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The parameters of carotid plaques' calcifications and clinical nature of the lesions in the context of revascularization treatment. The enlargement of calcifications is still the most important

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Abstract

Introduction: *It remains a challenge to determine criteria according to which patients with asymptomatic carotid stenosis could be properly qualified for revascularization treatment. Carotid calcification assessment seems to be here quite attractive. The aim of the study was the histological analysis of various parameters of calcifications in human carotid plaques in relation to the symptomatic/asymptomatic nature of the lesions.*

Material and methods: *The study involved carotid plaques taken from patients who have undergone endarterectomy of the internal carotid artery.*

Results: *Calcified plaques (with enlarged calcifications) were significantly more frequently asymptomatic than non-calcified plaques. The remaining calcification characteristics played no role. Calcified lesions disclosed the dominance of the fibrous component and the small lipid core significantly more frequently than non-calcified plaques. The percentage of females in the patients group with calcified lesions was significantly higher than in the group with non-calcified plaques. The percentage of males was lower. The former patients group used statins and angiotensin inhibitors significantly more frequently than patients with non-calcified plaques. Enlarged calcifications were independently associated with the asymptomatic nature of the carotid plaques.*

Conclusions: *The enlargement of calcifications in carotid plaques is the only calcification parameter important for the clinical outcome of carotid atherosclerosis. Patients with calcified carotid plaques have a significantly lower risk of ischemic stroke than patients with non-calcified lesions.*

Key words: *calcifications, carotid plaques, risk of ischemic stroke.*

Introduction

Carotid endarterectomy, or stenting, is an established method of ischemic stroke prevention in patients with symptomatic moderate to severe (50-99%) carotid artery stenosis [3,27]. Carotid artery revascularization in patients with asymptomatic stenosis raises considerable doubts, which stems from the low estimated risk of stroke related to asymptomatic lesions. This risk is often believed not to exceed the risk of stroke associated with revascularization therapy alone [8,18,22,31,32].

The detection of asymptomatic carotid stenosis has been on the rise, due to carotid artery ultrasound examinations, commonly performed for a variety of

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reasons. Hence, special attention should be given to the treatment of this patients group. It remains a challenge to determine criteria according to which patients with asymptomatic carotid stenosis could be properly qualified for revascularization treatment [6,17,23,29].

The risk of stroke associated with carotid atherosclerosis results not only from the degree of carotid stenosis, but also from the structure of the atherosclerotic lesions, i.e. their stability/instability. It is thus necessary to establish the parameters of high- and low-risk carotid lesions, easily available for regular assessment in clinical settings. Histological tests play a significant role in this context.

Inflammation within the carotid plaque leads to the destabilization of its structure if it dominates the reparative mechanisms [19,26,20,28]. It is considered as one of the features of potentially symptomatic lesions. Other features of such lesions are a thin fibrous cap with a large lipid-necrotic core and intraplaque haemorrhage [21]. We cannot tell, however, that these characteristics determine the symptoms of the carotid plaque. The capability to monitor them is curtailed by the limited availability of the appropriate methods [5,9,30].

Calcifications within carotid plaques are also worthy of attention. They seem to be more accessible to assessment than inflammation, based on techniques used in everyday practice [12]. There are some unresolved questions about calcifications and carotid atherosclerosis outcomes. Calcified plaques are thought generally as low-risk lesions; however, up to one fifth of them can be symptomatic [14,24,33]. Experimental researchers suggest that the location of calcifications in the plaque and calcification type might have a role [2,15,16]. We do not know if this is the case in humans.

We have decided to analyse various aspects of carotid calcifications in relation to carotid artery disease complications. Such analyses can be helpful in answering the above-mentioned questions and in discussing the potential usefulness of carotid calcification assessment in everyday practice.

The aim of the study was the histological analysis of calcifications in carotid plaques in relation to the symptomatic/asymptomatic nature of these plaques, including an assessment of other histological features of the lesions.

Material and methods

The study involved 202 carotid plaques taken from 202 patients (60 females and 142 males, aged between 40 and 92) who had undergone an endarterectomy of the internal carotid arteries. Patients after ischemic stroke (IS) or transient ischemic attack (TIA), or amaurosis fugax (AF) or with no history of cerebrovascular events, were analysed. They suffered from arterial hypertension (HA), type 2 diabetes (DM type 2), dyslipidemia, peripheral artery disease (PAD), and ischemic heart disease (IHD), occurring both separately and in combination with other conditions.

We excluded individuals after a possible or probable hemodynamic or cardioembolic stroke (the coexistence of hemodynamically significant heart failure, atrial fibrillation, sick sinus syndrome). We excluded patients with chronic diseases in which inflammatory and immunological factors played an evident role (systemic connective tissue diseases, viral hepatitis, cirrhosis, ulcerative colitis, Crohn's disease, multiple sclerosis, Hashimoto's thyroiditis, Graves' disease, proliferative diseases of the hematopoietic system, and other cancers), and patients with parathyroid-gland diseases. Other exclusion criteria were the taking of immunosuppressants (steroids/cytostatics), and hormone-replacement therapy and vitamin D3 supplementation within five years preceding enrolment into the study.

The plaques were divided into symptomatic and asymptomatic lesions. The first ones were plaques obtained from patients after ischemic stroke, TIA or AF, unilateral to the removed lesions, which occurred within 6 months preceding the endarterectomy [7,25]. The asymptomatic lesions were plaques obtained from patients who had never suffered from corresponding ischemic stroke, TIA, or AF, or the diseases had occurred more than 6 months before the endarterectomy.

The research was approved by the local Bioethical Commission. The study subjects gave informed consent to their participation.

In 13 cases, the nature of plaques could not be reliably determined, which left 189 carotid plaques for analysis of the relation of carotid plaque calcifications and other morphological features of the lesions to their symptomatic, asymptomatic character.

The microscopic evaluation of carotid plaques

The microscopic analysis involved all 202 intraoperatively harvested carotid plaques.

As it was described in our previous work [20], the plaques were fixed in a 10% formalin solution before they were divided into five parts, including the area

most affected by disease (the plaques' hot-spot). The material was then immersed in paraffin, sliced into 3-micrometer-thick fragments, and stained with hematoxylin and eosin (H&E).

Using the same criteria as previously [20], we assessed the following characteristics: calcifications, inflammatory infiltration, connective tissue elements, foam cells, lipid core, plaque vascularization, intraplaque haemorrhage, thrombi built into the plaque structure, and mural thrombi. We identified plaque shoulders (Sh), the central part of the fibrous cap (FC), and the central part of the plaque, including the lipid core (Core) [20].

1. Plaque calcifications:
   - the enlargement of plaque calcifications: considered large if calcifications occupied more than half of the plaque thickness at a magnification of 200× (calcified plaques),
   - the location of calcifications: I, mainly in the plaque shoulders; II, mainly in the central part of the fibrous cap; III, mainly in the core of the plaque; IV, comparably in all parts of the plaque,
   - the dominant calcifications type: A – small single or dispersed calcifications, B – nodules, C – chondro-osseus metaplasia,
   - the coexistence of various calcifications type: yes or no,
   - calcifications penetrate the plaque surface: yes or no.
2. Inflammatory infiltration:
   - the intensity of inflammation in the plaque shoulder and fibrous cap; the inflammatory cells were counted in four visual fields at a magnification of 400×, and infiltration was considered massive if we counted more than 100 cells,
   - the inflammatory cells in relation to connective tissue elements: the visual fields infiltrated by inflammatory cells to the fields with connective tissue dominance (without or with single inflammatory cells) at a magnification of 200×; A – inflammation dominance, B – connective tissue dominance.
3. The foam cells component, separately in plaque shoulders and the central part of the plaque's fibrous cap. The component was considered significant if the foam cells occupied more than one-third of the shoulder/fibrous cap thickness.
4. The lipid core (amorphous material containing cholesterol crystals): considered large if it occupied more than half of the plaque thickness.
5. Plaque vascularity: vessel sections counted in four visual fields at a magnification of 400×: 0, none; I, 1 to 9 vessel sections; II, > 9.
6. Intraplaque haemorrhage (separately in the fibrous cap, shoulder, and core): the area of erythrocytes within the plaque causing the disorganization of the plaque architecture or apparently organized haemorrhage with an accumulation of hemosiderin-laden macrophages or iron deposition within the plaque's connective tissue.
7. Intraplaque thrombus: the organized collection of fibrin and red blood cells within the plaque.
8. Mural thrombus: the organized collection of fibrin and red blood cells in the vessel lumen.

Evaluations of plaque morphology were performed at baseline and 3 months later. Both evaluations were performed by one researcher. The second analysis was masked from the first one. Both were performed without knowledge of the clinical data.

The plaques' hot-spot was used for further analysis.

The intra-observer agreement for plaque-morphology parameters ranged between 85 and 98 percent.

Statistical analysis

Nominal types of variables were assessed. Because of non-normality of the distributions between the variables, the results were presented as medians. Data were compared between groups using the nonparametric $\chi^{(2)}$ test for categorical variables/Fisher's exact test.

Multiple logistic regression models, including angiotensin inhibitors, statins usage, the fibrous-component integrity in the central part of the fibrous cap, inflammation vs fibrous component in the plaque shoulder, and the large lipid core, were used to identify independent predictors of asymptomatic carotid plaques. For each variable, odds ratio (OR), 95% confidence interval (95% CI) and statistical significance (p) were presented.

The statistical-significance threshold was set at $p < 0.05$. Calculations were performed with the use of STATISTICA 12 software.

Results

Calcifications in carotid plaques in relation to the symptomatic, asymptomatic character of the lesions

Calcified plaques (calcifications occupied more than half of the plaques' thickness) were significantly more frequently asymptomatic than the other (non-calcified) plaques ($p = 0.005$). The remaining

calcification characteristics, i.e. the location, the type, the penetrating of the plaque surface, and coexistence of various calcification types played no role (with appropriate *p* value of 0.28, 0.48, 0.29 and 0.93) (Table I).

Carotid plaque calcifications enlargement and other morphological features of the lesions

Calcified lesions disclosed the dominance of the fibrous component and the small lipid core significantly more frequently than the other group ($p = 0.03$ and 0.005, respectively). The mural thrombus and the thrombus built into the plaque were observed more rarely than among the non-calcified plaques, but without statistical significance (Table II).

Carotid plaque calcification enlargement in relation to the patients' age, sex, BMI, atherogenic risk factors, and medications taken

The percentage of females in the patients group with calcified lesions was higher than in the patients group with non-calcified plaques. The percentage of males was lower. The difference was significant ($p = 0.004$).

The patients of both group did not differ in age ($p = 0.92$), BMI ($p = 0.96$), or comorbidities known as atherogenic risk factors (p for PAD = 0.59, DM type 2 = 0.93, dyslipidemia = 0.76, HA = 0.73, IHD = 0.53), or smoking ($p = 0.90$). They differed because of the medications taken. The patients with calcified plaques used statins ($p = 0.02$) and angiotensin inhibitors ($p = 0.01$) significantly more frequently than the patients with non-calcified plaques (Table III).

Enlarged carotid plaque calcifications and other morphological features of the lesions

Multiple regression analysis disclosed the strongest correlation between enlarged calcifications ($p = 0.09$) and small lipid-necrotic component ($p = 0.03$) (Table IV).

Enlarged calcifications were independently associated with the asymptomatic nature of the carotid plaques ($p = 0.007$) (Table V).

Table I. Calcifications in carotid plaques in relation to the symptomatic and asymptomatic character of the lesions

	Symptomatic plaque 37 – total number of cases	Asymptomatic plaque 152 – total number of cases	*p*
Enlargement of carotid plaque calcifications			0.005
< 1/2 of plaque thickness	64.86% (37[a])	39.47% (152[a])	
> 1/2 of plaque thickness	35.14% (37[a])	60.53% (152[a])	
Main location of calcifications in the plaque			0.28
I	16.67% (36[a])	11.18% (152[a])	
II	47.22% (36[a])	34.87% (152[a])	
III	25.00% (36[a])	38.16% (152[a])	
IV	11.11% (36[a])	15.79% (152[a])	
Dominant calcifications type			0.48
A	40.54% (37[a])	36.18% (152[a])	
B	18.92% (37[a])	13.16% (152[a])	
C	40.54% (37[a])	50.66% (152[a])	
Calcifications penetrate plaque surface			0.29
No	58.33% (36[a])	43.92% (148[a])	
Yes	41.67% (36[a])	56.08% (148[a])	
Coexistence of various calcification types			0.93
No	75.68% (37[a])	75% (152[a])	
Yes	24.32% (37[a])	25% (152[a])	

[a]Number of cases with available assessment of particular morphological features, A – small single or dispersed calcifications; B – nodules; C – chondro-osseus metaplasia, I, mainly the central part of the fibrous cap; II, mainly the plaque shoulder; III, mainly the core of the plaque; IV, comparably in all parts of the plaque

Table II. Carotid plaque calcifications enlargement and other morphological features of the lesions

	Calcified carotid plaques 114 – total number of cases	Non-calcified carotid plaques 88 – total number of cases	*p*
Endothelium defect			
FC	33.33% (96[a])	24% (84[a])	0.72
Sh	31.63% (98[a])	30.49% (82[a])	0.52
Large component of foam cells in:			
FC	13.40% (97[a])	17.86% (84[a])	0.41
Sh	36.36% (99[a])	41.46% (82[a])	0.48
Massive infiltrations in:			
FC	10.64% (94[a])	43.37% (83[a])	0.32
Sh	29.90% (97[a])	30.12% (83[a])	0.06
Dominant inflammation vs fibrous component in:			
FC	31.58% (95[a])	30.12% (83[a])	0.83
Sh	65.31% (98[a])	49.40% (83[a])	0.03
Fibrous component integrity			
FC	71.13% (97[a])	53.57% (84[a])	0.01
Sh	37.37% (99[a])	25.30% (83[a])	0.08
Vascularity of FC			0.31
0	59.57% (94[a])	48.19% (83[a])	
I	24.47% (94[a])	30.12% (83[a])	
II	15.96% (94[a])	21.69% (83[a])	
Vascularity of Sh			0.95
0	20% (95[a])	19.23% (78[a])	
I	27.37% (95[a])	29.49% (78[a])	
II	52.63% (95[a])	51.28% (78[a])	
Vascularity of core			0.51
0	25.58% (86[a])	32.84% (67[a])	
I	18.60% (86[a])	13.43% (67[a])	
II	55.81% (86[a])	53.73% (67[a])	
Intraplaque haemorrhage			
FC	23.96% (96[a])	27.38% (84[a])	0.60
Sh	50.51% (99[a])	51.22% (82[a])	0.92
core	42.55% (94[a])	40.85% (71[a])	0.82
Cholesterol crystals	78.90% (109[a])	88.51% (87[a])	0.07
Large lipid core	48.57% (105[a])	67.05% (88[a])	0.005
Intraplaque thrombus	8.60% (93[a])	15.19% (79[a])	0.18
Mural thrombus	64.55% (110[a])	56.18% (89[a])	0.23

[a] number of cases with available assessment of particular morphological features
core – central part of the plaque; FC – central part of fibrous cap; Sh – plaque shoulder
0, no vessel sections counted in four visual fields at a magnification of 400×; I, 1 to 9 vessel sections; II, > 9

Carotid plaques calcifications in relation to the ultrasound assessment of the lesions

The calcified plaques shown on carotid Doppler ultrasonography were the most often hyperechogenic ($p = 0.006$). The non-calcified ones were seen as hypoechogenic lesions ($p = 0.03$) (Table VI).

Discussion

Atherosclerotic lesions result from an excessive, inflammatory-fibroproliferative response to injury to the artery wall. Accumulation of calcium deposits within the lesions is commonly observed. It is not clear whether the deposits are just a feature

Table III. Carotid plaque calcification enlargement in relation to the patients' age, sex, BMI, atherogenic risk factors, and medications taken

	Calcified plaques 114 – total number of cases	Non-calcified plaques 88 – total number of cases	*p*
Age, median (range) in years	70 (40-88)	71 (40-92)	0.92
Sex			0.004
Female	37.72% (114[a])	19.32% (88[a])	
Male	62.28% (114[a])	80.68% (88[a])	
BMI median (range)	26.43 (18.96-39.25)	26.1 (24.2-35.86)	0.96
Comorbidities			
PAD	38.94% (113[a])	35.23% (88[a])	0.59
DM type 2	30.09% (113[a])	29.55% (88[a])	0.93
Dyslipidemia	55.24% (105[a])	53.01% (83[a])	0.76
HA	85.84% (113[a])	84.09% (88[a])	0.73
IHD	52.21% (113[a])	47.73% (88[a])	0.53
Medications			
Angiotensin inhibitors	72.64% (106[a])	54.76% (84[a])	0.01
Statins	72.64% (106[a])	57.14% (84[a])	0.02
Acetylsalicylic acid	95.28% (106[a])	96.43% (84[a])	0.69
Smoking	42.99% (107[a])	43.90% (82[a])	0.90

[a] *number of cases with available data*
DM type 2 – type 2 diabetes mellitus; HA – arterial hypertension; IHD – ischemic heart disease; PAD – peripheral artery disease

Table IV. Enlarged carotid plaque calcifications and other morphological features of the lesions. Independent association with large lipid core (multiple regression analysis)

	Odds ratio	Confidence Interval –95	Confidence Interval +95	*p*
Angiotensin inhibitors	0.0389	0.7610	3.5951	0.2
Statins	1.8319	0.8335	4.0259	0.13
Fibrous component integrity in the central part of the fibrous cap	1.536	0.7223	3.2676	0.26
Inflammation vs fibrous component in the plaque shoulder	1.8934	0.9036	3.9673	0.09
Large lipid core	1.7038	1.0324	2.8117	0.03

Table V. Enlarged carotid plaque calcifications – independent association with asymptomatic character of the lesions (multiple regression analysis)

	Odds ratio	Confidence Interval –95	Confidence Interval +95	*p*
Angiotensin inhibitors	1.1221	0.4388	2.8691	0.81
Statins	1.1717	0.4468	3.0731	0.74
Fibrous component integrity in the central part of the fibrous cap	1.0728	0.4203	2.7383	0.88
Inflammation vs fibrous component in the plaque shoulder	0.6494	0.2531	1.6661	0.36
Large lipid core	1.0835	0.552	2.1146	0.81
Calcified plaque	0.2489	0.0900	0.6884	0.007

Table VI. Enlarged carotid plaques calcifications in relation to the ultrasound assessment of the lesions

	Calcified plaques	Non-calcified plaques	*p*
Hyperechogenic plaques	46.77% (62[a])	20.93% (43[a])	0.006
Hypoechogenic plaques	6.45% (62[a])	20.93% (43[a])	0.03
Heterogenic plaques	30.64% (62[a])	44.18% (43[a])	0.25

[a] *number of cases with available data*

of the process or whether they influence plaque stability.

Atherosclerotic plaques differ in respect of the enlargement of calcium deposits within them. They also differ in respect of the calcification type and their location within the plaques. The experimental researches suggest that all these three parameters might be important for the onset of complications associated with carotid plaques [2,16].

In our study, the enlargement of calcium deposits in plaques was the only calcification parameter significant for carotid plaque asymptomatic/symptomatic nature. Asymptomatic plaques were characterized by enlarged calcifications. The symptomatic ones were non-calcified. It should be stated that this parameter assessed in hot-spots was representative for the whole plaque. The enlargement of calcium deposits in the remaining parts of the analysed plaques was similar.

The location of calcifications in carotid plaques, the penetration of plaque surfaces, the calcification types, and the coexistence of various calcification types in one plaque, had no role in the symptomatic/asymptomatic nature of plaques.

The calcified plaques were characterized by the dominance of fibrous elements, the preserved integrity of the fibrous tissue, and the small lipid-necrotic element. Mural thrombus, thrombus built into the plaque, and a large foam-cell component, were more rarely observed, but without statistical significance. From the morphological point of view, such carotid atherosclerotic plaques are stable [1]. Considering the results described above, they are low-stroke-risk lesions. The enlarged calcifications within the plaques might be a marker of such lesions.

Calcified (stable, asymptomatic) carotid plaques were observed among women significantly more frequently than among men. Based on the literature, women, as a patients group, benefit less than men from the carotid revascularization procedure [4,13]. Considering our results, it might not be justified to expose them to the perioperative risk if they are predisposed to rather stable, low-risk carotid lesions. Taking angiotensin inhibitors and statins, i.e. medications with a proven stabilizing effect on the atherosclerotic plaque structure, can further reduce this risk [10,11]. The question is whether medical management with the medications mentioned above could be an alternative to them.

Our results have shown that enlarged carotid plaques calcification is independently associated with asymptomatic plaques nature. The assessment of the extent of calcium deposits in the carotid plaques could be used in patients with carotid artery stenosis as a prognostic marker for the risk of stroke.

The beneficial effect of pharmacotherapy vs revascularization, especially in women with asymptomatic carotid lesions, should be checked in additional researches with long-term follow-up.

Carotid ultrasound is an easily available and reliable method of carotid plaque calcification assessment [12]. In our work, calcified plaques were classified as ultrasonographically hyperechogenic lesions. Such lesions are discussed as lower-risk lesions than the hypo or heterogenic kind. This is, however, the weakest aspect of our work. Ultrasound results were not available for all patients. Not all patients were assessed in ultrasonography by the same person or using the same ultrasonograph.

Summing up, the enlargement of calcifications in carotid plaques is the only calcification parameter important for the clinical outcome of carotid atherosclerosis. Patients with calcified carotid plaques have a significantly lower risk of ischemic stroke than patients with non-calcified lesions. The lower risk among these patients relates to a stable plaque structure, with the dominance of a fibrous element and also a small atheromatous component.

Taking angiotensin inhibitors and statins is associated with low-risk carotid lesions, and seems to be significant for stroke prophylaxis among patients with carotid atherosclerosis.

Disclosure

The authors report no conflict of interest.

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