Reproducibility of Computer-Quantified Carotid Plaque Echogenicity: Can We Overcome the Subjectivity?

M.M. Sabetai, MD; T.J. Tegos, MD; A.N. Nicolaides, FRCS; S. Dhanjl, RVT; G.J. Pare, MD; J.M. Stevens, MD

Background and Purpose—We sought to assess the reproducibility, interobserver variability, and application to clinical studies of a new method for the quantitative assessment of carotid plaque echogenicity.

Methods—Carotid plaques were scanned with the use of ultrasound, and their images were stored in a computer. They were normalized by assigning certain gray values to blood and adventitia, and the gray scale median (GSM) was used to quantify their echogenicity. The variability between storage media, between degrees of magnification, and between probes was assessed. The method was applied to 232 asymptomatic carotid plaques causing 60% to 99% stenosis in relation to the presence of ipsilateral CT-demonstrated brain infarcts. In all parts of the study the plaque GSM was measured before and after normalization to evaluate its effect. Interobserver agreement for the scanning process was assessed.

Results—The GSM mean difference before and after normalization for variability studies of storage media, degrees of magnification, and probes was \(2\) and \(0.12\), \(2.24\) and \(1.68\), and \(8.3\) and \(0.7\), respectively. The median GSM of plaques associated with ipsilateral nonlacunar silent CT-demonstrated brain infarcts was 14, and that of plaques that were not so associated was 30 (\(P=0.003\)). The interobserver GSM difference was \(20.05\) (95% CI, \(-1.7\) to 1.6).

Conclusions—Our method decreases the variability between storage media and between probes but not the variability between degrees of magnification. It separates echomorphologically the carotid plaques associated with silent nonlacunar CT-demonstrated brain infarcts from plaques that are not so associated. (Stroke. 2000;31:2189-2196.)

Key Words: carotid stenosis ■ echogenicity ■ reproducibility ■ ultrasonography

Carotid plaque characterization by means of high-resolution ultrasound is used in clinical trials currently in progress.\(^{1,2}\) Different classifications have been used thus far that are subjective and qualitative.\(^{3-7}\) Several investigators have demonstrated the poor to moderate reproducibility of these classifications and emphasized the need for a standardized and quantitative method of assessment.\(^{6,8-11}\) It is suggested that such a method may strengthen the association between carotid plaque echogenicity and known risk factors for ischemic stroke and ultimately the incidence of ischemic stroke.\(^{8,12}\) Our group has developed a method for the objective and quantitative assessment of ultrasonic carotid plaque features.\(^{13}\) Computer-assisted carotid plaque characterization with B-mode image normalization aims at improving the comparability of such features. Initial experience has shown that by using this method it is possible to identify and quantify differences in the echogenicity of carotid plaques related to symptomatic and asymptomatic status and to different symptomatology (retinal and hemispheric).\(^{13,15}\)

Initial reproducibility studies performed by our group have shown good interobserver reproducibility in normalizing carotid plaque B-mode images (4 observers normalized images obtained using 2 different scanners)\(^{13}\) and improved comparability of carotid plaque echogenicity after normalization when different gains and different scanners were used.\(^{15}\)

The aim of the first part of this study was to assess the effect of B-mode image normalization on the comparability of carotid plaque echogenicity when the following were used: (1) 2 of the most commonly used storage media (super-VHS videotape and magneto-optical disk [MOD]); (2) 2 different degrees of magnification (zoom and high-definition zoom [HD zoom]); and (3) 2 different linear array probes (7 and 10 MHz).

The interobserver agreement in scanning a carotid plaque, resulting in an image suitable for normalization by following specific criteria, and the intraobserver agreement for normalizing images were evaluated.
The aim of the second part of the study was to examine the effect of B-mode image normalization on a possible association between carotid plaque computer-quantified echogenicity and the presence of ipsilateral nonlacunar silent CT-demonstrated brain infarcts in patients with significant asymptomatic carotid stenosis.

Subjects and Methods

Subjects
In the first part of the study, 19 patients with 26 carotid bifurcation plaques causing 50% to 99% diameter reduction were entered into the variability studies of storage media and degrees of magnification. To assess the variability when 2 different linear array probes were used, another sample of 25 patients with 30 carotid bifurcation plaques causing 50% to 99% diameter reduction was studied.

In the second part of the study, 186 patients (117 men and 69 women) with 232 asymptomatic carotid plaques causing 60% to 99% stenosis were studied with duplex scanning. The 60% cutoff point as an inclusion criterion was based on the results of the Asymptomatic Carotid Atherosclerosis Study (ACAS).16 In each patient the presence of such a plaque defined the side of interest (unit of the study). These patients were referred to our vascular laboratory for the evaluation of carotid stenosis in the presence of peripheral vascular disease, coronary artery disease, carotid bruit, or relevant risk factors. Patients with atrial fibrillation, valvular lesions, recent myocardial infarction, or severe congestive heart failure were excluded to eliminate cardioembolism as the cause of the cerebrovascular pathology. The cardiac diagnosis was based on clinical and ECG findings alone. No transcutaneous or transesophageal echocardiography was performed. All patients were examined by a neuroradiologist (T.J.T.) and underwent a brain CT scan, which was reported by an independent neuroradiologist (J.M.S.).

Grading of Carotid Stenosis
The degree of stenosis was calculated according to criteria established at an international consensus meeting on grading carotid stenosis.17 We measured the peak systolic velocity of the internal carotid artery18 and the ratio of the peak systolic velocity of the internal carotid artery to that of the common carotid artery,19,20 which has been validated by others. The ratio of the peak systolic velocity of the internal carotid artery to the end-diastolic velocity of the common carotid artery21,22 has been validated in our vascular laboratory with angiographic stenosis in relation to the distal internal carotid artery23,24 and applied previously.13,15

Carotid Duplex Settings
According to the recommendations of the international consensus concerning ultrasonic morphology and the stroke risk of carotid plaques,25 the choice of equipment, settings, and interobserver reproducibility should be considered.

Ultrasound scanning was performed with the ATL HDI 3000 scanner (Advanced Technologies Laboratories), and the following settings were selected: (1) maximum dynamic range (60 dB); (2) a 7-MHz linear array multifrequency probe (for the probes reproducibility study, the 7- and 10-MHz linear array probes were used); (3) gain setting was not set so high that the structural details of the far wall media-adventitia interface were concealed by introducing a great deal of noise in the vessel lumen (blood) and was not set so low (3) gain setting was not set so high that the structural details of the far wall media-adventitia interface were concealed by introducing a great deal of noise in the vessel lumen (blood) and was not set so low that the far wall intima-media interface could not be easily identifiable. This also ensured the availability of the 2 reference structures for image normalization: noiseless vessel lumen and an echo-dense area of adventitia in the vicinity of the plaque; (4) time gain compensation curve gently sloping but vertical through the lumen of the vessel. This ensured similar average gray scale levels of the tissues lying superficial and deep in relation to the artery and areas of anterior and posterior wall adventitia of nearly identical gray scale levels. The latter is essential for normalization of carotid plaque images with anterior and posterior components.

In addition, a linear postprocessing curve was selected because image normalization is achieved with linear scaling.

Carotid Plaque Recording Protocol
With the patient in the supine position, the carotid bifurcation was imaged in longitudinal (anterolateral, lateral, and posterolateral) and transverse projections. The transducer was kept parallel to the vessel so that the near and far wall adventitia was imaged at right angles. The aim was to obtain a carotid plaque B-mode image with a relatively noiseless vessel lumen, with an echo-dense area of adventitia in the vicinity of the plaque and with an echochogenic informative plaque that can be outlined easily. The projection that fulfilled these criteria was chosen. In the case of heterogeneous plaques (ie, those containing hypoechoic and hyperechoic areas), the projection that fulfilled the above criteria and provided the highest echogenicity was selected. Plaques included in an acoustic shadow were analyzed only if >50% of their area depicted real acoustic information, and only this area was subjected to analysis (the number of pixels in the area of interest was at least half of the total number of pixels in the area of the plaque, as calculated by the Adobe Photoshop software). Plaques for which we did not have any B-mode information because of acoustic shadowing were excluded from the study.

In the storage media reproducibility study, the most echochogenic informative longitudinal projection of the plaque was stored on videotape (Konica sVHS) and simultaneously digitized onto a MOD. The corresponding color Doppler plaque image was recorded on videotape and stored on a MOD to assist with the off-line delineation of the luminal plaque border. During the same scanning session, the same image was magnified ×1 and then ×3 with the HD zoom facility of the scanner. Both images were directly digitized onto a MOD. The corresponding color Doppler image was also digitized and stored onto a MOD. In the probes reproducibility study, the carotid plaque was imaged through the same longitudinal projection with the 2 linear array probes (7 and 10 MHz), and the B-mode and corresponding color Doppler images were directly digitized onto a MOD. All scans were done by 1 experienced operator (T.J.T.).

In the second part of the study, the aforementioned duplex settings and carotid plaque recording protocol were followed. All scans were performed by 2 experienced operators (T.J.T., S.D.) using the 7-MHz linear array probe and adjusting the gain and the degree of magnification according to their perception of the optimum image. Carotid plaque images were stored on super-VHS videotape and subsequently digitized to a personal computer with the use of a commercially available video grabber card or directly stored onto a MOD in digital form.

B-Mode Image Normalization
This method has been previously described.13 In brief, B-mode images are digitized and transferred to a personal computer. With the use of the software Adobe Photoshop (version 3.0 or later) and the “histogram” facility, the gray scale median (GSM) of the 2 reference points (blood and adventitia) in the original B-mode image is defined. Algebraic (linear) scaling of the image is performed with the “curves” option of the software so that in the resultant image the GSM of blood equals 0 to 5 and that of the adventitia equals 185 to 195. In this way, the gray scale values of all the pixels in the image are adjusted according to the input and output values of the 2 reference points (blood: input value = measured GSM before linear scaling; output value = 0 to 5; adventitia: input value = measured GSM before linear scaling; output value = 185 to 195) (Figure 1). The GSM of the plaque (the median of the frequency distribution of the gray levels of the pixels in the plaque) in the normalized image (adjusted image using linear scaling) is used to quantify its echogenicity (Figure 2).

CT-Demonstrated Brain Infarct Classification
Upon recruitment of the patients into the study, a CT brain scan was performed, and the presence of ischemic lesions in the middle and...
anterior cerebral artery territory was noted. Intracerebral hemorrhages and posterior circulation infarcts as well as ischemic lesions in sides with carotid bifurcation plaques causing <60% stenosis were not considered in this analysis.

The appropriate sides with CT-demonstrated brain infarctions were classified into group A (nonlacunar CT-demonstrated infarctions, cardioembolism excluded, mainly related to extracranial carotid atheroembolism), group B (lacunar CT-demonstrated infarctions and diffuse white matter low density changes, related to cerebral small-vessel disease), and normal CT scan. When both nonlacunar and lacunar CT-demonstrated brain lesions were present, the infarcts were classified as nonlacunar lesions. All CT brain scans were reported by an experienced neuroradiologist (J.M.S.) who was unaware of patients’ clinical characteristics.

Interobserver and Intraobserver Agreement

The application of the aforementioned settings should result in the optimum B-mode image for normalization, ie, one with a relatively noiseless vessel lumen, with an echo-dense area of adventitia in the vicinity of the plaque, and with an echoically informative plaque that can easily be outlined.

Fifty-two consecutive patients with 52 asymptomatic carotid bifurcation plaques were scanned during the same scanning session by 2 operators (S.D., M.M.S.) with varying degrees of experience. The 2 operators were unaware of each other’s carotid plaque scanning and the images obtained. Both were instructed to follow the carotid plaque scanning protocol described previously. B-mode and color Doppler images were stored onto a MOD and normalized off-line by 1 operator (M.M.S.) at a later time. In addition, 26 of the 52 B-mode images were normalized at 2 different times within a 6-month period by 1 operator (M.M.S.). The interobserver limits of agreement for following the carotid plaque scanning protocol and the intraobserver limits of agreement for normalizing images were evaluated according to the method proposed by Bland and Altman.

Statistical Analysis

The distribution of all variables was tested for normality with the Levene test for the homogeneity of variances. Normally distributed variables were expressed as mean and 95% CIs. To test whether the mean GSM differences were the same before and after image normalization, the paired-samples t test was used. The median and interquartile range (IQR) were used to describe the non–normally distributed variables. The nonparametric Wilcoxon Mann-Whitney test was used to compare the distributions between these variables.

To assess interobserver variability, the average of the paired measurements was plotted against their difference to examine whether the differences change in proportion to the corresponding average values. If the differences are relatively constant and normally distributed, 95% of these differences will lie between $\pm 2\text{SD}$ of the mean difference. This range will be referred as the interobserver “limits of agreement.” The frequency distribution of the average of the paired measurements of the GSM for each plaque was divided in quartiles, and the interobserver limits of agreement for each quartile were assessed.

To test the hypothesis that plaque echogenicity and/or the degree of stenosis (predictor variables) can predict the presence of nonlacunar or lacunar ipsilateral silent CT-demonstrated brain infarcts (dependent variable), logistic regression analysis was used. These calculations were performed with the statistical package SPSS for Windows (version 9.0). P values of $\leq 0.05$ were considered statistically significant.

Results

Table 1 shows the mean GSM difference of the plaques before and after normalization in the variability studies of storage media, degrees of magnification, and probes.

The prevalence of silent CT-demonstrated brain infarcts in group A was 27 of 232 (11.6%), and that in group B and the normal CT group was 205 of 232 (88.4%). In group A,
CT-demonstrated brain infarcts were identified in 6 of 46 patients (13%) with bilateral asymptomatic carotid stenosis (8/92 sides of interest). No hemorrhagic or watershed infarcts were identified in the hemispheres of interest in this group of patients. The median (IQR) GSM of plaques associated with (1) group A CT-demonstrated infarcts and (2) group B and normal CT scans before normalization was 11 (1 to 33) and 21 (9.5 to 32) ($P=0.165$) and after normalization was 14 (0 to

Figure 2. Hypoechoic plaque (GSM=9) with the histogram of the gray tone frequency distribution of the pixels in the selected area (plaque) of the normalized image. The colored image is used as a guide to outline the area of interest.
Table 1. Mean GSM Difference Before and After Normalization in the Three Parts of the Variability Study

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<th>Mean (95% CI) GSM Difference</th>
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<tr>
<td></td>
<td>Before Normalization</td>
<td>After Normalization</td>
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<tr>
<td>Super-VHS/MOD (n=26)</td>
<td>$-14.5$ ($-16.7$ to $-12.2$)</td>
<td>$-0.12$ ($-1.8$ to $1.6$)</td>
<td>$&lt;0.001$</td>
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<td>Zoom/HD zoom (n=26)</td>
<td>$2.24$ ($-0.75$ to $5.23$)</td>
<td>$1.68$ ($-2.37$ to $5.74$)</td>
<td>$0.59$</td>
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<tr>
<td>Linear probes (n=30)</td>
<td>$-8.3$ ($-12.3$ to $-4.5$)</td>
<td>$-0.7$ ($-2.4$ to $0.87$)</td>
<td>$&lt;0.001$</td>
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*Paired-samples t test.

26 (14 to 49), respectively ($P=0.003$, Wilcoxon Mann-Whitney test). Figure 3 illustrates the corresponding box and whiskers plots. The median (IQR) degree of stenosis of those associated with (1) group A CT-demonstrated infarcts and (2) group B and normal CT scans was 80% (65% to 90%) in both groups ($P=0.61$, Wilcoxon Mann-Whitney test). Logistic regression analysis revealed that plaque echogenicity after image normalization ($P=0.008$) and not the degree of stenosis ($P=0.82$) predicted the presence of group A CT-demonstrated brain infarcts as contrasted with the presence of group B and normal CT scans.

The mean GSM of the plaques entered into the interobserver and intraobserver reproducibility study was 26 (range, 0 to 79) and 16 (range, 0 to 54), respectively, which is a representative sample ranging from hypoechogenic to hyperechogenic plaques. Table 2 shows the interobserver mean difference (95% CI) and the limits of agreement for the full range and each quartile of average GSM. The interobserver mean difference was 1.7 ($0.5$ to $3.8$) and the limits of agreement $\pm 10.2$. Figure 4 shows the scatterplot of the mean GSM against the GSM difference for each plaque between the 2 observers. The limits of agreement are defined.

Discussion

An important consensus concerning the morphology and the stroke risk of carotid plaques emphasized the subjectivity of the existing classifications, the importance of reproducibility studies on carotid plaque echomorphology, and the need for uniform terminology. Computer-quantified echogenicity by means of GSM seeks to overcome the subjectivity but is subject to variability when different settings are used such as gain, storage media, linear array transducers, and display settings. Thus, algebraic (linear) scaling and 2 reference echo structures were used to adjust the brightness and contrast of the whole B-mode image to the same level before any GSM measurements.

Although there is agreement by most investigators regarding use of the vessel lumen as the reference structure for hypoechogenicity, there is no consensus on which one should be used for hyperechogenicity. The consensus report has suggested use of the mastoid muscle and the body of the cervical vertebra for isoechogenicity and hyperechogenicity, respectively. In agreement with others, we believe that the 2 reference structures should be vessel lumen for hypoechogenicity and the adjacent adventitia for hyperechogenicity. They are close to the plaque and thus easier to be included in the same projection with the most echogenic informative plaque and probably prone to lower interobserver variability. In addition, linear scaling is achieved between 2 points (the GSM of the 2 reference echo structures).

The Tromsø Study reported good intraobserver and interobserver (2 observers) agreement ($\kappa=0.73$ and $\kappa=0.69$, respectively) based on the Gray-Weale classification (type 1 to type 4 plaques, where type 1 denotes low and type 4 high echogenicity). They used 2 reference structures (vessel lumen and adventitia) and concluded that standardization of plaque echogenicity against well-defined reference structures is very important since lack of such structures is the major source of error. Two other studies reported good interobserver agreement ($\kappa=0.79$ and $\kappa=0.61$, respectively) although they are not directly comparable because they used different classifications, they did not use reference structures to compare plaque echogenicity, and 1 of them is based on B-mode images only.

The study by deBray et al reported the interobserver (9 observers) agreement on carotid plaque echogenicity for images collected in 4 different centers. They used the classification proposed by Geroulakos et al and 2 reference structures: the vessel lumen for hypoechogenicity and the intima-media complex for isoechogenicity. They reported fair interobserver agreement ($\kappa=0.31\pm0.14$) due to inadequate reference structure for hyperechogenicity and the subjective assessment of carotid plaque echogenicity. Similar results...
were obtained by Montauban van Swijndregt et al.\textsuperscript{11} \((\kappa = 0.38)\) using a modified Gray-Weale classification and the vessel lumen and adjacent adventitia as the echo structures of reference.

Arnold et al.\textsuperscript{10} using the 4-category Gray-Weale classification, reported fair to moderate interobserver (4 observers) agreement with fair agreement for the images stored in digital form and moderate for those stored on a hard copy (printed on paper). When the 2-category classification (homogeneous or heterogeneous) proposed by Reilly et al.\textsuperscript{3} was used, the agreement was moderate to good. The reference structures used were the vessel lumen and the adjacent adventitia or soft tissues. Furthermore, in a different study from the same group, it was reported that carotid plaques appearing hypoechoic using a transcutaneous 7-MHz probe may appear hyperechoic when they are scanned intraoperatively with a 10-MHz probe.\textsuperscript{43} They concluded that the difficulty in reproducing high levels of agreement previously published was due to the different and subjective classifications that exist, the variability in the storage and display media used, the variability in the image quality, and the statistics used.

In our study we have shown that the significant difference that exists in plaque echogenicity when the images are stored in super-VHS and MOD is eliminated after image normalization. B-mode images stored on videotape are subjected to compression and appear darker when reviewed off-line. This is more pronounced for images printed on paper.\textsuperscript{10} On the other hand, those stored on MOD, because they are not transformed from digital into another form, undergo the least possible compression and have the best achievable resolution, thus appearing brighter.

Significant differences in ultrasonic plaque character exist when different linear array probes are used, as shown in our study and studies by others.\textsuperscript{43} The better spatial resolution achieved when a higher-frequency probe is used, especially when superficial structures are scanned (such as carotid plaques), results in an image with lower contrast but more gray scale information. This may introduce significant variability in the assessment of plaque echogenicity, which is eliminated with B-mode image normalization because of the adjustment of contrast and brightness to predetermined GSM values of the reference echo structures.

Plaque echogenicity was the same before and after image normalization when 2 degrees of magnification (including HD zoom) were used. Excessive magnification (not used in this reproducibility study) may reduce the possibility of having available an echodense area of adventitia near the plaque and should be avoided.

Quantification by means of GSM allows us to use simple statistics to assess variability and to define easily the limits of experimental error. Additionally, we have shown that the carotid plaque recording protocol, which leads to images suitable for normalization, can be followed with high interobserver agreement. These findings may have significant implications in (1) reducing the variability of plaque characterization and (2) quantifying true changes (those outside the limits of experimental error) of plaque echogenicity in prospective multicenter studies.

The clinical usefulness of the method is shown in the association between asymptomatic carotid plaque echogenicity and the presence of nonlacunar CT-demonstrated brain infarcts, which becomes apparent only after image normalization. Although earlier studies by our group\textsuperscript{44} and others\textsuperscript{40} have shown an association between hypoechoic plaques by means of low GSM and CT-demonstrated brain infarcts, their results were based on a mixed group of symptomatic and asymptomatic plaques, the types of infarcts were not clearly defined, and the mean GSM values varied significantly because no normalization of B-mode images was applied.

The prevalence of 11.6% of nonlacunar silent CT-demonstrated brain infarcts in our study is similar to that reported by the ACAS trial (13%),\textsuperscript{16} although 2 other studies

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<th>Mean Interobserver GSM</th>
<th>Mean Difference (95% CI)</th>
<th>Limits of Agreement ±2 SD</th>
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<tr>
<td>0 to 79 (full range)</td>
<td>-0.05 (-1.7 to 1.6)</td>
<td>±12</td>
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<tr>
<td>0 to 7</td>
<td>0.78 (-0.4 to 1.96)</td>
<td>±4</td>
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<tr>
<td>8 to 20</td>
<td>-3 (-6.8 to 0.8)</td>
<td>±12</td>
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<tr>
<td>21 to 35</td>
<td>0.34 (-4.9 to 5.6)</td>
<td>±16</td>
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<tr>
<td>&gt;35</td>
<td>1.3 (-2.3 to 4.9)</td>
<td>±13</td>
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Figure 4. Scatterplot of the average GSM against the GSM difference for each plaque between the 2 observers with limits of agreement (dashed lines).
have reported a prevalence of 19% and 28%,5,46 The comparison is difficult, however, because of lack of uniformity in the classification of CT-demonstrated brain infarcts.

Hypoechoic plaques have been associated with the development of neurological events in several studies of asymptomatic carotid stenosis,4,8,47–49 systemic risk factors for atherosclerosis,39,42 and a tendency for higher embolic counts on transcranial Doppler.50 In addition, increased embolic counts on transcranial Doppler have been associated with a higher prevalence of nonlacunar CT-demonstrated brain infarcts.50 Because of the association of asymptomatic hypoechoic plaques with nonlacunar silent CT-demonstrated brain infarcts shown in this study, it is tempting to implicate carotid atheroembolism as the pathogenetic factor for these types of infarcts. On the other hand, although the presence of CT-demonstrated brain infarcts may predict the development of ischemic stroke in patients presenting with transient ischemic attacks51,52 and is associated with significantly worse prognosis after carotid endarterectomy,34,53,54 their role as a risk factor for atheroembolic stroke in patients with asymptomatic carotid stenosis is not certain and remains to be proven.

The limitations of our study were the lack of vascular imaging to detect carotid siphon or middle cerebral artery stenosis and the lack of assessment of the cerebral collateral circulation by means of transcranial Doppler. In addition, cardioembolism was excluded on the basis of medical history and electrocardiography alone.

In conclusion, we have shown that (1) it is possible to quantify carotid plaque echogenicity and to overcome the subjectivity of the existing classifications by using a simple computer-assisted method, (2) this method decreases the variability introduced when different storage media and different linear array probes are used, and (3) asymptomatic hypoechoic carotid plaques are associated with particular types of silent CT-demonstrated brain infarcts, possibly through an atheroembolic mechanism, in a cross-sectional study. This method can be applied in prospective clinical studies that seek to assess the association of carotid plaque echogenicity with other established risk factors for atherosclerosis and ischemic stroke.2

Acknowledgments

This study was supported in part by the CDER Trust UK and the Alexander S. Onassis Public Benefit Foundation. Dr Sabetai is the recipient of an Alexander Onassis fellowship.

References


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Stroke. 2000;31:2189-2196
doi: 10.1161/01.STR.31.9.2189
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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