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Carotid artery plaque structure in the context of symptomatic/asymptomatic nature of carotid artery stenosis

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SOUHRN

Cíl: V této studii jsme zkoumali vztah mezi charakteristikami aterosklerotického plátu a symptomatickým postižením karotických tepen.
Materiál a metody: Do studie bylo zařazeno 22 pacientů (13 mužů a 9 žen průměrného věku 60,2 ± 9,6 roku) po endarterektomii karotických tepen. Závažnost stenózy karotické tepny se pohybovala v rozmezí 75 % až 99 %. Osm pacientů bylo asymptomatických a zbývajících 14 symptomatických. Deset pacientů prodělalo cévní mozkovou příhodu. V anamnéze měl jeden pacient recidivující tranzitorní ischemické příhody, čtyři pacienti uváděli presynkopální stav a u jednoho byla stanovena diagnóza amaurosis fugax. Odstraněné pláty byly vyšetřeny skenující akustickou mikroskopií (scanning acoustic microscopy, SAM) a mikrovýpočetní tomografií (micro-computerized tomography, micro-CT).
Výsledky: U pacientů po cévní mozkové příhodě byly nalezeny nižší hodnoty akustické impedance ukazující na tvorbu plátů s vysokým obsahem kolagenu, zatímco u asymptomatických pacientů byly vyšší hodnoty akustické impedance spojeny s tvorbou plátů bohatých na vápník.
Závěry: Spíše než spoléhání se na závažnost stenózy u pacientů s postižením karotických tepen může při vyhledávání pacientů vyžadujících intervenci napomáhat určení charakteristik plátů moderními radiografickými metodami.

Keywords:
Atherosclerotic plaque characteristics
Carotid artery stenosis
Symptomatic disease

ABSTRACT

Objective: In this paper, we examined the relationship between atherosclerotic plaque characteristics and symptomatic carotid artery disease.
Material and methods: Twenty-two patients who underwent carotid endarterectomy were enrolled into the study. Thirteen patients were male and remaining 9 patients were female. The mean age was 60.2±9.6 years. The carotid artery stenosis ranged between 75% and 99%. Eight patients were asymptomatic and remaining 14 patients were symptomatic. Ten patients suffered stroke. One of them had recurrent transient ischemic attacks, 4 patients had dizziness, and one of them had amaurosis fugax. The extracted plaques were evaluated by scanning acoustic microscopy (SAM) and micro-computerized tomography (micro-CT).
Results: Smaller acoustic impedance values that reveal collagen-rich plaque formation were detected in patients who had stroke, while higher acoustic impedance values that reveal calcium-rich plaque formation were detected in the asymptomatic patients' plaques.
Conclusions: Determination of plaque characteristics with modern radiographic techniques, rather than relying on the degree of stenosis in patients with carotid artery disease, may be helpful to discriminate patients requiring intervention.

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Introduction

Carotid artery stenosis is one of the most important causes of stroke. With an increasing number of older age population, pathology is more frequently investigated.[1] It is revealed that 5–10% of population older than 65 years have over a 50% degree of stenosis in the proximal carotid artery.[2] However, every year, 0,3–2% of patients with asymptomatic carotid artery stenosis suffer an ischemic stroke event.[2]

In this era, the management of asymptomatic patients remains controversial. In European Society for Vascular Surgery (ESVS) guidelines, the certain indication of carotid endarterectomy is specified in patients who have a 70–99% carotid stenosis with carotid territory symptoms within the preceding 6 months and if procedural death/stroke rate is under 6%.[3] However, we know that asymptomatic patients may also experience ischemic stroke event.[2] At this point, the mechanism and risk factors of ischemic stroke event gain great importance,in the course of assessing the patients with carotid artery stenosis. In recent years, independent of the stenosis degree, vulnerability of atherosclerotic plaques has been considered as a potential risk factor. It is believed that the lipid rich necrotic core and thin fibrous cap makes plaque more vulnerable.[4,5]

The carotid artery stenosis causes symptoms due to embolism depending on an unstable plaque or hypoperfusion because of severe stenosis with poor collaterals. However, only 15% of the patients with asymptomatic disease have some signs before an ischemic cerebral event.[2]

The plaque nature in carotid artery disease is examined by investigators looking for if the plaque calcification has a role in determining the risk of ischemic stroke. In a histopathological study, calcifications divided as macrocalcifications and microcalcifications. Macrocalcifications were tended to be as a plaque stabilizer, while microcalcifications were instabilizing the plaque.[1] In addition, in asymptomatic cases, the plaque calcification, observed by computed tomography angiography, was found to be pronounced.[1] Doppler ultrasonography, computed tomography angiography, and magnetic resonance angiography are the most common imaging tools for determining carotid artery stenosis.[6] However, these methods are incapable in detecting the atherosclerotic plaque structure. Doppler ultrasonography is operator dependent and insufficient in determining the plaque components.[5] Computed tomography angiography may be useful in evaluating the volume of calcification, but it is not successful in detecting the microcalcification. Magnetic resonance imaging is better at revealing the intraplaque haemorrhage and plaque rupture.[7] Hence, other imaging techniques which may reveal the plaque nature are being investigated.

We previously reported that scanning acoustic microscopy may be a useful clinical tool for the diagnosis of vulnerable plaques.[8] In this report, we have enlarged our patients number and we have evaluated the atherosclerotic plaque-calcification volumes of the patients in the context of symptomatic or asymptomatic disease.

Patients and methods

This study was approved by Ethics Committee of Istanbul University Medical Faculty (No: 2018/952). All research was performed in accordance with relevant guidelines/regulations.

The study enrolled 22 patients with carotid artery stenosis who underwent carotid endarterectomy in Istanbul University Istanbul Medical Faculty, Department of Cardiovascular Surgery. Thirteen patients were male and the remaining 9 patients were female. The mean age was calculated to be 60.2±9.6 years. The carotid artery stenosis degree was between 75% and 99%. Seventeen people had hypertension history. Sixteen of them were smokers and 2 of them were ex-smokers. Diabetes mellitus was present in 15 of the patients. Eighteen patients had hypercholesterolemia with LDL cholesterol value above 160 mg/dl. Nine patients had undergone coronary artery stent procedure. Two patients had mitral valve replacement and one patient had aortic valve replacement. They have used warfarin since they had the operations. The administration of warfarin stopped 7 days before the surgery and administration of low molecule weight heparin started. When INR was under 1.5, the operations were performed in these patients. Five patients had coronary artery bypass grafting procedure history. Eight patients had bilateral carotid artery stenosis. In the patients who had bilateral carotid artery disease, the symptomatic in other words more stenotic side underwent surgery first and then the contralateral side underwent surgery after 1 week.

Eight patients were asymptomatic and the remaining 14 patients were symptomatic. Ten patients had suffered stroke; 6 of them had left hemiplegia and 4 of them had right hemiplegia. One of them had recurrent transient ischemic events, 4 patients had dizziness and one of them had amaurosis fugax concomitantly.

All patients used acetylsalicylic acid and the operations were performed under acetylsalicylic acid. Fifteen of them used clopidogrel. Administration of clopidogrel stopped 72 hours before the surgery except for one patient with recurrent ischemic events. This patient underwent carotid endarterectomy procedure immediately. The demographic features of the patients are summerized in Table 1.

Surgical technique

The operations were performed under deep and/or superficial regional anesthesia and infiltration anesthesia. The bupivacaine, lidocaine, and saline combination was used for the superficial cervical block. The 10 cc bupivacaine of 0.05%, 6 cc lidocaine of 2%, and 4 cc saline combination was injected at the lateral border of the sternocleidomastoid muscle subcutaneously. The transverse processes of cervical vertebrae C2, C3, and C4 were palpated to determine the level of deep cervical plexus block. Following negative aspiration result for blood, lidocaine hydrochloride and bupivacaine hydrochloride combination was performed for the deep cervical plexus block. The allowed total amount of bupivacaine hydrochloride was 2–3 mg/kg. The 50% mixture of prilocaine hydrochloride and bupivacaine hydrochloride was injected subcutaneously along the incision line for infiltration anesthesia. If the patients complained of pain or discomfort, additio-

Table 1 – Demographic features of 22 patients

Patient No	Symptoms
1	Stroke – TIA
2	Stroke
3	Stroke
4	Stroke
5	Stroke
6	Stroke
7	Stroke
8	Stroke
9	Stroke
10	Stroke
11	Dizziness
12	Dizziness, amaurosis fugax
13	Dizziness
14	Dizziness
15	Asymptomatic
16	Asymptomatic
17	Asymptomatic
18	Asymptomatic
19	Asymptomatic
20	Asymptomatic
21	Asymptomatic
22	Asymptomatic

nal doses were performed. A total dose of prilocaine of 5 mg/kg was allowed. During the procedure, remifentanil (0.025–0.05 mg/kg/min) was used to provide patient comfort. The patient's consciousness is quite necessary for the neurological function control in this procedure. Hence, sometimes, midazolam was added after de-clamping. Blood pressure control was achieved with continuous perfusion of nitroglycerin. Diltiazem or metoprolol was used as additional drugs in the management of blood pressure. Systemic heparin (100 IU/kg) was administered before clamping and was not antagonized after the procedure. In the patients who did not tolerate cross-clamping, the shunt was used. The surgical procedure began with a standard incision paralel to the sternocleidomastoid muscle and common carotid artery (CCA), internal carotid artery (ICA) and external carotid artery (ECA) were prepared and dissected. Following systemic heparin injection, the arteries were clamped. The patient's consciousness and response to verbal stimuli and ability to move contralateral side hand and foot were evaluated for the neurological examination at least 2–3 minutes prior to arteriotomy. In the patients who did not tolerate cross-clamping, endarterectomy was performed with insertion of a shunt. A longitudinal incision was performed from the common carotid artery to the internal carotid artery until the level of the plaque-free segment was observed. Thromboendarterectomy was performed with an elevator. The atherosclerotic plaque was removed and put into 2% formaldehyde for experimental investigation. The patchplasty was performed for the reconstruction of the arteries in all patients. After hemostasis, a drain was inserted to the surgical site and the tissues were sutured anatomically. In the patients who had bilateral carotid artery disease, the symptomatic in other words more stenotic side underwent surgery first and then the contralateral side underwent surgery.

Micro-computerized tomography (micro-CT)

Skyscan 1174v2 device (Bruker, Kontich, Belgium) with the following settings of 50 kV, 800 µA, 1024×1304 resolution, filter of 0.5 mm aluminum, rotation step of 0.9° and 40 W power was used for scanning the plaques fixed within 2% formaldehyde. After adjusting a micro-focal spot and high-resolution detectors for X-rays, for each plaque, full-scan mode 360° was performed in about 50 min scan period and then the images were created via Ctvox and ctan programs. 480 raw images in TIFF format were reconstructed using NRecon Software (Bruker, Kontich, Belgium) which generated 755 horizontal sections in BMP format.

Scanning acoustic microscopy (SAM)

Methods in SAM experiments were applied in accordance with Bogazici University Institutional Review Board for Research with Human Subjects. The plaques were charac-

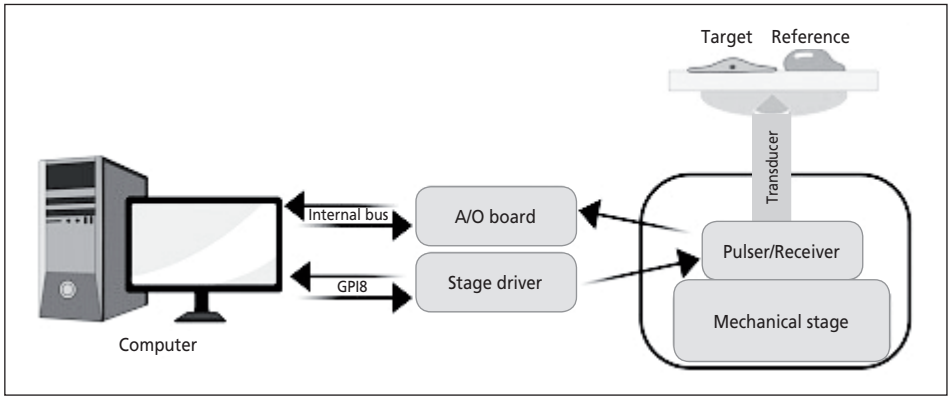

Fig. 1 – SAM schematic in AI mode.

terized by scanning acoustic microscope (AMS-50SI) which was developed by Honda Electronics (Toyohashi, Japan). The schematic of SAM setup in acoustic impedance (AI) mode is shown in Figure 1. It is composed of a transducer with quartz lens, a pulser/receiver, an oscilloscope, a computer and a display monitor. 80 MHz transducer has a spot size of 17 μm and a focal length of 1.5 mm. It generates single pulses of width of 5 ns with a repetition rate of 10 kHz and also collects the reflected acoustic waves, therefore, acts as a pulser/receiver. Distilled water is the coupling medium between the quartz lens and the substrate. X-Y stage scans the transducer. The signals reflected from both the reference and target material are analysed by the oscilloscope. Finally, with a lateral resolution of approximately 20 μm, acoustic intensity and impedance maps of the region of interest with 300×300 sampling points are visualized.

In Figure 1, the target is the plaque sample and the reference is distilled water. The target signal can be written as

- where is the generated signal by the 80 MHz transducer, is the acoustic impedance of the plaque andis the acoustic impedance of the polystyrene substrate,which is 2.37 MRayl. The plaque's acoustic impedance is calculated by comparing the reflected signal from the plaque with the one from the reference. The signal reflected from the reference can be written as
- where is distilled water's acoustic impedance, which is 1.50 MRayl. Consequently, the plaque's acoustic impedance is

Statistical analysis

Statistical analysis was performed using SPSS (SPSS for Windows, SPSS Inc, Chicago, IL, US) 15.0 computer software. Data were expressed as mean ± standard deviation for continuous variables and counts and frequencies for categorical variables. Conformity of normal distribution and homogeneity were tested with the Kolmogorov–Smirnov test for the analysis of continuous variables. Normally distributed data were compared with Student t Test. Mann–Whitney U was was used for the analysis of data which do not show normal distribution. Categorical values were evaluated with Chi-square test. Parametric values were evaluated with Independent Samples T-Test. A *p*-value of less than 0.05 was considered statistically significant.

Results

All patients underwent conventional carotid endarterectomy procedure under regional and infiltration anesthesia. During the procedure neurological deterioration did not occur in any patients hence the shunt was not needed. The mean operation time was 64.22±29.04 minutes.

Perioperatively, in 10 patients, the atherosclerotic lesions were quite rigid under macroscopic inspection, which supports calcium-rich plaque formation. These patients were asymptomatic or had dizziness and were stroke-free. Plaque rupture was detected in 6 patients with stroke history. One patient with recurrent transient ischemic event had a severe ulcerative plaque. Remaining 5 patients with cerebral ischemic event had soft plaque formation. The stenosis degree was not significantly different in soft or though plaques. However, dizziness was more common in patients with stenosis degree above 90%.

Postoperatively, all patients had been taken to the intensive care unit. The mean drainage was 24.50±17.64 cc. Hematoma occured in 2 patients. One of them was the patient who underwent operation immediately under clopidogrel therapy. The hematomas resolved spontaneously and reoperation was not required in any patient. Erythrocyte suspension replacement was not required in any patient. After one day in intensive care unit, the patients were taken to the ward. The mean hospital stay after the operation was 36.40±13.46 hours. Minor neurologic deficit confined to the nerves of platysma and hypoglossal nerve occured in 10 patients and resolved spontaneously during follow up. The patients were discharged from the hospital under clopidogrel and acetylsalicylic acid dual therapy, except the patients with mechanical valve replacement history. They are followed under warfarin and acetylsalicylic acid. In the six-month follow-up period, the mortality, major or minor bleeding did not occur in any patients. The control computed tomography angiography revealed successfull procedure without restenosis in all patients.

Micro-CT results

Each plaque, fixed with gauze inside a tube full of 2% formaldehyde, was first monitored with micro-CT, to visualize calcifications spread through, as can be seen in Figure 2. The calcific regions had been specified before the plaques were prepared for SAM studies.

Fig. 2 – Micro-CT image of a plaque sample in which the calcifications were monitored clearly in 3 dimensions. Darker regions belong to calcifications.

SAM results

The plaques had cylinder-like structures of a height of around 2 cm and radius of approximately 1 cm. We prepared smaller cylindrical cross sections of a height of 2 mm and radius of 1 cm with plain facets by slicing them with a lancet, for SAM studies.We obtained the images of plaques by using AI mode of SAM. Figure 3 shows the acoustic impedance map of one sample which was constructed using the acoustic reflections from both surfaces of distilled water and the plaque cross-section on the polystyrene substrate (Fig. 1). The acoustic impedance distribution indicated different components with different elasticity within the atherosclerotic plaques.The carotid artery plaques were examined in the context of morpho-

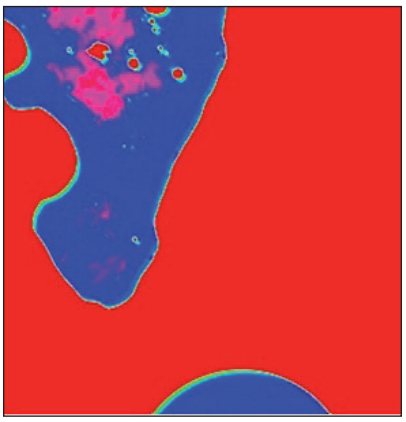

Fig. 3 – Acoustic impedance map of one atherosclerotic plaque obtained by comparing the reflected signals from distilled water and the sample. Upper nonuniform shape belongs to the plaque sample, having collagen-rich and calcific regions specified with different colors, and the lower uniform shape belongs to distilled water. The scanning area is 4.8 mm × 4.8 mm.

Table 2 – Mean acoustic impedance values of collagen-rich and calcific regions of plaques of all patients

Patient No	Mean acoustic impedance value (MRayl)	Symptoms
1	1.542–1.723	Stroke – TIA
2	1.540–1.719	Stroke
3	1.510–1.732	Stroke
4	1.503–1.654	Stroke
5	1.507–1.724	Stroke
6	1.506–1.694	Stroke
7	1.513–1.751	Stroke
8	1.501–1.765	Stroke
9	1.538–1.729	Stroke
10	1.521–1.686	Stroke
11	1.561–1.744	Dizziness
12	1.510–1.751	Dizziness, amaurosis fugax
13	1.521–1.714	Dizziness
14	1.560–1.627	Dizziness
15	1.514–1.700	Asymptomatic
16	1.547–1.832	Asymptomatic
17	1.544–1.772	Asymptomatic
18	1.511–1.724	Asymptomatic
19	1.577–1.760	Asymptomatic
20	1.559–1.761	Asymptomatic
21	1.501–1.743	Asymptomatic
22	1.614–1.814	Asymptomatic

logical features. The high acoustic impedance value is associated with calcium-rich plaque morphology. The relationship between plaque morphology and symptomatic/asymptomatic disease and the risk of stroke was evaluated. The acoustic impedance values of al lpatients were summarized in Table 2.

As it is seen in the Table 2, the smaller acoustic impedance values are associated with collagen-rich regions, while the higher values are associated with calcific regions within the plaques. These results show that symptomatic disease is not correlated with formation of high density of calcification.Table 2 presents acoustic impedance microscopy results in collagen-rich and calcific regions.

Discussion

Carotid artery stenosis is accused of being one of the major causes of the stroke in the world.[1] Under current guidelines, carotid endarterectomy is performed in serious cases with high degree of stenosis to eliminate stroke risk.[9] However, the stroke risk is not associated with only the degree of carotid stenosis, the structure of the atherosclerotic plaque has also an important role in their stability or instability.[10,11] Hence, a clinical practice is crutial to predict the high-risk lesions even in asymptomatic patients.

In histological examinations, inflammation leads to destabilization and causes potentially symptomatic lesions. Besides, the lesions with thin fibrous cap, large lipid necrotic core, and intraplaque haemorrhage may cause instable plaques.[10] However, because of the limited availibility of the imaging methods, the relationship between the structure of the atherosclerotic lesions and symptomatic disease is still unclear. Calcified plaques are considered as low risk lesions because of the stable nature[12] but one fifth of them may be symptomatic.[10]

In clinical practice, Doppler ultrasound (USG) and computed tomographic angiography (CTA) are most commonly used to reveal the degree of carotid artery stenosis.[6] Although USG is generally the first choice imaging tool; it is subjective depending on the operator and is not enough to specificity tissue composition.[5] The CTA has an advantage of revealing the exact localization and extent of the stenosis, the calcium volume; however, it has some disadvantages such as iodinated contrast agents and radiation exposure. Besides, it is not appropriate for showing hemodynamics, flow abnormalities or detect micro-calcifications.[1,6] The magnetic resonance angiography (MRI) is another option for screening in clinical practice. MRI is superior to CTA in assessing the intraplaque hemorrhage and ulceration of atherosclerotic plaque.[7]

The degree of stenosis is evaluated with the cerebral symptomatology and the carotid endarterectomy procedure is decided in the patients who have beneficial potentials.[1,13] Carotid artery stenting is another option in appropriate patients but the surgical procedure is still the gold standard.[14] In some centers, the symptomatic disease is the only indication for carotid endarterectomy.[1]

Intraplaque hemorrhage and ulceration have been known to be associated with plaque vulnerability by leading instability.[1] Intraplaque hemorrhage induces inflammation and is considered as one of the biomarkers of the plaque instability. Plaque ulceration is another instability factor because it causes fibrous cap rupture and leads to ischemic cerebrovascular events. Hence, the lesion is more

common in symptomatic patients than asymptomatic patients.[2] When intraplaque hemorrhage is together with plaque ulceration, recurrent ischemic events may occur. In many series, calcification is thought to be protective for ischemic events[15] and the patients with collagen-rich plaque had more cerebral stroke.[2,6] In contrary to these, there have been some studies mentioning that calcification may increase plaque vulnerability depending on calcification sizes and locations.[7] The calcifications that are thinner and closer to the surface may suffer high stress concentration and lead to intraplaque hemorrhage and plaque rupture. However, the deeper calcification has lesser plaque stress and acts like a barrier for preventing intraplaque hemorrhage formation.[7] Also, there have been some studies suggesting that the incidence of preoperative neurological symptoms and histological complications are not so different in patients with or without plaque calcification, in addition to this, cerebral ischemic events may be seen more frequently in the presence of more calcifications.[12]

Because of requiring predictor risk factors of the atherosclerotic plaque, the examinations have been focused on the stenosis degree. Stenosis degree is considered as one of the risk factors. Although previous studies reported that high degree stenosis has a role in symptomatic disease, recent studies showed that there is no relation between stenosis degree and ischemic cerebral event.[2] Ipsilateral hypoperfusion is assigned as another risk factor for symptomatic disease. In this content, the brain perfusion may have an utmost importance for triggering the symptomatic disease.[2]

In recent years, with an increasing number of studies that reveal the importance of components in atherosclerotic plaque for determining the risk potential; the clinicians examine the imaging tools that highlight plaque structure. Intravascular ultrasound (IVUS), near-infrared spectroscopy (NIRS), and optical coherence tomography (OCT) have been used in coronary circulation and are found useful to evaluate plaque morphology. While IVUS and OCT are useful methods for atherosclerotic lesions, NIRS can detect lipid components.[16] In a study, Agatston calcium score was assumed to be a predictor factor for plaque vulnerability.[15] The higher scores meant lower symptoms ipsilateral to the carotid lesion. Hence, in the asymptomatic group, there had been significantly more carotid calcification than in symptomatic group.[15]

As we previously reported, scanning acoustic microscopy (SAM) may be considered as another optional imaging tool obtaining useful information about structural and mechanical information of atherosclerotic plaque.[8,16] SAM provides differentiation between collagen-rich areas and calcified regions, so it may be beneficial to determine high-risk lesions in clinics. In our study, we have enlarged the patient number and evaluated the plaque structure in the context of symptomatic or asymptomatic disease. The patients with plaques of less acoustic impedance, which had more collagen-rich regions, suffered stroke more than the patients with plaques of high acoustic impedance, which had more calcification. This result is correlated with previous reports that claim the calcification is protective for stroke by providing a stable plaque. Also, we have revealed that when the calcification region is increased, the number of patients complaining about the dizziness increases. We think that this result may be the consequence of hypoperfusion depending on the increased calcification.

In this study, we concluded that the components of the carotid plaque are strongly associated with the stroke rates. In a daily clinical practice, the stenosis degree is thought as the most important indicator of the high risk pathology. Recent studies have started to change this opinion with patient series. Plaque morphology takes attention for the determination of high risk lesions, therefore new imaging tools are required. According to our results, the symptomatic disease is not correlated with the formation and high density of calcification. However, supporting the previous studies that promote calcium-rich plaque formation as a stabilizer, our results reveal that the stroke risk is associated with collagen-rich plaque formation. Our major limitation is a small number of the patients. The larger number and multicenter series are necessary to make examination of the morphological features of the carotid artery plaques a standard procedure in clinical practice to differentiate high-grade lesions and discriminate which patients require intervention for the atherosclerotic carotid artery disease.

Conflict of interest
None.

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References

1. Diab HMH, Rasmussen LM, Duvnjak S, et al. Computed tomography scan based prediction of the vulnerable carotid plaque. BMC Med Imaging 2017;17:61.
2. Park MS, Kwon S, Lee MJ, et al. Identification of High Risk Carotid Artery Stenosis: A Multimodal Vascular and Perfusion Imaging Study. Front Neurol 2019;10:765.
3. Naylor AR, Ricco JB, de Borst GJ, et al. Editor's Choice – Management of Atherosclerotic Carotid and Vertebral Artery Disease: 2017 Clinical Practice Guidelines of the European Society for Vascular Surgery (ESVS). Eur J Vasc Endovasc Surg 2018;55:3–81.
4. Munemitsu T, Ishii A, Okada E, et al. Ex Vivo Assessment of Various Histological Differentiation in Human Carotid Plaque with Near-infrared Spectroscopy Using Multiple Wavelengths. Neurol Med Chir (Tokyo) 2019;59:163–171.
5. Sheahan M, Ma X, Paik D, et al. Atherosclerotic Plaque Tissue: Noninvasive Quantitative Assessment of Characteristics with Software-aided Measurements from Conventional CT Angiography. Radiology 2018;286:622–631.
6. Seyman EE, Bornstein N, Auriel E, et al. Assessment of carotid artery ultrasonography in the presence of an acoustic shadow artifact. BMC Neurol 2019;19:178.
7. Yang J, Pan X, Zhang B, et al. Superficial and multiple calcifications and ulceration associate with intraplaque hemorrhage in the carotid atherosclerotic plaque. Eur Radiol 2018;28:4968–4977.
8. Bilen B, Sener LT, Albeniz I, et al, Determination of Ultrastructural Properties of Human Carotid Atherosclerotic Plaques by Scanning Acoustic Microscopy, Micro-Computer Tomography, Scanning Electron Microscopy and Energy Dispersive X-Ray Spectroscopy. Sci Rep 2019;9:679.

9. Azar D, Torres WM, Davis LA, et al. Geometric determinants of local hemodynamics in severe carotid arterystenosis. Comput Biol Med 2019;114:103436
10. Masztalewicz M, Rotter I, Nowacki P, et al. 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. Folia Neuropathol 2019;57:63–71.
11. Kolodgie FD, Yahagi K, Mori H, et al. High-risk carotid plaque: lessons learned from histopathology. Semin Vasc Surg 2017;30:31–43.
12. Pini R, Faggioli G, Fittipaldi S, et al. Relationship between Calcification and Vulnerability of the Carotid Plaques. Ann Vasc Surg 2017;44:336–342.
13. Dharmakidari S, Bhattacharya P, Chaturvedi S. Carotid Artery Stenosis: Medical Therapy, Surgery, and Stenting. Curr Neurol Neurosci Rep 2017;17:77.
14. Ugurlucan M, Akay HT, Erdinc I, et al. Anticoagulation strategy in patients with atrial fibrillation after carotid endarterectomy. Acta Chir Belg 2019;119:209–216.
15. Yoon WJ, Crisostomo P, Halandras P, et al. The Use of the Agatston Calcium Score in Predicting Carotid Plaque Vulnerability. Ann Vasc Surg 2019;54:22–26.
16. Bilen B, Gokbulut B, Kafa U, et al. Scanning Acoustic Microscopy and Time-Resolved Fluorescence Spectroscopy for Characterization of Atherosclerotic Plaques. Sci Rep 2018;8:14378.