Article

Stratified gray-scale median analysis and color mapping of the carotid plaque: correlation with endarterectomy specimen histology of 28 patients

SZTAJZEL, Roman, *et al*.

Abstract

To determine whether a stratified gray-scale median (GSM) analysis of the carotid plaque combined with color mapping could predict plaque histology better than an overall GSM measurement.

Stratified Gray-Scale Median Analysis and Color Mapping of the Carotid Plaque: Correlation With Endarterectomy Specimen Histology of 28 Patients

R. Sztajzel, S. Momjian, I. Momjian-Mayor, N. Murith, K. Djebaili, G. Boissard, M. Comelli and G. Pizolatto

Stroke. 2005;36:741-745; originally published online February 10, 2005;
doi: 10.1161/01.STR.0000157599.10026.ad
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0039-2499. Online ISSN: 1524-4628

Stratified Gray-Scale Median Analysis and Color Mapping of the Carotid Plaque

Correlation With Endarterectomy Specimen Histology of 28 Patients

R. Sztajzel, MD; S. Momjian, MD; I. Momjian-Mayor, MD; N. Murith, MD; K. Djebaili, MD; G. Boissard; M. Comelli; G. Pizolatto, MD

Background and Purpose—To determine whether a stratified gray-scale median (GSM) analysis of the carotid plaque combined with color mapping could predict plaque histology better than an overall GSM measurement.

Methods—Thirty-one carotid plaques derived from 28 patients undergoing carotid endarterectomy were investigated by ultrasound. GSMs of the whole plaque were used as measurement of echogenicity. A profile of the regional GSM as a function of distance from the plaque surface could be generated. Plaque pixels were further mapped into 3 different colors depending on their GSM value.

Results—Plaques with large calcifications presented the highest GSM values, and those with large hemorrhagic areas or with a predominant necrotic core exhibited the lowest. Fibrous plaques had intermediate GSM values. A necrotic core located in a juxtalumenal position was associated with significantly lower GSM values ($P=0.009$) and with a predominant red color (GSM <50) at the surface ($P=0.0019$). With respect to the thickness of the fibrous cap and the position of the necrotic core, the sensitivity and specificity of the predominant red color of the whole plaque was respectively 45% and 67% and 53% and 75%; considering the predominant red color of the surface, the sensitivity and specificity increased to 73% and 67% and 84% and 75%, respectively.

Conclusions—The stratified GSM measurement combined with color mapping showed a good correlation with the different histopathological components and further allowed identification with good accuracy of determinants of plaque instability. This approach should be investigated in a prospective, natural history study. **(*Stroke*. 2005;36:741-745.)**

Key Words: carotid artery plaque ■ pathology ■ ultrasonography

Besides degree of stenosis, plaque morphology, defined by its structure and surface characteristics, is thought to play an important role in the pathogenesis of stroke. An unstable carotid plaque is associated with a thinning of the fibrous cap, infiltration of inflammatory cells leading to surface ulceration, and plaque rupture.[1,2] Recent studies also indicate that the proximity of the necrotic core to the lumen is associated with an increased risk of clinical ischemic events.[3] An early identification of these features suggesting plaque instability would therefore be very useful for treatment. Recent ultrasound studies based on a computerized measurement of the gray-scale median (GSM) value of the carotid plaque demonstrated that a low GSM value, reflecting hypoechoic lesions, was associated with an increased risk of cerebrovascular events and may represent a good predictor of carotid plaque behavior.[4] Although characterization of the internal structure of the plaque by computer-assisted image analysis correlates closely with clinical symptoms,[4–9] little is known about the relationship between this computerized analysis of the plaque and the corresponding histopathological findings. The few studies performed so far showed conflicting results.[6,10–12] GSM analysis represents a median value of the whole atherosclerotic area and therefore may not necessarily reflect the presence of particular regional components. Therefore the aim of our work was to determine whether a stratified GSM assessment, analyzing each millimeter from the surface to the bottom of the lesion, combined with color mapping could predict plaque histology better than the usual overall GSM measurement.

Patients and Methods

We investigated 31 endarterectomy specimens (plaques or segments of plaques) derived from 28 patients with a mean age of 74 years (57 to 92) of whom 13 were symptomatic (6 transient ischemic attacks [TIAs] and 7 strokes). All patients presented a carotid stenosis between 60% and 90% (North American Symptomatic Carotid Endarterectomy Trial [NASCET] criteria) as established by ultrasound and magnetic resonance angiography. These investigations were performed within a mean delay of 4 weeks before endarterec-

Received September 2, 2004; final revision received October 29, 2004; accepted December 2, 2004.

From the Departments of Neurology (R.S., I.M.-M.), Neurosurgery (S.M.), Cardiovascular Surgery (N.M., K.D.), and Pathology (G.P.), University Hospital Geneva, Switzerland; the Department of Statistics (M.C.), University of Pavia, Italy; and Consultant in Biostatistics (G.B.), Pavia, Italy.

Correspondence to Dr R. Sztajzel, Neurosonology Unit, Department of Neurology 24, rue Micheli-du-Crest 1211 Geneva 14, Switzerland. E-mail Roman.Sztajzel@hcuge.ch

Stroke **is available at http://www.strokeaha.org** **DOI: 10.1161/01.STR.0000157599.10026.ad**

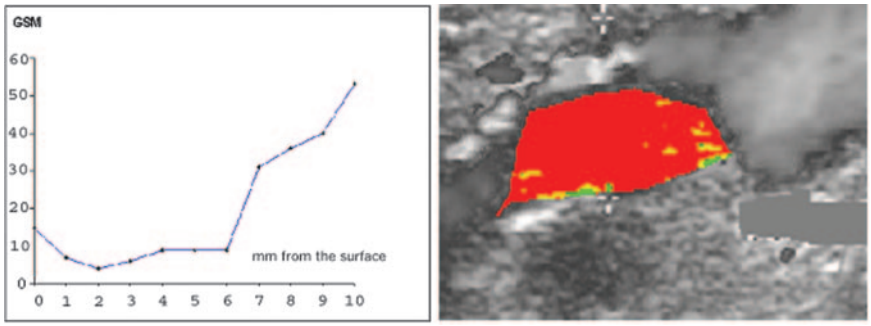

Figure 1. Stratified GSM analysis (left) showing a plaque of 11 mm, with the corresponding GSM values at each millimeter (from 0 to 10) and color mapping (right) of the same plaque with the predominant red color of the whole plaque and of its surface (GSM <50; arrow). Some small yellow and green areas within the plaque suggest the presence of GSM values >50 and 80, respectively.

tomy for the symptomatic patients and within a mean delay of 10 days for the asymptomatic ones. Ultrasound investigations (7.5-MHz probe; Acuson Sequoia Apparatus) included for the establishment of degrees of stenosis longitudinal and transverse sections by color-Duplex imaging and by power mode. Peak systolic velocities at the level of the stenosis as well as the ratio between peak systolic velocities in the internal carotid artery and the common carotid artery were assessed. The cardiovascular risk factors were determined according to the clinical and the laboratory findings (data not shown).

Only plaques presenting a similar luminal contour and spatial arrangement of the plaque segments, identified on a simultaneous visual assessment by confrontation of the ultrasound image and the histology specimen, were included in the study. This selection was performed before any GSM or histological examination of the internal structure of the plaque.

GSM Analysis

The video signal from the ultrasound device was converted to a digital image format by a personal computer, and the images were analyzed with a 2- to 3-fold increase of the initial size. GSM measurements were performed by 2 independent investigators. The GSM of the frequency distribution of gray-scale values of the pixels within the whole plaque or a region of it was used as measurement of the echogenicity. A program written in-house (S.M.) in MATLAB (Mathworks) was used to perform the following steps. All carotid plaques were first normalized by automatic linear scaling after the examiner was requested to outline a blood region and an adventitia region. The normalized gray scale was 0 for blood and 195 for adventitia.[5,13,14] After normalization, plaque was outlined in its longitudinal section to obtain a binary map. The luminal margin was then outlined again to provide to the program the precise location of plaque surface. The program then calculated the shortest distance in millimeters between each plaque pixel and the plaque surface along with the normalized gray-scale value at that pixel. The distance was quantified in millimeters according to the resolution of the ultrasound scanner (144 pixels/inch). For each plaque, the plaque pixels were binned according to their distance from the plaque surface. The GSM value of all pixels at each millimeter increment of distance (ie, stratum) was calculated. A profile of the regional GSM as a function of distance from the plaque surface could be generated, realizing a stratified determination of the GSM. The following stratums for each plaque were chosen for statistical analysis: level 0 (GSM 0), and level 30 and 50 (GSM 30 and 50), corresponding respectively to the GSM values obtained at 30% and at 50% of the thickness of the plaque. GSM measures were compared with the values obtained for the whole plaque (total GSM value).

Color Mapping of the Normalized Gray-Scale Plaques

The plaque pixels were mapped into 3 different colors, namely red, yellow, and green, depending on their gray-scale value. Thresholds were chosen as: lowest gray-scale values (<50 mapped in red), intermediate values (between 50 and 80 mapped in yellow), and highest values (>80 mapped in green; Figure 1). We determined for each plaque the predominant color present on the surface, which was defined as the upper third part of the lesion, and the predominant color of the whole plaque or plaque segment. In case of presence of 2 or 3 colors on the whole plaque or on the surface, predominance was decided according to the corresponding GSM value of the whole plaque and to the corresponding stratified GSM values of levels 0 and 30 for the surface. The plaque was considered homogeneous when only 1 predominant color was present on at least two thirds of the lesion and heterogeneous when at least 2 different colors were equally present. All color mappings of the plaques were evaluated by 2 independent investigators (I.M. and R.S.).

Histological Examination

After endarterectomy, fresh specimens were rinsed briefly in normal saline solution to remove the surface blood, were immersed in 4% formalin fixative, and subsequently decalcified to be sectioned. Each block was processed to paraffin and then sectioned at 5 μm in a longitudinal plane. Slices were then stained in sequence with hematoxylin and eosin, Masson trichrome, Miller, Congo red, phosphotungstic acid hematoxylin, and Perls stains. All sections were examined by an experienced pathologist (G.-P.P.) for the presence of the different plaque components. Fibrosis, hemorrhage, calcification, or necrotic/lipid core were respectively expressed as large or small if they occupied >50% or <50% of the total area of the plaque. Thrombus was either present or not. The fibrous cap was measured using an ocular micrometer; a value of <80 μm corresponded to a thin and >80 μm to a thick fibrous cap.[15] The necrotic/lipid core was considered near the surface, in a juxtalumenal position (not covered by the fibrous cap), or distant from the surface of the plaque (covered by the fibrous cap, whatever its thickness).

Statistical Analysis

Statistical analysis was performed using the Wilcoxon rank sum test, allowing nonparametric comparisons and the Fisher exact test. A P value of <0.05 was chosen as the level of significance.

Results

A total of 31 endarterectomy specimens (28 patients) were included in the study. For each patient, only the largest side of the plaque was considered for analysis, except for 3 patients who presented 2 different segments of similar size. Thirteen patients were symptomatic (6 TIAs and 7 strokes) and 15 asymptomatic. The histological characteristics of the plaques or plaque segments were as follows: 39% (12 of 31) with a predominant fibrotic tissue; 71% (22 of 31) with a large necrotic core; 19% (6 of 31) with a large hemorrhagic area; and 13% (4 of 31) with large calcifications. A thick fibrous cap (>80 μm) was found in 29% (9 of 31) and a thin (<80 μm) or ruptured fibrous cap in 71% (22 of 31) of the plaques or plaque segments. The necrotic lipid core was covered by the fibrous cap in 39% (12 of 31) and not covered in 62% (17 of 31) of the plaques. Although symptomatic patients presented more frequently a thin fibrous cap (10 of

Correlation Between Stratified GSM Analysis and Color Mapping of 31 Plaques or Plaque Segments and 2 Determinants of Plaque Instability (fibrous cap and lipid necrotic core)

	Thick Fibrous Cap (>80 μm) n=9 of 31, 29%	Thin Fibrous Cap (<80 μm) n=22 of 31, 71%	Lipid Necrotic Core Distant From Surface (covered by fibrous cap) n=12 of 31, 39%	Lipid Necrotic Core in Juxtalumenal Position (not covered by fibrous cap) n=19 of 31, 61%
Mean GSM 0	48.9 (SD 25.4)	33.7 (SD 14.5) P=0.078	49.5 (SD 22.2)	30.9 (SD 13.1) P=0.009
Mean GSM 30	66.1 (SD 48.1)	44.7 (SD 20.5) P=0.12	66.0 (SD 41.2)	41.4 (SD 19.9) P=0.024
Mean GSM 50	64.3 (SD 40.9)	50.3 (SD 24.6) P=0.35	62.3 (SD 36.6)	49.3 (SD 25.1) P=0.31
Mean overall GSM value	55.0 (SD 32.1)	43.8 (SD 20.2) P=0.09	57.6 (SD 28.0)	40.4 (SD 19.5) P=0.013
Predominant color of whole plaque	Red 3 of 9 (33%); green and yellow 6 of 9 (67%)	Red 10 of 22 (45%); green and yellow 12 of 22 (54%); P=0.5	Red 3 of 12 (25%); green and yellow 9 of 12 (75%)	Red 10 of 19 (53%); green and yellow 9 of 19 (47%); P=0.12
Predominant color of the plaque surface	Red 3 of 9 (33%); green and yellow 6 of 9 (67%)	Red 16 of 22 (73%); green and yellow 6 of 22 (27%) P=0.056	Red 3 of 12 (25%); green and yellow 9 of 12 (75%)	Red 16 of 19 (84%); green and yellow 3 of 19 (16%); P=0.0019
Homogeneous plaques	2 of 9 (22%)	5 of 22 (23%)	2 of 12 (17%)	5 of 19 (26%)
Hetereogeneous plaques	7 of 9 (78%)	17 of 22 (77%); P=1.0	10 of 12 (83%)	14 of 19 (74%); P=0.68

13 [77%]) and a necrotic core located at the surface (10 of 13 [77%]), the difference with histological findings of asymptomatic patients (thin fibrous cap 9 of 15 [60%]; necrotic core located at the surface 7 of 15 [47%]) was not significant (P=0.1 for both variables). Patients with a history of stroke or TIA presented lower mean regional GSM values than asymptomatic patients, in particular at the surface level (GSM 0); however, the difference showed only a trend toward statistical significance (P=0.07). Color mapping of the plaques also revealed that symptomatic patients, compared with the asymptomatic ones, presented an increased frequency of the red color predominance on the surface (11/14, 79% versus 8/17, 47% P=0.06). No difference was observed between the 2 groups of patients regarding the color distribution of the whole plaque (6 of 14 [43%] versus 7 of 17 [47%]; P=0.9) and also regarding a homogeneous versus a heterogeneous pattern.

Thin or interrupted fibrous caps were associated with lower GSM values, although without statistical significant difference (P=0.07 for the mean GSM 0 level and P=0.09 for the mean overall GSM measurement; Table). Plaques containing a necrotic core located in a juxtalumenal position also presented lower GSM values; however, in this case, the difference turned out to be highly significant for the GSM value at the surface level (GSM 0; P=0.009) as well as for the GSM value of the total plaque (P=0.013; Table). Furthermore, color mapping demonstrated a predominance of the red color (GSM <50) at the surface of the plaques containing a necrotic core located near the surface (P=0.0019) or a thin fibrous cap (P=0.056; Table). The sensitivity and specificity of the predominant color of the whole plaque with respect to the thickness of the fibrous cap and the position of the necrotic core were respectively 45% and 67% and 53% and 75%. When considering the predominant color of the surface, the sensitivity and specificity increased to 73% and 67% for the thickness of the fibrous cap and 84% and 75% for the position of the necrotic core, respectively.

The correlation between the different plaques or plaque segments and the stratified GSM values are shown in Figure 2. Plaques with large calcifications presented the highest GSM values at any level (from 49 to 75), and plaques with large hemorrhagic areas or with a predominant necrotic core exhibited the lowest ones, in particular, at the surface level, with a GSM value of 30 and 34, respectively. Predominantly, fibrotic plaques presented intermediate GSM values (from 42 to 53).

There was a very good agreement between the stratified GSM method and color mapping. The κ values for color mapping were 0.76 for the color of the whole plaque and 0.73 for the color of the surface.

Discussion

Several multicenter studies have demonstrated that carotid endarterectomy is an effective treatment decreasing the risk of subsequent stroke in symptomatic patients affected by carotid stenosis.[16–19] However, whereas the risk-benefit ratio is very much in favor of surgery for the ≥70% symptomatic stenosis, this is much less the case for the asymptomatic lesions. It has actually been estimated for this latter group of patients that ≈20 operations would have to be performed to prevent 1 stroke. Therefore, degree of stenosis alone may not be sufficient to evaluate the risk of stroke, and additional markers are needed to better characterize the patients who

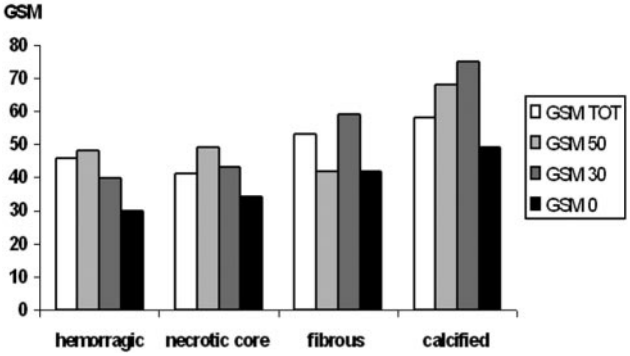

Figure 2. Stratified GSM values of the different histological plaque types of 31 carotid plaques or plaque segments: predominant fibrotic tissue n=12 (39%); large hemorrhagic areas n=6 (19%); large calcifications n=4 (13%); and large necrotic core n=22 (71%). TOT indicates total.

would benefit most from surgery. Recent trials demonstrated that anechogenic plaques are also associated with the occurrence of cerebrovascular symptoms[20] and that plaque morphology characterization may play an important role in the identification of subsets of high-risk patients. The echogenicity of the plaque was classified initially as bright (hyperechoic) or dark (hypoechoic).[21] To provide more objective descriptions, more detailed classifications were developed;[22,23] however, with a weak interobserver agreement and only little correlation with the histopathological findings.[22–30] An alternative approach was quantification of the echogenicity of the plaque by means of a computer-assisted analysis. As mentioned previously, the GSM is a global measure of total plaque brightness and, as such, does not reflect regional variations within the plaque.

Our study showed that a quantitative, stratified analysis of plaque echogenicity by means of GSM measurement provided a good correlation with histological findings (Figure 2) and also allowed identification of some characteristics suggesting plaque instability such as the thickness of the fibrous cap or the juxtalumenal position of the necrotic core. Plaques with a necrotic core located near the surface were identified correctly by GSM analysis, and a significant correlation was obtained with the GSM 0 level ($P=0.009$) as well as with the total plaque GSM measurement ($P=0.013$). Color mapping of the plaque also demonstrated a highly significant correlation between the predominant red color at the surface, corresponding to GSM values of <50 and the presence of determinants of unstable plaques. In contrast, no correlation was found between the predominant red color of the whole plaque and features of plaque instability, suggesting in this case the superiority of a GSM assessment restricted to the surface level compared with the overall GSM measurement (Table).

Stratified GSM analysis and color mapping are in fact 2 complementary methods of plaque analysis (Figure 1). In case of competition between 2 different colors, for example, which may be present either on the whole plaque or on the surface, predominance is finally decided on ground of the numeric values given by the stratified method. Furthermore, a precise delineation of the surface area (defined as the upper third of the plaque) may be sometimes difficult, and again, the numeric values corresponding to the levels 0 and 30 render this evaluation more reproducible. Finally, stratified analysis gives information of the GSM values at different levels but does not allow a visual representation of the distribution of these GSM variations within the plaque; this aspect is best shown with the color mapping method (Figure 1).

A wide range of threshold values, going from 32 to 74, are proposed in the literature to distinguish anechogenic from echogenic plaques by means of GSM measurement.[4,5,10,31] These various cut-off points may be attributable to different choices for the standardization of the reference values.[5,12] For the purpose of the present study, we decided to choose the threshold value of 50 by using the usual recommended reference values for the blood and the adventitia. However, we believe that other cut-off points should also be further investigated to determine the most sensitive threshold value.

Conclusions

Ultrasound analysis of the carotid plaque combining a stratified GSM measurement and color mapping showed a good correlation with the different histopathological plaque types and further allowed identification of determinants of plaque instability, with a sensitivity of 73% for the thickness of the fibrous cap and 84% for the juxtalumenal position of the necrotic core. Therefore, this combined approach should be investigated in a prospective, natural history study.

References

1. Golledge J, Greenhalgh RM, Davies AH. The symptomatic carotid plaque. *Stroke*. 2000;31:774–781.
2. Bassiouny HS, Davis H, Massawa N, Gewertz BL, Glagov S, Zarins CK. Critical carotid stenoses: morphologic and chemical similarity between symptomatic and asymptomatic plaques. *J Vasc Surg*. 1989;9:202–212.
3. Bassiouny HS, Sakaguchi Y, Mikucki SA, McKinsey JF, Piano G, Gewertz BL, Glagov S. Juxtalumenal location of plaque necrosis and neoformation in symptomatic carotid stenosis. *J Vasc Surg*. 1997;26: 585–594.
4. Gronholdt ML, Nordestgaard BG, Schroeder TV, Vorstrup S, Sillesen H. Ultrasonic echolucent carotid plaques predict future strokes. *Circulation*. 2001;104:68–73.
5. Elatrozy T, Nicolaides A, Tegos T, Zarka AZ, Griffin M, Sabetai M. The effect of B-mode ultrasonic image standardisation on the echodensity of symptomatic and asymptomatic carotid bifurcation plaques. *Int Angiol*. 1998;17:179–186.
6. Tegos TJ, Sohail M, Sabetai MM, Robless P, Akbar N, Pare G, Stansby G, Nicolaides AN. Echomorphologic and histopathologic characteristics of unstable carotid plaques. *AJNR Am J Neuroradiol*. 2000;21: 1937–1944.
7. Tegos TJ, Sabetai MM, Nicoloaides AN, Elatrozy TS, Dhanil S, Stevens JM. Patterns of brain computed tomography infarction and carotid plaque echogenicity. *J Vasc Surg*. 2001;33:334–339.
8. Sabetai MM, Tegos TJ, Clifford C, Dhanjil S, Belcaro G, Kakkos S, Kalodiki E, Stevens JM, Nicolaides AN. Carotid plaque echogenicity and types of silent CT-brain infarcts. Is there an association in patients with asymptomatic carotid stenosis? *Int Angiol*. 2001;20:51–57.
9. El-Barghouty N, Nicolaides AN, Tegos T, Geroulakos G. The relative effect of carotid plaque heterogeneity and echogenicity on ipsilateral cerebral infarction and symptoms of cerebrovascular disease. *Int Angiol*. 1996;15:300–306.
10. El-Barghouty N, Levine T, Ladva S, Flanagan A, Nicolaides A. Histological verification of computerized carotid plaque characterisation. *Eur J Vasc Endovasc Surg*. 1996;11:414–416.
11. Lal BK, Hobson RW II, Pappas PJ, Kubicka R, Hameed M, Jamil Z, Padberg FT Jr, Haser PB, Duran WN. Pixel distribution analysis of B-mode ultrasound scan images predicts histologic features of atherosclerotic carotid plaques. *J Vasc Surg*. 2002;35:1210–1217.
12. Matsagas MI, Vasdekis SN, Gugulakis AG, Lazaris A, Foteinou M, Sechas MN. Computer-assisted ultrasonographic analysis of carotid plaques in relation to cerebrovascular symptoms, cerebral infarction, and histology. *Ann Vasc Surg*. 2000;14:130–137.
13. El-Barghouty N, Geroulakos G, Nicolaides A, Androulakis A, Bahal V. Computer-assisted carotid plaque characteristaion. *Eur J Vasc Endovasc Surg*. 1995;9:389–393.
14. Sabetai MM, Tegos TJ, Nicolaides AN, Dhanjil S, Pare GJ, Stevens JM. Reproducibility of computer-quantified carotid plaque echogenicity: can we overcome the subjectivity? *Stroke*. 2000;13:2189–2196.
15. Lammie GA, Wardlaw J, Allan P, Ruckley CV, Peek R, Signorini DF. What pathological components indicate carotid atheroma activity and can these be identified reliably using ultrasound? *Eur J Ultrasound*. 2000;11: 77–86.
16. European Carotid Surgery Trialists' Collaborative Group. Randomised trial of endarterectomy for recently symptomatic stenosis: final results of MRC European Carotid Surgery Trail (ECST). *Lancet*. 1998;351: 1379–1387.
17. North American Symptomatic Carotid Endarterectomy Trial Collaborators. Beneficial effect of carotid endarterectomy in symptomatic patients with high-grade carotid stenosis. *N Eng J Med*. 1991;325: 445–453.

18. Barnett HJM, Taylor DW, Eliasziw M; North American Symptomatic Carotid Endarterectomy Trial Collaborators. Benefit of Carotid endarterectomy in patients with symptomatic moderate or severe stenosis. *N Eng J Med*. 1998;339:1415–1425.
19. Executive Committee of the Asymptomatic Carotid Atherosclerosis Study. Endarterectomy for asymptomatic carotid artery stenosis. *J Am Med Assoc*. 1995;273:1421–1428.
20. Mathiesen EB, Bonaa KH, Joakimsen O. Echolucent plaques are associated with high risk of ischemic cerebrovascular events in carotid stenosis: the Tromso Study. *Circulation*. 2001;103:2171–2175.
21. Reilly LM, Lusby RJ, Hugues L, Ferrell LD, Stoney RJ, Ehrenfeld WK. Carotid plaque histology using real-time ultrasonography: clinical and therapeutic implications. *Am J Surg*. 1997;113:1352–1358.
22. Johnson JM, Kennely M, Decesale D, Morgan S, Sparrow S. Natural history of asymptomatic plaque. *Arch Surg*. 1985;120:1010–1012.
23. Gray-Weale AC, Graham JC, Burnett JR, Byrne K, Lusby RJ. Carotid artery atheroma: comparison of preoperative B-mode ultrasound appearance with carotid endarterectomy specimen pathology. *J Cardiovasc Surg*. 1988;29:676–681.
24. Widder B, Paulat K, Hachspacher J. Morphological characterization of carotid artery stenoses by ultrasound duplex scanning. *Ultrasound Med Biol*. 1990;16:349–354.
25. Geroulakos G, Ramaswami G, Nicolaides A. Characterisation of symptomatic and asymptomatic carotid plaques using high resolution real time ultrasound. *Br J Surg*. 1993;80:1274–1277.
26. Arnold JA, Modaresi KB, Thomas N, Taylor PR, Padayachee TS. Carotid plaque characterization by duplex scanning: observer error may undermine current clinical trials. *Stroke*. 1999;30:61–65.
27. Joakimsen O, Bonaa KH, Stensland-Bugge E. Reproducibility of ultrasound assessment of carotid plaque occurrence, thickness, and morphology. The Tromso Study. *Stroke*. 1997;28:2201–2007.
28. Mayor I, Momjian S, Lalive P, Sztajzel R. Carotid plaque: comparison between visual and grey-scale median analysis. *Ultrasound Med Biol*. 29:961–966.
29. Montauban van Swijndregt AD, Elbers HR, Moll FL, de Letter J, Ackerstaff RG. Ultrasonographic characterization of carotid plaques. *Ultrasound Med Biol*. 1998;24:489–493.
30. Hartmann A, Mohr JP, Thompson JL, Ramos O, Mast H. Interrater reliability of plaque morphology classification in patients with severe carotid artery stenosis. *Acta Neurol Scand*. 1999;99:61–66.
31. Biasi GM, Sampaolo A, Mingazzini P, De Amicis P, El-Barghouty N, Nicolaides AN. Computer analysis of ultrasonic plaque echolucency in identifying high risk carotid bifurcation lesions. *Eur J Vasc Endovasc Surg*. 1999;17:476–479.