ORIGINAL ARTICLE

CONTENTS

Modern approaches to ultrasonographic assessment of carotid plaque in terms of its potential instability

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ABSTRACT

Aim: To identify potentially adverse characteristics of atherosclerotic carotid plaque in terms of stroke risk.

Materials and Methods: The study is based on the analysis of US data obtained from 96 patients aged 47 to 84 years diagnosed with carotid stenosis. The patients were divided into 2 groups depending on the presence or absence of ischemic events. Examination methods: clinical, duplex US, brain MRI, statistical. In addition to the standard US criteria for the evaluation of carotid stenoses, superb microvascular imaging (SMI) and shear wave elastography (SWE) are used.

Results: Statistically significant association of the following characteristics of plaques with the presence of ipsilateral ischemia focus was established: maximum thickness ($p = 0,04$), presence of microvascularization according to SMI data ($p = 0,02$), degree of carotid stenosis (statistically significant in the right carotid circulation [$p = 0,04$]), stiffness of the plaque according to SWE data (significantly in the right carotid circulation [$p = 0,001$]), type of plaque echogenicity according to Gray-Weale-Geroulakos classification (on the right carotid artery; $p = 0,04$).

Conclusions: The presence of microvascularization according to SMI data and the low stiffness of the plaque according to SWE data can characterize the potential instability of the carotid plaque. These criteria should be added to the traditional US assessment of carotid plaques.

KEY WORDS: carotid plaque, carotid stenosis, ultrasound, superb microvascular imaging, shear wave elastography

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INTRODUCTION

Among radiological methods of diagnosing carotid stenoses, the leading role belongs to an ultrasonographic (US) study, and not only as the first link or screening in the assessment of this pathology. The complex of US criteria formed today allows to carry out quantitative and qualitative characterization of the arterial lesion area and its blood supply, as well as determine the indications for revascularization. Indications for carotid revascularization are based on the assessment of the patient's symptomatic status and the degree of ipsilateral stenosis. According to various prospective studies, ipsilateral 50-99% carotid stenosis was responsible for ischemic stroke in 8%-12.5% of patients [1]. Recently, it is believed that the degree of luminal stenosis is not the best predictive indicator of stroke risk, as the morphology of the plaque plays a significant role. The clinical guidelines of the European Society of Vascular Surgery note the importance of assessing plaque vulnerability [1].

Histopathological factors of unstable atherosclerotic plaque (AP) are thinning of the fibrous cap, ulceration and violation of the cap with the formation of blood clots on the surface, the predominance of atheromatosis over fibrosis, the presence of a lipid (necrotic) nucleus, hemorrhage in the plaque, the presence of neoplastic vessels. In vivo detection of this kind of signs a priority of new diagnostic methods [2].

According to US criteria, echo-transparent AP (hypoechogenic plaques of types 1 and 2 according to Gray-Weale classification) are traditionally considered to be unstable, as they occur more frequently in patients with symptomatic carotid stenosis. The irregularity of the surface of the plaque, the presence of ulceration, are also suggested as an ultrasound marker of an unstable plaque and an increased risk of stroke [3].

The recently proposed Plaque-RADS classification presents a system for reporting the composition and morphology of carotid plaques obtained through various imaging techniques such as US, CT and MRI. The categories of plaque evaluation include maximum wall thickness, fibrous cap, intraplaque hemorrhage, lipid-saturated/lipid-rich necrotic nucleus, ulcerated plaque, as well as "ancillary features", namely plaque inflammation and neovascularization, plaque burden, positive carotid remodeling, stenosis progression, and calcification. Of the 4 categories of plaque severity, categories from 2 to 3a can be diagnosed by US, the rest are determined with the help of MRI and CT [4]. However, new US techniques are able to complement the traditional assessment of AP.

Neovascularization in atherosclerotic plaque plays an important role in plaque vulnerability and is a major source of intra-plaque hemorrhage. Imaging of neogenic intra-plaque vessels has recently been considered to be a possible factor of its instability [5]. Conventional Doppler examinations filter out low-flow signals and prevent small vessel imaging. Contrast-enhanced ultrasound (CEUS) is useful in assessing the neovascularization of the carotid plaque as recommended by the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB, 2017), but requires the use of a contrast agent. Superb microvascular imaging (SMI) is a novel technique which can depict microvascular blood flow signals without the use of contrast agents [2]; the SMI technique is available in certain US diagnostic systems (Canon Medical Systems Corporation Otawara, Japan), demonstrated good sensitivity compared to CEUS as well as correlation with clinical symptoms. AP with higher SMI levels showed correlation with more neovessels in histological examination, as well as with hypoechogenic lesions compared to echogenic ones [5, 6].

Shear Wave Elastography (SWE) is a potential tool for the improvement of detection of vulnerable plaques and therefore it can improve the stratification of atherothrombotic stroke risk. Studies show good reproducibility and the potential clinical benefit of the Young's modulus (YM), its correlation with the qualitative assessment of the B-mode plaque (Gray-Weale classification), which helps to improve the diagnostic characteristics of the vulnerability of carotid plaques [7]. It was demonstrated that a lower mean YM is associated with focal neurological symptoms: transient ischemic attack (TIA), transient mononuclear blindness or stroke. Additionally, significantly lower YM was found in plaques where intra-plaque hemorrhage or thrombus was present, and in plaques with increased amount of foam cells [8]. Studies have revealed a lower mean YM for vulnerable plaque, although values vary between studies, e.g. 50 kPa versus 79 kPa for vulnerable and stable AP [8]; 62 kPa vs. 88 kPa respectively [2]; as well as 81 kPa vs 115 kPa [9].

Thus, advanced US SMI and SWE techniques can be promising in non-invasive identification of unstable carotid plaque and stratification of patients at risk of ischemic events.

AIM

To identify potentially adverse ultrasonographic characteristics of atherosclerotic carotid plaque in terms of stroke risk.

MATERIALS AND METHODS

The study included 96 patients aged 47 to 84 years, (22 women, 74 men), who underwent examination and treatment at the State Institution «National Scientific Center «The M.D. Strazhesko Institute of Cardiology, Clinical and Regenerative Medicine» of the National Academy of Medical Sciences of Ukraine» ("Sonolife" Medical Center) and the Municipal Non-Profit Enterprise of Kyiv Regional Council "Kyiv Regional Clinical Hospital." The set of studies included the assessment of clinical and anamnestic data, results of brain MRI, ultrasound of the main vessels of the neck and head using Toshiba Aplio 400 and Canon i800 ultrasound systems. All patients were diagnosed with carotid stenosis. Attention was paid to internal carotid artery (ICA) stenoses, assessment was carried out according to the standard duplex scanning procedure with determination of the degree of stenosis according to the NASCET protocol [1].

Morphological assessment of the types of ASP was carried out according to Gray-Weale-Geroulakos classification *[10]* 5 types of AP were evaluated: type 1 – hypoechogenic plaques with a thin hyperechogenic cap, type 2 – mainly hypoechogenic plaques with small hyperechogenic areas, type 3 – mainly hyperechogenic plaques with small hypoechogenic areas, type 4 – homogeneous hyperechogenic plaques, type 5 – plaques that cannot be classified due to strong calcification and acoustic shadows.

We applied new techniques for estimating AP in addition to traditional gray scale, color and spectral Doppler modes. The technique of SMI, which is in the software of the ultrasound systems we used, is aimed at finding newly formed vessels in the structure of the plaque. We used SMI in all the patients in two modes: monochrome (shades of gray) and color. To obtain high-quality images, image parameters were corrected: the speed scale was set to less than 2.5 cm/s, color gain was increased to improve sensitivity, smoothing time was increased, which improved temporal resolution due to the accumulation of blood flow signals [11].

SWE technique was also used to determine the density of the plaque, a Jung modulus of kPa was estimated. To obtain optimal visualization, the proposed recommendations were followed Standardized scanner settings for SWE: color range (0-180 kPa); acoustic power (0,0); gain (70-90%); The SWE Area of Interest (ROI) was adjusted to cover the entire plaque with carotid artery

Table 1. US characteristics of carotid stenoses in groups

Criterion	Groups, number of patients		Comparison in groups: U; p
	Group 1	Group 2	
AP thickness, mm, Me (IQR) on the right on the left	 3.3 (2.5–3.9), n=49 3.0 (2.3–3.7), n=48	 2.7 (2.1–3.3), n=36 3.0 (2.3–3.4), n=43	 U=680.0; p=0.02 U=959.5; p=0.56
Degree of stenosis, % Me (IQR) on the right on the left	 45 (30-60), n=49 35 (25-60), n=48	 30 (20–50), n=36 35 (25–50), n=43	 U=601.5; p=0.01 U=944.0; p=0.48

Table 2. US assessment of plaque structure in patient groups

Components	Groups				Comparison in groups
	Group 1		Group 2		
	right	left	right	left	χ^2; U; p;
Gray-Weale-Geroulakos AP type, quantity (%)					
1	4 (8.0)	5 (10.4)	1 (2.5)	0	right
2	16 (32.0)	13 (27.1)	2 (5.0)	5 (11.6)	χ^2=19.4; p=0.001
3	18 (36.0)	13 (27.1)	14 (35.0)	14 (32.6)	left
4	10 (20.0)	15 (31.3)	23 (57.5)	24(55.8)	χ^2=12.4; p=0.01
5	2 (4.0)	2 (4.2)	0	0	
Surface ulceration, n/N, quantity (%)	6/49 (12.2)	7/48 (14.6)	2/36 (5.6)	2/43 (4.7)	right χ^2=1.1; p=0.29; left χ^2=2.5; p=0.11
Presence of microvascularization n/N, quantity (%)	30/49 (61.2)	29/48 (60.4)	14/36 (38.8)	17/43 (39.5)	right χ^2=4,2; p=0.04 left χ^2=4,0; p=0.05
AP stiffness, kPa, Me (IQR)	70.0 (57.0–80.0)	72.0 (60.0–79.0)	82.0 (77.5–89.0)	85.0 (74.0–94.0)	Right U=390; p=0.001 left U=380; p=0.001

wall and soft tissue nearby at about two to three times the size of the plaque. To evaluate the SWE color map, the following options were selected: the blue color on the elastogram displayed a low YM (soft), the red color showed a high YM (hard). For the assessment, a film loop with about 10 frames was used. To stabilize the SWE image and facilitate inter-frame variability, the first two SWE frames were discarded due to operator arm movements, five frames were randomly selected to evaluate the plaque YM [7]. Thus, the assessment of carotid plaques was carried out according to a set of criteria: the maximum thickness of AP in mm, the degree of stenosis of ICA in% (NASCET protocol), the presence of local changes in the linear blood flow rate (CBF rate) in the stenosis zone, the morphological assessment of AP types in accordance with Gray-Weale-Geroulakos classification, presence of ulcers, presence of microvascularization according to SMI data, stiffness assessment according to SWE data. The observation group did not include patients with heart rhythm disorders in order to exclude the cardioembolic subtype of ischemic stroke.

Statistical analysis was carried out with the help of MS Office and Statistica 6 software package. The statistical significance of the difference between the groups was assessed taking into account the discrepancy between the data with the parameters of the normal distribution; for the comparison of quantitative indicators – according to the Mann-Whitney U-criterion, for qualitative indicators – according to the χ^2 criterion. The association between two binary variables was assessed by φ coefficient. The relative risk (RR) (with 95 % confidence interval [CI]) of the factor influence on the development of the event was determined. The level of significance was estimated as $p<0.05$.

The research was carried out in accordance with the principles of the Helsinki Declaration of the World Medical Association (1964-2000) and the "Council of Europe Convention on Human Rights and Biomedicine" within the framework of the dissertation research program approved by the Ethics Commission of the Shupyk National Healthcare University of Ukraine.

Table 3. Characteristics of carotid stenosis according to the formation of ipsilateral foci of ischemia

Characteristics of carotid stenosis	Right carotid circulation		Left carotid circulation	
	Foci of ischemia		Foci of ischemia	
	Are present	Not detected	Are present	Not detected
Degree of stenosis, %; Me (IQR)	50.0 40.0-60.0	35.0 25.0-55.0	40.0 30.0-60.0	35.0 20.0-60.0
U; p	169.0; 0.04		174.5; 0.07	
AP thickness, mm; Me (IQR)	3.7 2.9-4.0	3.0 2.5-3.5	3,3 2.5-3.7	2,4 2.1-3.0
U; p	180.5; 0.04		166.0; 0.04	
AP stiffness, kPa; Me (IQR)	59.0 53.0-71.0	80.0 70.5-87.5	70.5; 57.5-77.8	74.0 67.0-83.5
U; p	62.0; 0.001		109.0; 0.14	
Gray-Weale-Geroulakos AP type, χ^2; p	9.78; 0.04		8.88; 0.06	
Presence of microvascular quantity (%)	21(75.0)	8(42.1)	20 (74.1)	7(36.8)
χ^2; p; φ; RR (95% CI)	5.18; 0.02; 0.33; 1.8 (1.0-3.1)		6.38; 0.01; 0.37; 2.0 (1.1-3.8)	
Increasing blood velocity in stenosis zone, quantity (%)	15 (53.6)	5 (23.8)	13 (48.1)	5 (22.7)
χ^2; p; φ; RR (95% CI)	4.40; 0.04; 0.30; 2.3 (1.0-5.2)		3.40; 0.07	
AP Ulceration, quantity (%)	5 (17.9)	1 (5.3)	5(18.5)	1 (5.3)
χ^2; p	1.61; 0.20		1.73; 0.19	

RESULTS

According to the results of the clinical and instrumental data analysis, patients were divided into 2 age-matched groups. Group 1 included of 51 patients with symptomatic carotid stenosis; patients` age was (median [Me], interquartile range [IQR]) 68 (61-74) years. Patients of group 1 showed signs of ischemic events in the carotid circulation during the last 6 months: ischemic stroke – in 49 cases, transient ischemic attack in 2 observations. The ischemic focus (larger than 1.5 cm) in the right carotid circulation occurred in 22 patients, in the left – in 21, and in both carotid blood supply territories – in 6. Group 2 was formed by 45 patients with asymptomatic carotid stenosis, patients' age was (Me [IQR]) 67 (56-74) years. According to MRI, signs of discirculatory changes in the brain predominated in group 2. In all patients studied, II-III degree arterial hypertension (AH) was established: in group 1 – II degree AH in 15 (29.4%) observations, III degree – in 36 (70.6%), in group 2 – II degree AH in 23 (51.1%), and III degree – in 22 (48.9%). Diabetes mellitus in group 1 occurred in 10 (19.6%) patients, in group 2 – in 6 (13.3%).

ICA stenoses in the vast majority of patients were bilateral; in the group 1 only right-sided stenosis occurred in 3 patients, only left-sided in 2, in group 2 only right-sided stenosis was observed in 2 cases, only left-sided – in 9. The maximum thickness of the AP in group 1 was statistically significantly greater, reliable difference in the observation groups was established by the right ICA (U = 680.0; p=0.02). Comparison in the groups indicates a higher percentage of ICA stenosis in the group of patients with ischemic events, a significant difference in the right ICA was noted (U = 601.5; p=0.01) (Table 1). Group 1 showed a higher number of observations with a stenosis degree of ≥50% accompanied with increasing of blood velocity in the stenosis zone, (right 23 (46.9%) against 10 (27.8%), left 19 (39.5%) against 12 (27.9%), but with an unreliable difference in the groups (right $\chi^2 = 3.21$; p=0.07; left $\chi^2 = 1.4$; p = 0.24) (Table 1).

When evaluating the morphological structure of AP in gray scale mode, according to 5 types according to the Gray-Weale-Geroulakos classification, the predominance of heterogeneous AP of type 2 and 3 was established in group 1, and AP of type 3 and type 4 were more often detected in group 2. The occurrence of conventionally unstable plaques of low echogenicity (type 1) was small and was 8.1% in group 1 – 10.4% on the right and left respectively, 2.8% in group 2 only on the right. The difference in groups by type of AP was statystically significant in the right ($\chi^2 = 19.4$; p = 0.001) and left ICA ($\chi^2 = 12.4$; p = 0.01) (Table 2).

Complications of AP in the form of surface ulceration did not reveal significant differences in the groups. In the 1st group, ulceration was registered in only 6 and

7 plaques respectively on the right and left, in the 2nd group – in 2 cases on the right and left, ($\chi^2 = 1.1$; p = 0.30 in the right ICA and $\chi^2 = 2.5$; p = 0.11 in the left ICA). It should be noted that the use of the SMI mode significantly improves the assessment of the plaque surface and ulceration.

Evaluation of SMI mode data revealed the presence of microvascularization mainly in type 2 and 3 plaques, microvascular loci were less common in type 4 AP. A significant prevalence of findings in the group of patients with ischemic events ($\chi^2 = 4.2$; p = 0.04 in the right ICA and $\chi^2 = 4.0$; p = 0.05 in the left ICA). In calcified AP behind calcification sites, a flicker artifact was recorded that simulated the presence of microvascularization, so we selected areas without calcification for evaluation.

AP stiffness determination with the help of SWE made it possible to isolate plaques with YM from 34 to 102 kPa. We did not use SWE to evaluate plaques with pronounced calcification (5 type by Gray-Weale) and thickness less than 2 mm. The level of stiffness was significantly lower in the group of patients with ischemic events (Me = 70.0, IQR: 57,0–80,0 kPa in right ICA, Me = 72.0, IQR: 60.0–79.0 in left ICA) compared to the asymptomatic stenosis group (Me=82.0, IQR: 77.5–89.0 kPa in right ICA, p = 0.001; Me = 85.0, IQR: 74.0–94.0 in left ICA, p = 0.001) (Table 3).

Thus, significant differences in the characteristics of the AP in the observation groups were established according to the following indicators: plaque thickness and stenosis degree (in the right ICA), types of plaques according to the Gray-Weale-Geroulakos classification, the presence of microvascularization according to SMI data, plaque stiffness according to SWE data.

Further data analysis was carried out in the direction of comparison of the nature of carotid stenosis on the side of ischemia lesions (foci) formation according to MRI ("symptomatic" stenosis) compared to stenoses on the side without ischemia foci ("asymptomatic" stenosis) corresponding to the definition of a pathogenetic subtype of stroke according to TOAST classification criteria. Thus, the influence of qualitative and quantitative parameters of AP on the development of an ischemic event was determined. The analysis was perfomed in a group of patients with a stroke, there were foci on the right carotid territory in 28 cases, on the left carotid territory in 27, including patients with ischemic foci in both carotid circulations. The data are presented in Table 3.

Based on the results of statistical data processing, a significant association of characteristics of AP with the presence of ipsilateral ischemic focus ("symptomatic AP") was established. Among the quantitative parameters, the maximal thickness of the plaque (p=0,04 in the right and left carotid circulation), the degree of stenosis (statistically significant on the right side, p=0.04, close to significant on the left side, p=0.07), the stiffness of the plaque according to SWE data (statistically significant only on the right side, p=0.001), were determined. Among the qualitative characteristics are the presence of local disorders of blood flow velocity in the stenosis zone (on the right ICA, p=0.04, RR increases by 2.3 times), the presence of microvascularization according to SMI data (p=0,02 i p=0,01 according to the right and left sides, RR increases by 1.8-2,0 times), as well as types of plaques according to the Gray-Weale-Geroulakos classification (p=0,04 in the right ICA, p=0,06 in the left ICA).

The characteristics of carotid plaques that demonstrated a valid association with ischemic events can be taken into account in predicting stroke risk in a complex of other clinical indicators, as well as applied to a personalized approach in the planning of treatment tactics.

DISCUSSION

In recent years, many studies have been presented that have tried to identify the characteristic ultrasonic signs of unstable atherosclerotic plaque. However, it was not possible to determine any separate characteristic that could reliably predict the instability and risk of ipsilateral stroke. In our study, we analyzed both the traditional Doppler and gray-scale characteristics of plaques, as well as the signs found with modern ultrasound techniques such as SMI and SWE.

According to traditional parameters (degree of stenosis according to NASCET, plaque thickness, hemodynamic disorders in the stenosis zone, structure of the plaque according to the Gray-Weale-Geroulakos classification), we obtained the expected results, namely a significant difference in the groups with symptomatic and asymptomatic carotid stenosis.

Our use of the new SMI ultrasound technique demonstrated a statistically significant predominance of signs of plaque microvascularization on the ipsilateral stroke side. These results correlate with literature data and reflect the association of the presence of neovessels with potential plaque instability and risk of cerebral ischemic events [2]. Based on meta-analysis data from Zhou Y et al. it is possible to confirm the feasibility of using SMI to detect intra-plaque neovascularization [12]. When assessing the stiffness of the plaque from SWE data, we obtained significant differences in the observation groups. Our results showed a severe stiffness of plaques in patients with stroke compared to the non-stroke group, which corresponds to the literature [2, 8, 9, 13]. However, when assessing the stiffness of the symptomatic and asymptomatic plaque, significant differences were obtained only in the right carotid basin.

The absence of a statistically significant difference in the other carotid basin may be due to the systemic nature of the inflammatory process and the presence of soft carotid plaques in symptomatic patients on both sides, as well as a small number of observations.

A further prospect of our development is to combine a number of clinical data and the above-mentioned factors of unstable plaque into a prognostic model in order to stratify patients at risk of cerebral ischemic events.

CONCLUSIONS

1. Statistically significant differences in the characteristics of symptomatic and asymptomatic carotid plaques were found: maximum plaque thickness p=0,04, stenosis degree according to the NASCET protocol (p=0.04 in the right ICA), types of plaques echogenicity according to the Gray-Weale-Geroulakos classification (p=0,04 in the right ICA), the presence of microvascularization according to SMI data (p=0,02 i p=0,01 according to the right and left sides), the plaque stiffness indicator according to SWE data (p=0.001 in the right ICA).
2. The presence of microvascularization according to SMI data and the low stiffness of the plaque according to SWE data can characterize the potential instability of the AP. These criteria should be added to the traditional US assessment of carotid plaques.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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