technique. ECG triggering was used in T1W, T2W and PDW. For T1 and PD weighted images, ECG triggering was one cardiac cycle (time for one R-R interval), and for T2 weighted images, it was two cardiac cycles. Fat suppression (FS) to reduce the signal from subcutaneous adipose tissue was used in T1W, T2W, T1 3D Cube in all patients and in PDW in some patients. For each sequence, the signal of the adjacent sternocleidomastoid muscle was used as a reference zone, which compares the signal intensity of the major plaque component. Atherosclerotic plaques were classified into 8 types based on the modified MR classification of the American Heart Association (6).

Statistical analysis

The data were analyzed through the statistical software IBM SPSS version 27 (2020) and Medcalc version 21.1 (2021). Continuously measured variables were described through the mean values and standard deviations when normality was observed, and between group comparisons were performed using the t-test for independent samples. Variables that were not normally distributed were described with the medians and interquartile range (IQR) and compared between groups through the Mann-Whitney U test. Potential connections between biomarkers and different types of atherosclerotic plaques were examined through Spearman rank-order correlation analysis. Binary and categorical data were presented in frequencies and percentages and associations were established through the Chi-square test and Fisher's

exact test. Receiver operating characteristic (ROC) curve was used to establish the potential of biomarkers in the diagnosis of different atherosclerotic plaque. All statistical tests were two-tailed and performed at level of significance *alpha* = 0.05.

RESULTS

Demographic and clinical data

The study involved 120 patients with carotid atherosclerosis (CA) of mean age 67.10±9.80 years and 33 patients without CA of mean age 57.75±10.52, with a significant age difference ($p < 0.001$) (Table 1). The sex distribution was similar with no significant differences between the patients with CA and without CA ($p = 0.077$). A significantly higher proportion of the patients with CA had hypertension ($p = 0.039$) and cardiovascular diseases ($p = 0.005$) in comparison with the patients without CA. The groups did not differ in the proportions of atrial fibrillation ($p = 0.202$), diabetes ($p = 0.590$) and stroke ($p = 0.434$).

The serum hs-CRP level was significantly higher in the patients with CA compared to the patients without CA, $p < 0.001$ (Figure 1a). The proportion of patients with risk levels of hs-CRP > 3mg/l was 60.00% in the CA group versus 36.40% in the group without CA, $p = 0.018$. Significantly higher fibrinogen levels were observed in the patients with CA, $p=0.012$ (Figure 1b). The proportion of the patients with fibrinogen > 4g/l was 54.00% in the group with CA versus 27.27% in the patients without CA, $p = 0.026$. ICAM-1 and VCAM-1 did not show a significant association with the presence of CA.

ROC curve analysis showed an acceptable level of diagnostic accuracy of hs-CRP in distinguishing patients with CA from patients without CA (AUC = 0.702, $p < 0.001$). The optimum criterion cut-off value was estimated as hs-CRP > 4.13 mg/l, associated with 59.33% sensitivity and 78.79% specificity. Fibrinogen showed a similar level of diagnostic potential (AUC = 0.793, $p < 0.001$), criterion value > 3.61 g/l, sensitivity 56.77% and specificity 84.00% (Table 2 and Figure 2).

Carotid artery stenosis

The percentage of carotid artery stenosis ranged between 5% and 100%, with a mean value of 40.70±28.09% and a median of 40%. No significant association was observed between the percentage of carotid vessel stenosis and the serum levels of the biomarkers: hs-CRP ($r^s = 0.001$, $p = 0.993$); fibrinogen ($r^s = -0.010$, $p = 0.920$); ICAM-1 ($r^s = - 0.025$, $p = 0.820$); VCAM-1 ($r^s = -0.014$, $p = 0.901$) (Figure 3).

Biomarkers versus the different types of carotid plaques determined by ultrasound and MRI diagnostic methods

Gray-Weale/Gerolacus classification

In the group with CA, the Gray-Weale/Gerolacus US plaque types showed the following distribution: Type I - 4.2% (n =5); Type II - 15.8% (n=19); Type III - 40% (n=48); Type IV- 26.70% (n = 32); Type V - 13.30% (n=16). None of the biomarkers showed a significant association with the Gray-Weale/Gerolacus types: hs-CRP ($r^s = 0.028$, $p = 0.760$); fibrinogen ($r^s = -0.078$, $p = 0.468$); ICAM-1 ($r^s = -0.073$, $p = 0.479$); VCAM-1 ($r^s = -0.202$, $p = 0.066$) (Figure 4).

AHA MRI classification

AHA MRI classification of the carotid plaques was performed for 30 CA patients, 18 male and 12 female, yielding the following distribution of plaque types: Type I & II – 3.30% (n = 4); Type III – 1.7o% (n = 2); Type IV & V – 9.20% (n = 11); Type VI – 3.30% (n = 4); Type VII – 5.00% (n = 6); Type VIII – 2.50% (n=3).

The serum levels of the target biomarkers did not show a significant association with the AHA plaque types: hs-CRP (rs = -0.075, $p = 0.697$); fibrinogen (rs = 0.199, $p = 0.300$); ICAM-1 (rs = - 0.293, $p = 0.122$); VCAM-1 (rs = -0.262, $p = 0.171$) (Figure 5).

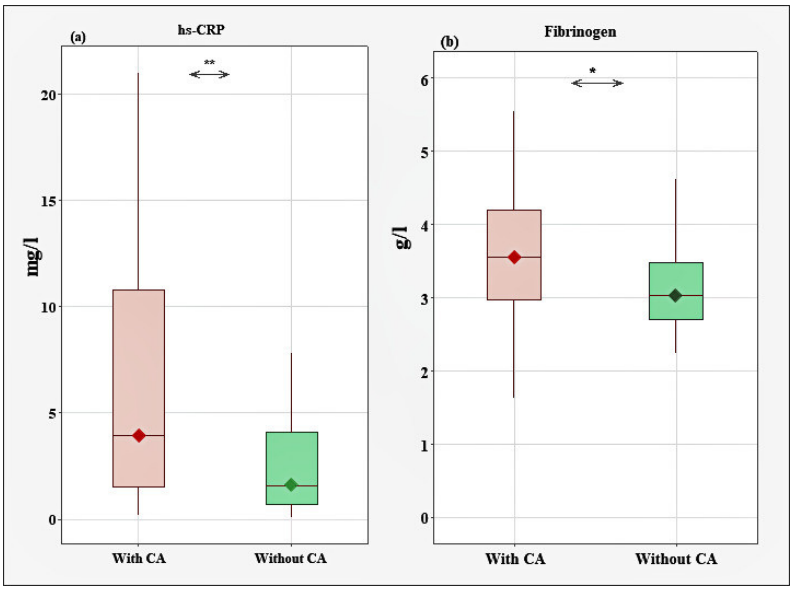
Table 1. Demographic and clinical data

Variables	Group		p
	With carotid atherosclerosis (n = 120)	Without carotid atherosclerosis (n = 33)	
Age			
o Mean (SD)	67.10 (9.80)	57.75 (10.52)	$< 0.001^t$
o Min.-Max.	41 to 88	38 to 78	
Sex			
o Male	69 (57.50%)	13 (39.40%)	0.077^f
o Female	51 (42.50%	20 (60.60%)	
Hypertension n (%)	105 (87.50%)	24 (73.00%)	$0.039^{\chi 2}$

Variables	Group		p
	With carotid atherosclerosis (n = 120)	Without carotid atherosclerosis (n = 33)	
Atrial fibrillation n (%)	23 (19.00%)	3 (9.00%)	0.202 χ^2
Cardiovascular diseases n (%)	62 (51.70%)	8 (24.20%)	0.005 χ^2
Diabetes n (%)	40 (33.30%)	9 (27.30%)	0.590 χ^2
Stroke n (%)	65 (54.16%)	15 (45.45%)	0.434 χ^2
hs-CRP mg/l			
○ Median (IQR)	3.93(9.27)	1.57(3.38)	0.005 U
○ Min.-Max.	0.21 to 314.78	0.11 to 52.84	
○ n(%) > 3mg/l	72 (60%)	12 (36.40%)	0.018 f
Fibrinogen g/l			
○ Median (IQR)	3.56 (1.22)	3.03 (0.78)	
○ Min.-Max.	1.63 to 7.32	0.66 to 5.23	0.012 U
○ n(%) > 4g/l	65 (54.00%)	9 (27.27%)	0.026 f
ICAM-1			
○ Median (IQR)	457.50 (201.25)	417.00 (133.50)	0.168 U
○ Min.-Max.	164.00 to 981.00	176.00 to 671.00	
VCAM-1			
○ Median (IQR)	1032.50 (1267.50)	1020.00 (1246.25)	0.319 U
○ Min.-Max	395.00 to 7705.00	300.00 to 2465.00	

t- t-test for independent samples; f – Fisher's exact test; $\chi 2$ – chi-square test; U – Mann-Whitney

Figure 1: Significantly higher hs-CRP levels (**panel a**) and fibrinogen levels (**panel b**) in the patients with CA (**b**)



** - p < 0.01; *- p < 0.05

Table 2. ROC curve results for the diagnostic potential of hs-CRP and fibrinogen

Biomarker	AUC 95% CI	SE	p	Criterion value	Sensitivity	Specificity
hs-CRP	0.702 (0.623 to 0.774)	0.05	<0.001	>4.13mg/l	59.33%	78.79%
Fibrinogen	0.703 (0.616 to 0.789)	0.05	<0.001	> 3.61 g/l	56.77%	84.00%

Figure 2. Roc curves for the diagnostic potential of serum hs-CRP (panel a) and fibrinogen (panel b) in distinguishing patients with CA from patients without CA

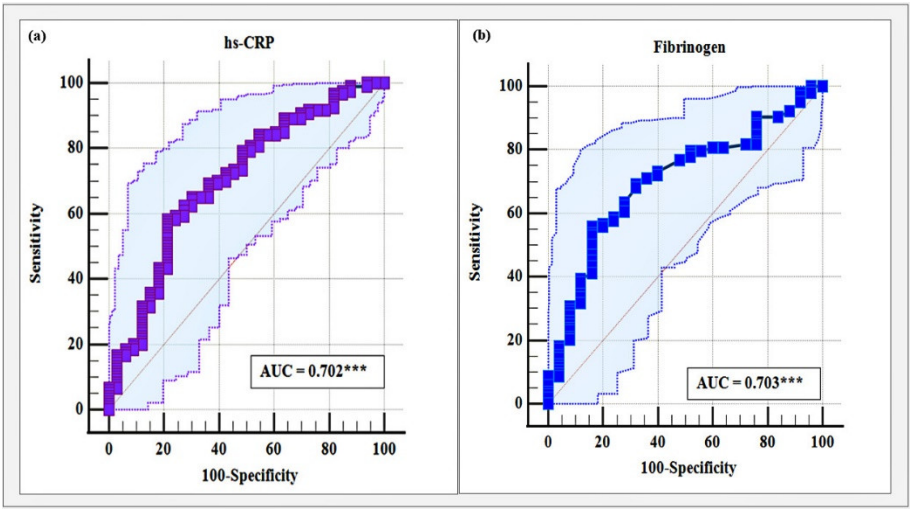


Figure 3. Scatter plots show no association between % stenosis and serum levels of hs-CRP (**panel a**), Fibrinogen (**panel b**), ICAM-1 (**panel c**) and VCAM-1 (**panel d**)

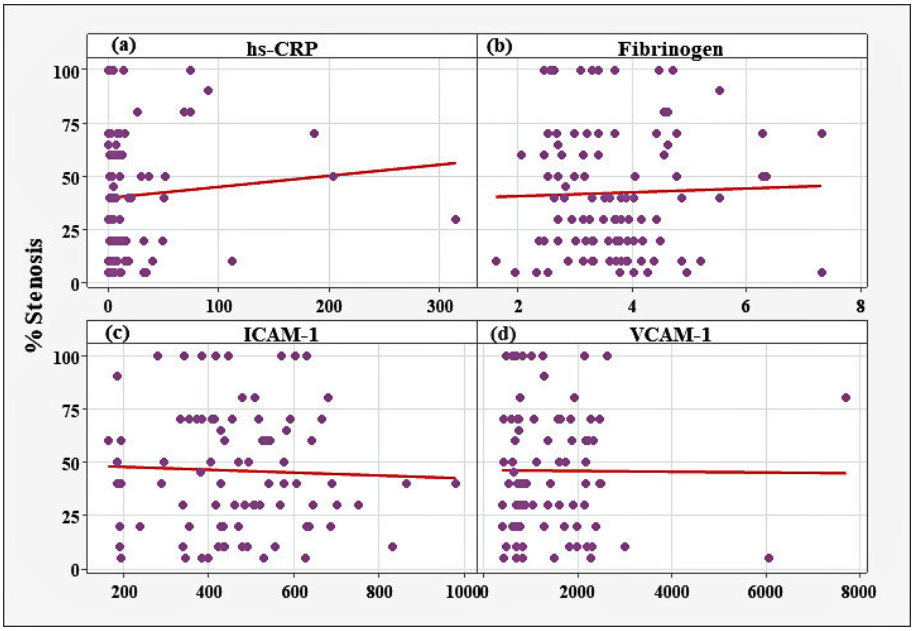


Figure 4. Scatter plots show no association between Gray-Weale/Gerolacus US plaque types and serum levels of hs-CRP (**panel a**), Fibrinogen (**panel b**), ICAM-1 (**panel c**) and VCAM-1 (**panel d**)

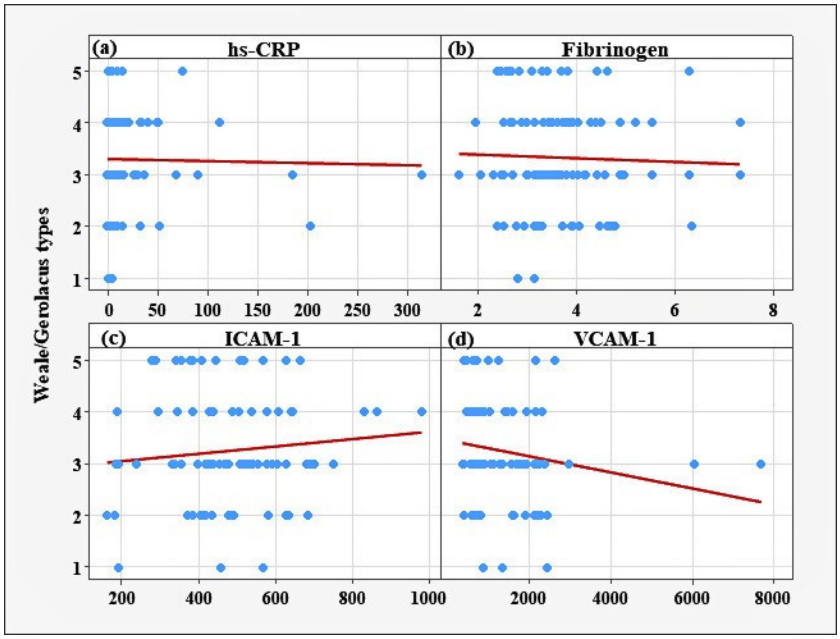
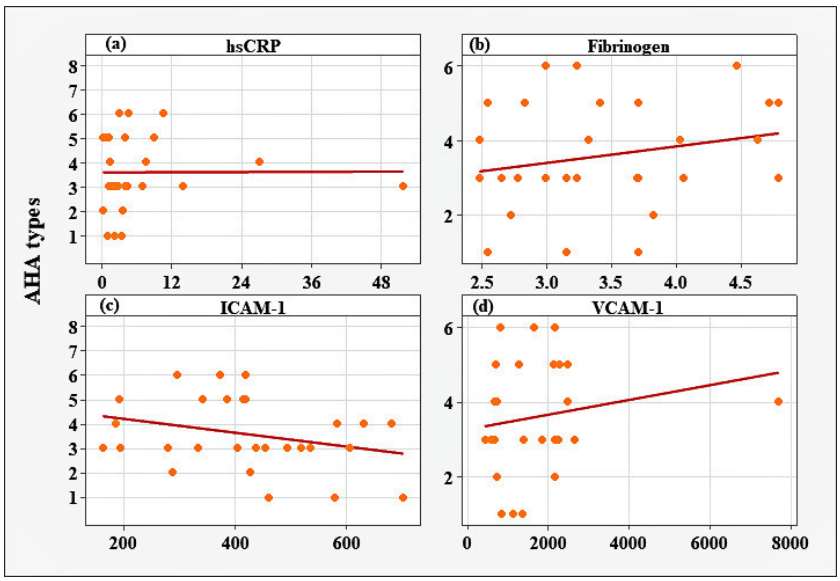


Figure 5: Scatter plots show no association between AHA MRI plaque types and serum levels of hs-CRP (**panel a**), Fibrinogen (**panel b**), ICAM-1 (**panel c**) and VCAM-1 (**panel d**)



DISCUSSION

The demographic data in our study showed that the mean age of patients with CA is significantly higher compared to patients without CA. We connect this fact with the aged- related changes. With increasing of age, the plaque burden and the incidence of atherosclerosis-related events accelerates and the morphology of atherosclerotic plaques changes toward vulnerable plaques (7, 8).

About the comorbidity we found that a significantly higher proportion of the patients with CA had hypertension and cardiovascular diseases (ischemic heart disease, cardiomyopathy, heart failure, valve defects, etc.). We think that further investigations should be done to analyze this connection.

In our study we chose to investigate several serum biomarkers (hs-CRP, fibrinogen, ICAM-1, VCAM-1) associated with the processes of vascular dysfunction and atherosclerosis (9-11). To determine their relationship with carotid atherosclerosis we performed investigations in several directions. First we performed a comparative analysis between patients with established CA and without CA on the level of hs-CRP, fibrinogen, ICAM-1 and VCAM-1. The serum levels of hs-CRP ($p < 0.001$) were significantly higher in the patients with CA. These data confirm the results obtained by other authors for the association of hs-CRP with carotid atherosclerosis (12-14). Most of the cited studies used the approved by the American Heart Association hs-CRP value above 3 mg / l. In their study, Puz et al [14] determined hs-CRP above 5 mg / l as a marker for the presence of carotid atherosclerosis. Studies in carotid atherosclerosis and ischemic stroke also confirmed the importance of hs-CRP as a diagnostic biomarker, but no standardized value was determined (15, 16, 17). If we used the proposed by AHA risk level we found that the proportion of patients with hs-CRP > 3mg/l was 60.00% in the CA group versus 36.40% in the group without CA ($p = 0.018$). We used the ROC curve analysis to find an acceptable level of diagnostic accuracy for hs-CRP in distinguishing patients with CA from patients without CA (AUC = 0.702, $p < 0.001$). The level of hs-CRP > 4.13 mg/l showed 59.33% sensitivity and 78.79% specificity.

Fibrinogen was the other biomarker that was significantly increased in patients with CA ($p=0.012$). The analyze about the distribution of patients with fibrinogen values > 4.0 g / l also showed a higher proportion in patients with atherosclerosis (54.00%) compared to those without atherosclerosis (27.27%). Increased fibrinogen levels could be observed in atherosclerosis with different localization - coronary heart disease, peripheral arterial insufficiency and carotid atherosclerosis (18). In order to find an acceptable value for fibrinogen associated with the presence of CA we again used the ROC curve analysis. Fibrinogen showed a level of diagnostic potential (AUC = 0.793, $p < 0.001$) with criterion value > 3.61 g/l (sensitivity 56.77% and specificity 84.00%).

Cell adhesion molecules (CAM) are immunoglobulin-like structures with a proven role in endothelial dysfunction and atherosclerosis (11, 19, 20). Increased expression was found in ischemic heart disease and in particular in acute coronary syndrome [21]. Debing et al. [12] studied the serum VCAM-1 levels in 180 patients with carotid atherosclerosis and compared the data with healthy controls and looked for additional dependencies with symptomatic carotid stenosis. The results of the study revealed that VCAM-1 could serve as markers for carotid atherosclerosis but not to identify plaque at risk for symptomatic conversion. Our analyzes of the potential association between ICAM-1, VCAM-1 and carotid atherosclerosis showed that CAM levels varied between patients, without a statistically significant trend. The measured serum values of ICAM-1 and VCAM-1 did not show a connection with the presence of carotid atherosclerosis.

In our study no significant association was observed between the percentage of carotid stenosis and the serum levels of the selected biomarkers. Lack of such connection was also reported by Puz et al. (14), and Debing et al. (12). In a study of 104 patients, Benbir et al. (22) found a connection between hs-CRP and the severity of carotid atherosclerosis. In the study, patients were divided into 4 categories - patients without plaques, with increased IMT, with plaques below 50% stenosis and with plaques above 50% stenosis. According to the authors, increased hs-CRP values and more pronounced atherosclerotic changes were independent risk factors and their interaction increased the risk of cerebrovascular accident. Musialek at al. (23) investigated several circulating biomarkers in 300 patients with ultrasound assessed carotid stenosis ≥50% who were referred for potential revascularization. They observed that the levels of high-sensitivity C-reactive protein were higher ($p=0.04$ and $p=0.07$, respectively) in the symptomatic stenosis group compared to the asymptomatic stenosis group. Sabeti et al. (24) monitored fibrinogen levels in 1,268 patients with atherosclerotic plaques of the carotid arteries. Patients performed periodic ultrasound assessment at 3 and 6 month intervals, and were divided into 6 groups depending on the percentage of arterial stenosis (from 0% to 29%, from 30% to 49%, from 50% to 69%, from 70% up to 89%, from 90% to 99% and total occlusion). In order to determine the activity of inflammatory processes in atherosclerosis, a comparison was made with other inflammatory markers. The results of the study showed increased fibrinogen levels with the progression of atherosclerosis, but this association seemed to be nonspecifically related to the extent of the inflammatory process in atherosclerotic disease rather than to specific properties of fibrinogen.

The morphological characteristics of the plaque are important in determining their instability and risk of causing cerebrovascular accidents (25, 26). In our study, we used established Gray-Weale/Gerolacus ultrasound scale (5) and the Modified AHA Classification for MRI (6). With these diagnostic methods, plaque structures such as calcification, fibrous cap, plaque hemorrhage and lipid-rich necrotic nucleus could be identified with moderate to good sensitivity and specificity (27). Weiss and al. (28) explored MRI properties of large arteries an their association with serum markers of inflammation. The authors thought that MRI of large arteries could provide a new approach to investigate the contribution of inflammation in atherogenesis. van Dijk and al. (29) investigated the association between fibrinogen and fibrinogen γ' and atherosclerotic plaque morphology in symptomatic carotid artery stenosis. Presence of plaque ulceration, intra plaque hemorrhage (IPH) volume and lipid-rich necrotic nuclei (LRNC) volume was determined by Multidetector-Row Computed Tomography and Magnetic Resonance Imaging. The author found that fibrinogen and fibrinogen γ' were inversely associated with IPH volume and LRNC volume, independent of inflammation.

The results about the hs-CRP are controversial. Puz et al. (14) found no correlation between hs-CRP values and ultrasound morphological characteristics of unstable carotid

plaques (hypoechoic, with an irregular surface and stenosis above 70%). Lombardo et al. (30) observed higher values of hs-CRP in ultrasound assessed complex atherosclerotic plaques with the following characteristics - heterogeneous with irregular and ulcerated surface.

There are a small number of studies comparing CAM and the imaging characteristics of atherosclerotic plaques. Rohde et al. (31) monitored the levels of CAM in patients with carotid atherosclerosis, finding a positive relationship between their serum concentrations and the ultrasound index IMT. Shindo et al. (32) studied patients with unstable atherosclerotic plaques of the carotid arteries diagnosed by magnetic resonance imaging. They found a positive correlation between the magnetic resonance characteristics of unstable plaques and the serum values of several pro-inflammatory factors, one of which is VCAM-1.

In our study no significant association was observed between the levels of hs-CRP, fibrinogen and ICAM-1 and VCAM-1 and different types of atherosclerotic plaques determined by the Gray-Weale / Gerolacus ultrasound classification and the modified MP classification of the American Heart Association.

CONCLUSIONS

Our data showed significantly higher serum levels of hs-CRP and fibrinogen in the patients with carotid atherosclerosis. A criterion value for hs-CRP > 4.13mg/l and for fibrinogen > 3.6 g/l can be applied in the diagnosis of carotid plaques in patients with accuracy of 70%. We found no correlation between serum levels of selected biomarkers and arterial stenosis and plaque morphology presented by ultrasound and MR diagnostic methods.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocol was approved by the Local Ethics Committee (31.03.2016/protocol №2). All subjects provided written informed consent prior to their participation in the study.

CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

FUNDING

None.

REFERENCES

1. Grau A, Weimar C, Buggle F, Heinrich A, Goertler M, Neumaier S, et al. Risk factors, outcome and treatment in subtypes of ischaemic stroke: the German stroke data bank. Stroke. 2001;32:2559-66.
2. Ammirati E, Moroni F, Norata G, Magnoni M, Camici P. Markers of inflammation associated with plaque progression and instability in patients with carotid atherosclerosis. Mediators Inflamm. 2015;2015:718329.
3. Stary H, Chandler A, Dinsmore R, Fuster V, Glagov S, Insull W, et al. A Definition of Advanced Types of Atherosclerotic Lesions and a Histological Classification of Atherosclerosis. A Report From the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Circulation. 1995; 92:1355-74.
4. Osborn E, Jaffer F. Imaging atherosclerosis and risk of plaque rupture.Curr Atheroscler Rep. 2013; 15(10):359.
5. Gray-Weale A, Graham J, Burnett J, Byrne K, Lusby R. Carotid artery atheroma: comparison of preoperative B-mode ultrasound appearance with carotid endarterectomy specimen pathology. J Cardiovasc Surg. 1988;29:676-81.
6. Cai J, Hatsukami T, Ferguson M, Small R, Polissar N, Yuan C. Classification of human carotid atherosclerotic lesions with in vivo multicontrast magnetic resonance imaging. Circulation. 2002;106:1368-73.
7. van Oostrom O, Velema E, Schoneveld A, de Vries J, de Bruin P, Seldenrijk C, et al. Age-related changes in plaque composition: a study in patients suffering from carotid artery stenosis. Cardiovasc Pathol. 2005;14(3):126-34.
8. Pelisek J, Wendorff H, Wendorff C, Kuehnl A, Eckstein H. Age-associated changes in human carotid atherosclerotic plaques. Ann Med. 2016;48(7):541-51.
9. Pasceri V, Willerson J, Yeh E. Direct proinflammatory effect of C-reactive protein on human endothelial cells.Circulation. 2000;102:2165-8.
10. Levenson J, Giral P, Razavian M, Gariepy J, Simon A. Fibrinogen and silent atherosclerosis in subjects with cardiovascular risk factors. Arterioscler Thromb Vasc Biol. 1995;15:1263-8.
11. Chia M. The role of adhesion molecules in atherosclerosis. Crit Rev Clin Lab Sci. 1998;35(6):573-602.
12. Debing E, Peeters E, Demanet C, De Waele M, Van den Brande P. Markers of inflammation in patients with symptomatic and asymptomatic carotid artery stenosis: a case-control study. Vasc Endovascular Surg. 2008;42(2):122-27.
13. Hashimoto H, Kitagawa K, Hougaku H, Shimizu Y, Sakaguchi M, Nagai Y, et al. C-reactive protein is an independent predictor of the rate of increase in early carotid atherosclerosis. Circulation. 2001;104(1):63-7.
14. Puz P, Lasek-Bal A, Ziaja D, Kazibutowska Z, Ziaja K. Inflammatory markers in patients with internal carotid artery stenosis. Arch Med Sci. 2013;9(2):254-60.
15. Rost N, Wolf P, Kase C, Kelly-Hayes M, Silbershatz H, Massaro J, et al. Plasma concentration of C-reactive protein and risk of ischemic stroke and transient ischemic attack: the Framingham study. Stroke. 2001;32(11):2575-9.
16. Di Napoli M, Elkind M, Godoy D, Singh P, Papa F, Popa-Wagner A. Role of C-reactive protein in cerebrovascular disease: a critical review. Expert Rev Cardiovasc Ther. 2011;9(12):1565-84.

17. Peycheva M, Deneva T, Zahariev Z.High-sensitive CRP in ischemic stroke patients-from risk factors to evolution. Cesk Slov Neurol N. 2019;82(5): 549-55.
18. Surma S, Banach M. Fibrinogen and Atherosclerotic Cardiovascular Diseases-Review of the Literature and Clinical Studies. Int J Mol Sci. 2021;23(1):193.
19. Papagianni A, Kalovoulos M, Kirmizis D, Vainas A, Belechri A, Alexopoulos E, et al. Carotid atherosclerosis is associated with inflammation and endothelial cell adhesion molecules in chronic haemodialysis patients. Nephrol Dial Transplant. 2003;18(1):113-9.
20. Hulthe J, Wikstrand J, Mattsson-Hultén L, Fagerberg B. Circulating ICAM-1 (intercellular cell-adhesion molecule 1) is associated with early stages of atherosclerosis development and with inflammatory cytokines in healthy 58-year-old men: the Atherosclerosis and Insulin Resistance (AIR) study. Clin Sci (Lond). 2002;103(2):123-9.
21. Best P, Gersh B. Cell adhesion molecules and inflammation in acute coronary syndromes: markers and emerging risk factors. Eur Heart J. 2001;22(14):1155-9.
22. Benbir G, Bozluolcay M, Ince B. Is the level of C-reactive protein correlated with the extent of carotid atherosclerosis? Acta Neurol Belg. 2005;105(2):73-80.
23. Musialek P, Tracz W, Tekieli L, Pieniazek P, Kablak-Ziembicka A, Przewlocki T, et al. Multimarker approach in discriminating patients with symptomatic and asymptomatic atherosclerotic carotid artery stenosis. J Clin Neurol. 2013;9(3):165-75.
24. Sabeti S, Exner M, Mlekusch W, Amighi J, Quehenberger P, Rumpold H, et al. Prognostic impact of fibrinogen in carotid atherosclerosis: nonspecific indicator of inflammation or independent predictor of disease progression? Stroke. 2005;36(7):1400-4.
25. Brinjikji W, Rabinstein A, Lanzino G, Murad M, Williamson E, DeMarco J, et al. Ultrasound Characteristics of Symptomatic Carotid Plaques: A Systematic Review and Meta-Analysis. Cerebrovasc Dis. 2015;40(3-4):165-74.
26. Moreno P. Vulnerable plaque: definition, diagnosis, and treatment. Cardiol Clin. 2010;28(1):1-30.
27. den Hartog A, Bovens S, Koning W, Hendrikse J, Luijten P, Moll F, et al. Current status of clinical magnetic resonance imaging for plaque characterisation in patients with carotid artery stenosis. Eur J Vasc Endovasc Surg. 2013;45(1):7-21.
28. Weiss C, Arai A, Bui M, Agyeman K, Waclawiw M, Balaban R, at al. Arterial wall MRI characteristics are associated with elevated serum markers of inflammation in humans. J Magn Reson Imaging. 2001;14(6):698-704.
29. van Dijk A, Donkel SJ, Zadi T, Sonneveld M, Schreuder F, Chohan M, et al. Association between fibrinogen and fibrinogen γ' and atherosclerotic plaque morphology and composition in symptomatic carotid artery stenosis: Plaque-At-RISK study. Thromb Res. 2019;177:130-5.
30. Lombardo A, Biasucci L, Lanza G, Coli S, Silvestri P, Cianflone D, et al. Inflammation as a possible link between coronary and carotid plaque instability. Circulation. 2004;109(25):3158-63.
31. Rohde L, Lee R, Rivero J, Jamacochian M, Arroyo L, Briggs W, et al. Circulating cell adhesion molecules are correlated with ultrasound-based assessment of carotid atherosclerosis. Arterioscler Thromb Vasc Biol. 1998;18(11):1765-70.
32. Shindo A, Tanemura H, Yata K, Hamada K, Shibata M, Umeda Y, et al. Inflammatory biomarkers in atherosclerosis: pentraxin 3 can become a novel marker of plaque vulnerability. PLoS One. 2014;9(6):e100045.


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