Original article

Folia Neuropathologica

Carotid atheromatous plaques' instability. Practical implication of morphologic assessment

Marta Masztalewicz[1], Przemysław Nowacki[1], Anna Bajer-Czajkowska[1], Katarzyna Kotfis[2], Jowita Biernawska[2], Maciej Żukowski[2], Piotr Gutowski[3]

[1]Department of Neurology, Pomeranian Medical University, Szczecin, Poland, [2]Department of Anaesthesiology and Intensive Care, Pomeranian Medical University, Szczecin, Poland, [3]Department of General Vascular Surgery and Angiology, Pomeranian Medical University, Szczecin, Poland

Folia Neuropathol 2012; 50 (2): 159-165

Abstract

The only method giving the possibility of a thorough assessment of plaques instability, is a histologic examination. Three plaques categories were distinguished: unstable, potentially unstable and stable. The distribution of particular types of plaques was similar in symptomatic and asymptomatic patients. Over three quarters of lesions which could correspond to stable plaques in a macroscopic assessment, microscopically fulfilled the criteria for unstable or potentially unstable ones (the number of confirmed stable lesions vs the number of unconfirmed ones, 13 vs. 51 respectively, $p < 0.0001$). In a microscopic assessment made for all the plaques altogether, 52 plaques (58.4%) were considered unstable; 18 (20.2%) fulfilled the criteria for potentially unstable ones. The remaining 19 plaques (21.4%) were classified as plaques of stable structure. Unstable plaques constituted a significant majority (unstable vs potentially unstable and unstable vs. stable, $p = 0.0006$ and $p = 0.0008$ respectively).

Due to the fact that majority of carotid atheromatous plaques appear to be unstable or potentially unstable because of the inflammation and related mechanisms, the role of the inflammatory-immunologic component of atherosclerosis should be used in prophylaxis of stroke and the new therapeutic concepts worked out.

Key words: *carotid plaques, instability, morphologic assessment.*

Introduction

In a majority of cases, an ischaemic stroke happens as a result of arteriosclerosis in arteries supplying the brain. Research conducted over the years shows that not only the presence of plaques but also their character creates the risk of an acute cerebral episode [5,6,8,24]. Carotid atheromatous plaques' instability as a predictor and risk factor of ischaemic stroke appears to be very important from the point of view of stroke prophylaxis [8,15]. Hence there have been many attempts at establishing ultrasound criteria of carotid plaque instability. So far the suggested criteria and results obtained have not been clear-cut. Hypoechogenicity of atheromatous plaques in carotid arteries seems to be a parameter which could indicate their potential instability, referring to the lipid-necrotic component or intra-plaque haemorrhages [7,9,18]. How-

Communicating author:

Marta Masztalewicz, The Department of Neurology, Pomeranian Medical University, Unii Lubelskiej 1, 71-252 Szczecin, Poland, e-mail: nowiczontko@poczta.onet.pl

ever, research with the use of more advanced ultrasound analyses prove that broadly available ultrasound techniques, used every day, do not give a full possibility to assess this parameter [8]. Approximately 37 to 58% of ultrasonographically assessed carotid plaques seem to be higher risk lesions [1,4,25]. It should also be stressed that the above-mentioned features are not the only or sufficient ones to distinguish unstable or potentially unstable atheromatous lesions [2].

A more accurate answer considering carotid plaque stability/instability can be obtained based on gross examination of the lesion. It can be carried out only in patients who undergo endarterectomy [12,26].

The aim of this paper was to estimate the frequency of higher risk plaques, when we take into consideration patients with advanced carotid disease, thinking about appropriate treatment of this patient group.

Material and methods

Ninety-one atheromatous plaques collected from 91 patients who underwent endarterectomy of carotid arteries were put through a morphological analysis. The examination was carried out in 27 women (29.67%) and in 64 men (70.33%), aged 44 to 85 years (average age 67.7 years), with arterial hypertension and/or diabetes type 2, and/or ischaemic heart disease and/or dyslipidaemia, and/or peripheral artery disease. Exclusion criteria included pathologies with underlying inflammatory or immune mechanisms (acute and chronic infections, liver cirrhosis, systemic connective tissue disease, multiple sclerosis, autoimmune neuropathies, Crohn's disease, ulcerative colitis, Hashimoto's thyroiditis, Graves' disease, neoplasms).

Every plaque was submitted to a morphological assessment, comprising macroscopic and microscopic examination. Conformity of the macroscopic and microscopic plaque evaluation was compared taking into consideration indications of its instability.

Macroscopic evaluation

Intraoperatively the plaque surface (smooth, ulcerated, with a parietal thrombus) and colour (white, yellow, coloured by a thrombus) were evaluated. A plaque with a parietal thrombus and/or ruptures of the interior membrane and/or its exfoliation, unconnected with the perioperative injury, was considered unstable. A plaque which showed ulceration on the surface and/or was coloured by a thrombus, was yellow with soft structure (a large lipid core), without a parietal thrombus or ruptures of the intima, was described as potentially unstable. A white plaque with a smooth surface, without the features mentioned before, was considered stable.

Microscopic evaluation

Plaques collected intraoperatively were fixed in an 8% solution of formalin and then three parts were taken from each of them, including from the most pathologically significant place. The material was embedded in paraffin and, having been cut into 3 µm thick slices, they were stained with haematoxylin and eosin as well as with the use of PAS and van Gieson's methods. The evaluation included inflammatory infiltrations, plaque vascularization, presence of intra-plaque haemorrhages and a parietal thrombus, assessment of elements of connective tissue, foam cells, and presence of cholesterol crystals. A plaque with a parietal thrombus, fibrous cap ruptures and also containing a large lipid core and/or intra-plaque haemorrhages and/or thrombi embedded in the structure of the plaque, massive/disseminated inflammatory infiltrations, rich vascularization, many foam cells and a mixed structure of fibres was considered microscopically unstable. A plaque was considered potentially unstable if it lacked parietal thrombi and ruptures of the fibrous cap, and showed coexistence of at least four of the following features: cholesterol crystals, massive/disseminated inflammatory infiltrations, rich vascularization, many foam cells, mixed structure of fibres. A plaque lacking the above-mentioned features, without clearly disturbed integrity of the fibrous elements, was described as stable. The assessment was made on the basis of the criteria established by the American Heart Association and the analysis of the data available from the literature, especially those which refer to carotid arteries [2,8,13,19,22].

Both analyses (macroscopic and microscopic) were blind in reference to each other and to clinical data.

Statistical analysis

In the analysis of measurable variables the following ones were presented: median (Me), minimum value (Min), maximum value (Max), standard deviation (SD). The measurable variables showed distributions significantly departing from a normal distribution (Shapiro-Wilk test, $p < 0.05$), which is why non-parametric tests were used. To show the significance of differences among more than two groups, Kruskal-Wal-

lis ANOVA was used, and to compare two groups of patients the Mann-Whitney U test was used. Nominal variables were compared using the chi-square test or its modifications with Yates' correction or the precise two-sided Fisher's test (for 2 × 2 tables). For a one-factor and then multi-factor analysis of an odds ratio (OR) with 95% confidence interval (95% CI), logistic regression was applied. As a statistical significance threshold $p < 0.05$ was assumed. Statistical calculations were performed using the program Statistica 7.1.

The research was conducted on the basis of the consent of the Local Bioethical Commission (resolution no. BN-001/36/06). All subjects gave informed consent.

Results

In 51 cases plaques were collected from patients after an ischaemic stroke or transient ischaemic attack (TIA), corresponding to assessed lesions (*symptomatic plaques*). In the remaining 38 cases an endarterectomy procedure was performed in patients without a previous stroke episode (*asymptomatic plaques*). Demographic data concerning the patients are shown in Table I.

Macroscopic assessment of atheromatous plaques

Among 89 assessed plaques, both symptomatic and asymptomatic (in 2 patients description of macroscopic lesions was not obtained) 13 (14.6%) were considered unstable, and another 12 (13.5%) fulfilled the criteria of potentially unstable plaques ($p = 0.881$). A majority (71.9%) of lesions assessed perioperatively corresponded to plaques of a stable character (stable vs. unstable and stable vs potentially unstable $p < 0.0001$). The distribution of particular types of plaques in a macroscopic evaluation taking into consideration the division into symptomatic and asymptomatic plaques was similar. In patients with a stroke, unstable plaques constituted 15.7%, potentially unstable plaques 11.8%, and stable plaques 72.5%. Among asymptomatic patients this percentage was 13.3%, 15.6% and 71.1% respectively ($p = 0.8931$).

Microscopic assessment of atheromatous plaques

In a microscopic assessment made for all the plaques altogether, 52 plaques (58.4%) were considered unstable; 18 (20.2%) fulfilled the criteria for potentially unstable ones. The remaining 19 plaques (21.4%) were classified as plaques of stable structure (Figs. 1A-C, 2). Unstable plaques constituted a significant majority (unstable vs potentially unstable and unstable vs. stable, $p = 0.0006$ and $p = 0.0008$ respectively).

No significant difference between the group of symptomatic and asymptomatic plaques for the above-described features was found. In patients with a stroke, unstable plaques constituted 54.9%, potentially unstable 21.6%, and stable 23.5%. Among asymp-

Table I. Demographic data of patients qualified for examinations, including symptomatic and asymptomatic plaques

Symptomatic character of lesions[1]	Yes	No
Number of patients (*n*)[2]	52	39
Age (years)[3]	67.9	67.7
Sex (*n* /%)[4]:		
Women	15 (28.85)	12 (30.77)
Men	37 (71.15)	27 (69.23)
Coexisting diseases (%)[5]:		
Arterial hypertension	90.2*	68.4
Diabetes type 2	29.4	26.3
Ischaemic heart disease	21.6	60.3*
PAD	23.5	28.9
Dyslipidaemia	28.9	13.2
Smoking (%)[6]	70	65
Medications (%)[7]:		
Antiaggregants	75	64.1
Statins	65.4	51.3
ACE inhibitors	53.8	51.3
Degree of stenosis (median)[8]	80%	80%

[1]Symptomatic character of lesions: yes – plaques collected from patients after an ischaemic stroke or transient ischaemic attack (TIA); no – plaques collected from patients without a previous stroke episode. [2]Number of patients (n) – number of patients assigned to particular groups (with symptomatic and asymptomatic plaques). [3]Age (years) – age, in years, of patients assigned to particular groups (with symptomatic and asymptomatic plaques. [4]Sex (n/%) – sex of patients assigned to particular study groups (number/percentage). [5]Coexisting diseases (%) – percentage of patients with particular comorbidities in group with symptomatic and asymptomatic plaques. [6]PAD – peripheral artery disease. [7]ACE inhibitors – angiotensin-converting enzyme inhibitors. [8]Degree of stenosis (median) – median stenosis of operated carotid arteries, revealed by preoperative ultrasonography
** Significantly more frequently (p < 0.05)*

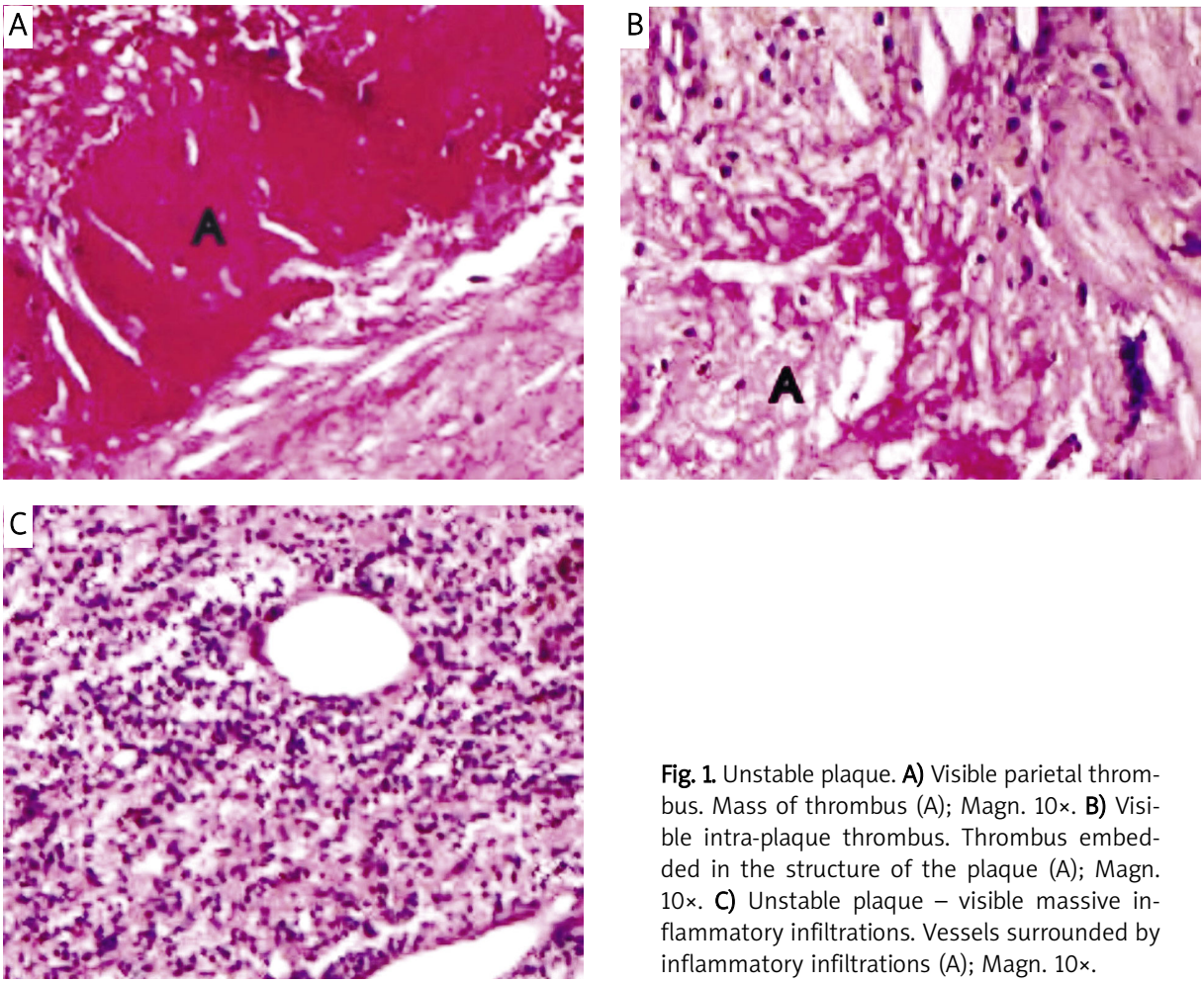

Fig. 1. Unstable plaque. **A)** Visible parietal thrombus. Mass of thrombus (A); Magn. 10×. **B)** Visible intra-plaque thrombus. Thrombus embedded in the structure of the plaque (A); Magn. 10×. **C)** Unstable plaque – visible massive inflammatory infiltrations. Vessels surrounded by inflammatory infiltrations (A); Magn. 10×.

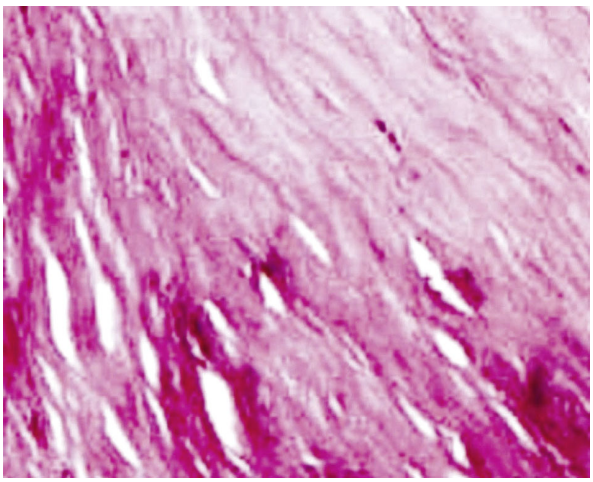

Fig. 2. Stable plaque with preserved integrity of the fibrous elements and small calcifications; Magn. 10×.

tomatic patients the percentages were 63.2%, 18.4% and 18.4% respectively ($p = 0.7186$).

Although individually none of the microscopically analysed plaque parameters determined a symptomatic character of lesions (Table II), rich plaque vascularization (*vasc*) and intensive inflammatory infiltrations (*inf*) were revealed as features characterizing potentially unstable and unstable lesions (stable vs. potentially unstable, stable vs unstable, potentially unstable vs unstable: $p^{vasc} = 0.02$ and $p^{inf} = 0.0003$; $p^{vasc} = 0.001$ and $p^{inf} < 0.0000$; $p^{vasc} = 0.8972$ and $p^{inf} = 0.1982$ respectively).

Both these parameters significantly influenced the occurrence of parietal coagulation (plaques with parietal thrombus vs plaques without thrombus: $p^{vasc} = 0.0333$, $p^{inf} = 0.0000$).

As it appears from the above, there were significant differences in assessment of the plaque type in a ma-

croscopic and a microscopic examination (the number of consistent assessments vs the number of inconsistent assessments, 26 vs. 63 respectively, $p < 0.0001$). The divergence especially concerned classification to the group of unstable plaques, and to a lesser degree potentially unstable plaques. Over three quarters of lesions, considered stable in a macroscopic examination, fulfilled microscopic criteria of instability or potential instability (the number of confirmed stable lesions vs the number of unconfirmed ones, 13 vs. 51 respectively, $p < 0.0001$). Conformity of macroscopic and microscopic assessments of atheromatous plaques of carotid arteries is presented in Table III.

Discussion

Due to the fact that endarterectomy was performed in patients after a stroke as well as in individuals without previous cerebral vascular episodes, the above analysis considers both primary and secondary stroke prophylaxis [10,11,27]. From a clinical point of view, the unstable or potentially unstable character of plaques is particularly important [6,18,24]. Criteria of instability of plaques comprise a number of lesions that are visible only under microscopic examination. They are: an active inflammatory process within atheromatous lesions, rich vascularization of plaques, intra-plaque haemorrhages, a large lipid-necrotic core, an injury of the fibrous cap, and parietal coagulation [2,22].

Our microscopic examination revealed the intensive inflammatory component within the analysed lesions. It is accepted as a main factor for plaque instability. Considering data from the literature concerning pathogenesis of atherosclerosis it also seemed justified to include occurrence of cholesterol crystals in the criteria of instability, and to evaluate the character of fibres [21,22]. On the basis of the assumed microscopic criteria, the percentage of unstable and potentially unstable lesions in our material increased considerably, from about 30% in a macroscopic evaluation to 80% in a microscopic one. It should be noted that none of the microscopic parameters of plaque instability taken into consideration separately determined the occurrence of a stroke episode, and so did not determine the symptomatic character of stenosis caused by the plaque [3,24]. Hence, a single microscopic parameter of lesion instability cannot be considered as a predictor of potential stroke. It seems that coexistence of many microscopic criteria leads to instability. Our results confirm the significant role of plaque vascularization and inflammatory infiltrations [2,17,18,23]. Both these parameters influenced the occurrence of parietal thrombosis, an unquestionable sign of plaque instability.

Discussing the atherosclerotic process, systemic factors should also be taken into consideration. They may influence changes in the arterial wall, leading to destabilization of developing lesions [16]. Patients with disorders where inflammatory and immune factors play

Table II. Parameters of microscopic assessment of atheromatous plaques (logistic regression)

Microscopic parameter	Odds ratio (OR)	*p*
Crystals[1]	1.5299	0.3913
Fibres[2]	1.8298	0.1811
Foam cells[3]	1.9427	0.1779
Inflammatory infiltrations[4]	0.6240	0.2745
Vessels[5]	0.8771	0.7347
Intraplaque haemorrhages[6]	0.5586	0.3472
Thrombus[7]	0.8318	0.7278

[1]Crystals – cholesterol crystals within plaques. [2]Fibres – plaque fibres. [3]Foam cells – foam cells within plaques. [4]Inflammatory infiltrations – inflammatory component of assessed carotid plaques (infiltration by inflammatory cells). [5]Vessels – vascularization of assessed carotid plaques. [6]Intraplaque haemorrhages – haemorrhages within assessed carotid plaques. [7]Thrombus – parietal thrombus

Table III. Conformity of macroscopic and microscopic assessments of atheromatous carotid plaques

	Microscopically stable	Microscopically potentially unstable	Microscopically unstable
Macroscopically stable	13	14	37
Macroscopically potentially unstable	4	3	5
Macroscopically unstable	2	1	10

Microscopically stable – plaques classified as stable in microscopic assessment. Microscopically potentially unstable – plaques classified as potentially unstable in microscopic assessment. Microscopically unstable – plaques classified as unstable in microscopic assessment. Macroscopically stable – plaques classified as stable in macroscopic assessment. Macroscopically potentially unstable – plaques classified as potentially unstable in macroscopic assessment. Macroscopically unstable – plaques classified as unstable in macroscopic assessment

a role were excluded from the study. Hence it can be stated that plaque destabilization in our patients did not result from involvement of such extravascular inflammatory-immune factors. Nevertheless, our histopathological study, taking into account the numerous criteria of instability, revealed a significantly larger percentage of potentially unstable or unstable carotid atheromatous plaques than might be expected based on ultrasound analyses [1,4,25] or even gross examination after endarterectomy. It additionally justifies the use of statins as pleiotropic drugs in stroke prophylaxis. It also constitutes a significant reason for intensification of anti-inflammatory components [14,20,28].

Importantly, the distribution of plaque types in the groups of symptomatic and asymptomatic plaques was similar. It is possible that if endarterectomy had not been performed, the appearance of neurological complications in the patients would have only been a matter of time.

Summing up, each patient with advanced carotid disease (including those not qualified for endarterectomy) should be treated as a patient with potentially higher risk plaques.

It should also be underlined that pathogenesis of stroke connected with the potentially unstable plaques and especially unstable ones seems to be more complex.

While in the case of stable plaques antiaggregation treatment can be sufficient, in the remaining two cases one should consider using drugs with properties for stabilizing a plaque, including those with an anti-inflammatory action.

Conclusions

Due to the fact that the majority of carotid atheromatous plaques appear to be unstable or potentially unstable because of the inflammation and related mechanisms, apart from antiaggregants and anticoagulants, inhibitors or agents limiting the role of the inflammatory-immunological component of atherosclerosis should be used in prophylaxis of stroke, and new therapeutic concepts should be developed.

References

1. AbuRahma AF, Wulu JT, Crotty B. Carotid plaque ultrasonic heterogeneity and severity of stenosis. Stroke 2002; 33: 1772-1775.
2. Alsheikh-Ali AA, Kitsios GD, Balk EM, Lau J, Ip S. The vulnerable atherosclerotic plaque: scope of the literature. Ann Intern Med 2010; 153: 387-395.
3. Baroncini LAV, Filho AP, Ramos SG, Martins AR, Murta LO. Histological composition and progression of carotid plaque. Thromb J 2007; 5: 4.
4. Brevetti G, Sirico G, Giugliano G, Lanero S, De Maio JI, Luciano R, Laurenzano E, Chiariello M. Prevalence of hypoechoic carotid plaques in coronary artery disease: relationship with coexistent peripheral arterial disease and leucocyte number. Vascular Medicine 2009; 14: 13-19.
5. Executive Committee for the Asymptomatic Carotid Atherosclerosis Study (ACAS). Endarterectomy for asymptomatic carotid artery stenosis. JAMA 1995; 273: 1421.
6. Golledge J, Greenhalgh RM, Davies AH. The symptomatic carotid plaque. Stroke 2000; 31: 774-781.
7. Gray-Weale AC, Graham GC, Burnett JR, Byrne K, Lusby RJ. Carotid artery atheroma: comparison of preoperative B-mode ultrasound appearance with carotid endarterectomy specimen pathology. J Cardiovasc Surg 1998; 29: 676-681.
8. Grogan JK, Shaalan WE, Cheng H, Gewertz B, Desai T, Schwarze G, Glagov S, Lozanski L, Griffin A, Castilla M, Bassiouny HS. B-mode ultrasonographic characterization of carotid atherosclerotic plaques in symptomatic and asymptomatic patients. J Vasc Surg 2005; 42: 435-441.
9. Gronholdt M-LM. Ultrasound and lipoproteins as predictors of lipidrich, rupture-prone plaques in the carotid artery. Arterioscler Thromb Vasc Biol 1999; 19: 2-13.
10. Halliday A, Harrison M, Hayter E, Kong X, Mansfield A, Marro J, Pan H, Peto R, Potter J, Rahimi K, Rau A, Robertson S, Streifler J, Thomas D; Asymptomatic Carotid Surgery Trial (ACST) Collaborative Group. 10-year stroke prevention after successful carotid endarterectomy for asymptomatic stenosis (ACST-1): a multicentre randomised trial. Lancet 2010; 376: 1074-1084.
11. Hellings WE, Moll FL, de Vries JP, Ackerstaff RGA, Seldenrijk KA, Met R, Velema E, Derksen WJM, De Kleijn DPV, Pasterkamp G. Atherosclerotic plaque composition and occurrence of restenosis after carotid endarterectomy. JAMA 2008; 299: 547-554.
12. Hobson RW, Mackey WC, Ascher E, Murad MH, Calligaro KD, Comerota AJ, Montori VM, Eskandari MK, Massop DW, Bush RL, Lal BK, Perler BA. Management of atherosclerotic carotid artery disease: clinical practice guidelines of society for vascular surgery. J Vasc Surg 2008; 48: 480-486.
13. Katsuda S, Kaji T. Atherosclerosis and extracellular matrix. J Atheroscler Thromb 2003; 10: 267-274.
14. Kunte H, Amberger N, Bush MA, Ruckert RI, Meiners S, Harms S. Markers of instability in high-risk carotid plaques are reduced by statins. J Vasc Surg 2008; 47: 513-522.
15. Lovett JK, Gallagher PJ, Hands LJ, Walton J, Rothwell PM. Histological correlates of carotid plaque surface morphology on lumen contrast imaging. Circulation 2004; 110: 2190-2197.
16. Lutgens E, Suylen R-J, Faber BC, Gijbels MJ, Eurlings PM, Bijnens AP, Cleutjens KB, Heeneman S, Daemen MJAP. Atherosclerotic plaque rupture: local or systemic process? Arterioscler Thromb Vasc Biol 2003; 23: 2123-2130.
17. Mofidi R, Crotty TB, McCarthy P, Sheehan SJ, Mehigan D, Keaveny TV. Association between plaque instability, angiogenesis and symptomatic carotid occlusive disease. B J Surg 2001; 88: 945-950.

18. Redgrave J-E, Lovett JK, Gallagher PJ, Rothwell PM. Histological assessment of 526 symptomatic carotid plaques in relation to the nature and timing of ischemic symptoms. Circulation 2006; 113: 2320-2328.
19. Redgrave JN, Gallagher P, Lovett J, Rothwell PM. Critical cap thickness and rupture in symptomatic carotid plaques. The Oxford Plaque Study. Stroke 2008; 39: 1722-1729.
20. Schölkens BA, Landgraf W. ACE inhibition and atherogenesis. Can J Physiol Pharmacol 2002; 80: 354-359.
21. Shaalan WE, Cheng H, Gewertz B, McKinsey JF, Schwartz LB, Katz B, Cao D, Desai T, Glagov S, Bassiouny HS. Degree of carotid plaque calcification in relation to symptomatic outcome and plaque inflammation. J Vasc Surg 2004; 40: 262-269.
22. Stary HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Insull W Jr, Rosenfeld ME, Schwartz CJ, Wagner WD, Wissler RW. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from Committee on Vascular Lesions of the Council on Atherosclerosis, American Heart Association. Circulation 1995; 92: 1355-1374.
23. Staub D, Patel MB, Tibrewala A, Ludden D, Johnson M, Espinosa P, Jaeger KA, Feinstein SB. Vasa vasorum and plaque neovascularization on contrast-enhanced carotid ultrasound imaging correlates with cardiovascular disease and past cardiovascular events. Stroke 2010; 41: 41-47.
24. TegosTJ, Sohail M, Sabetai MM, Robless P, Akbar N, Pare G, Stansby G, Nicolaides AN. Echomorphologic and histopathologic characteristics of unstable carotid plaques. Am J Neuroradiol 2000; 21: 1937-1944.
25. Topakian R, King A, Kwon SU, Schaafsma A, Shipley M, Markus HS. Ultrasonic plaque echolucency and emboli signals predict stroke in asymptomatic carotid stenosis. Neurology 2011; 77: 751-758.
26. Virmani R, Ladich ER, Burke AP, Kolodgie FD. Histopathology of carotid atherosclerotic disease. Neurosurgery 2006; 59 (5 suppl 3): 219-227.
27. Xiong L, Deng Y, Bi X, ZHU Y, Shentu W, Yu F, Zhang Y. Evaluation of carotid atherosclerotic plaque stability with contrast-enhanced ultrasonography. J Huazhong Univ Sci Technol 2008; 28: 724-726.
28. Yamada K, Yoshimura S, Kawasaki M, Enomoto Y, Asano T, Minatoguchi S, Iwama T. Effects of atorvastatin on carotid atherosclerotic plaques: a randomized trial for quantitative tissue characterization of carotid atherosclerotic plaques with integrated Backscatter ultrasound. Cerebrovasc Dis 2009; 28: 417-424.