B-mode ultrasonographic characterization of carotid atherosclerotic plaques in symptomatic and asymptomatic patients

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Objective: To identify features on B-mode ultrasonography (US) prevalent in symptomatic plaques and correlate these findings with histopathologic markers of plaque instability.

Method: Carotid endarterectomy (CEA) plaques from symptomatic and asymptomatic patients with critical stenoses (>70%) were qualitatively assessed using preoperative B-mode US for echolucency and calcific acoustic shadowing. US echolucency was quantitated ex vivo using computerized techniques for gray-scale median (GSM) analysis. Histopathologic correlates for US plaque echolucency (percentage of necrotic core area) and acoustic shadowing (percentage of calcification area) were determined.

Results: Fifty CEA plaques were collected from 48 patients (46 unilateral and two bilateral); 26 of these plaques were from symptomatic patients. Age, degree of stenosis, and atherosclerotic risk factors were similar for the symptomatic and asymptomatic patients. Using preoperative B-mode US, 58%, 35%, and 7% of symptomatic plaques and 18%, 41%, and 41% of asymptomatic plaques were found to be echolucent, echogenic, and calcific, respectively (P < .05). Using ex-vivo B-mode US and GSM analysis, symptomatic plaques were more echolucent (41 ± 19) than asymptomatic plaques (60 ± 13), P < .03. A strong inverse correlation was found between the percent plaque necrotic area core and GSM (R = −0.9, P < .001). Percentage of calcification area in plaques with acoustic shadowing was 66% and only 27% in those without acoustic shadowing (P < .05).

Conclusions: Using B-mode US, symptomatic plaques are more echolucent and less calcified than asymptomatic plaques and are associated with a greater degree of histopathologic plaque necrosis. Such features are indicative of plaque instability and should be considered in the decision-making algorithm when selecting patients with high-grade asymptomatic carotid stenosis for intervention. (J Vasc Surg 2005;42:435-41.)

Currently, operative or endovascular intervention for carotid bifurcation disease is largely predicated on the severity of internal carotid artery (ICA) arteriographic stenosis in both symptomatic and asymptomatic patients.1-3 However, most patients with high-grade asymptomatic ICA stenoses will remain stroke free on long-term follow-up.3,4 In this patient cohort with >60% ICA stenosis, the 5-year risk of disabling stroke without any premonitory symptoms is 11% to 12%.5-7 These findings indicate that for a given critical stenosis, other variables such as plaque structural morphology and instability play an important role in the natural history of carotid occlusive disease. Previously, we have shown that unstable and disrupted plaques exhibit increased lipid concentration and plaque necrotic core formation in association with macrophage/foam cell infiltration in the ablumenal fibrous cap.8,9 We have also demonstrated that the degree of plaque calcification is associated with plaque stability and a lower risk of clinical ischemic events.10

Studies correlating such carotid plaque histopathologic characteristics with B-mode ultrasonography (US) suggest that the degree of plaque echolucency is attributed to plaque necrosis or hemorrhage.11-16 Steffen et al16 and others17-21 have demonstrated that for all degrees of hemodynamically significant stenoses, patients with echolucent carotid plaques are at increased risk of symptomatic thromboembolic ischemic cerebrovascular events and cerebral infarction on computed tomography (CT). Computer-assisted plaque characterization using standardized B-mode US imaging and digital post-processing now allows the quantitative evaluation of differences in plaque echolucency between symptomatic and asymptomatic plaques using a gray-scale median (GSM) score.18,20,22,23 To further define the role of B-mode US in characterizing unstable carotid bifurcation plaques, we studied symptomatic and asymptomatic plaques with similar degrees of stenosis to determine differences in the prevalence of echolucency, echodensity, and acoustic shadowing. After carotid endarterectomy (CEA), GSM scores were calculated from cross-sectional images of the excised plaques and correlated with histomorphometric measures of plaque necrosis. Additionally, the presence of preoperative B-mode acoustic...
shadowing was related to the degree of carotid plaque calcification.

METHODS

Patient population and CEA plaques. Fifty carotid bifurcation plaques were collected from 48 consecutive patients who underwent CEA for high-grade ICA stenosis. These plaques were also studied in a previous study that reported the degree of calcification and plaque inflammation in relation to symptomatic outcome. Risk factors for atherosclerotic disease, including tobacco use, hypertension, diabetes mellitus, coronary artery disease, peripheral artery disease, and hypercholesterolemia, were recorded for all patients. Indications for CEA were transient ischemic attack (16 plaques), stroke (six plaques), and amaurosis fugax (four plaques) within 6 months of CEA, and high-grade asymptomatic carotid stenosis (24 plaques). Plaques were endarterectomized using a standard semi-eversion technique that allowed removal of the intact specimen with preservation of plaque structural integrity and minimized the possible disruption of the plaque luminal surface.

Preoperative color duplex US (CDU). Preoperative noninvasive evaluation of bilateral carotid arteries was performed for every patient using an ATL HDI 3000, ATL HDI 5000 (Advanced Technology Laboratories, Bothell, Wash) or Acuson Sequoia 512 (Acuson Corp., Mountain View, Calif) with a linear array 4-7 MHz transducer. All scans were performed by one experienced registered vascular technologist (R.V.T.). The study included longitudinal and transverse views. Also included were pulsed Doppler waveform samples of the subclavian, vertebral, and external carotid arteries; two samples of the common carotid artery (CCA) (mid and distal), and three samples of the ICA (proximal, mid and distal). All Doppler waveform samples were recorded from the midstream of the vessel being interrogated. The angle of insonation was kept at 60 degrees. Color flow mapping was additionally employed to identify any suspect regions of hemodynamic disturbance. Specific flow parameters calculated included peak systolic velocity (PSV), end diastolic velocity (EDV), the ratio of PSV in the ICA and CCA (ICA/CCA ratio), and the presence of spectral broadening or turbulence due to stenosis. Gray-scale (B-mode) imaging was used as an adjunct tool to detect the presence, location, extent of disease, and the degree of stenosis when present. Plaque morphologic characteristics were subjectively evaluated, and each plaque was graded as predominantly (>50% area of plaque images) as echolucent = hypoechogenic or echodense = hyperechoic (Fig 1A and B). The presence of acoustic shadowing and interference with pulsed Doppler sampling was indicative of a calcific plaque (Fig 1C) intraobserver reproducibility of plaque morphology by B-mode US was assessed, with acceptable results (κ = 0.83). Strandness velocity criteria were used for the diagnosis and grading of carotid disease in this study. Additionally, the degree of stenosis based on B-mode image measurements was recorded.

Ex vivo B-mode US. Ex vivo B-mode US imaging of intact endarterectomy plaques was performed to quantitate plaque echodensity. Carotid bifurcation plaques were embedded in a gelatin basin with a 5-mm interval echogenic floor grid (Fig 2A). B-mode US scans were performed using an Acuson Sequoia 512 ultrasound scanner with a 10-MHz foot probe. B-mode scan settings were standardized using a dynamic range to a linear post-processing curve and a high frame rate and persistence. The probe was adjusted so that the ultrasonic beam was at a right angle to the surface of the plaque. Images were magnified using the high-definition zoom feature and the time gain compensation (TGC) curve, and overall gain was adjusted to achieve an optimum image of the near and far walls. All settings were saved as a preset in the US machine for use with each examination. All B-mode US scans were performed by R.V.T. Each plaque was subjected to cross-sectional B-mode imaging at 2- to 3-mm intervals. Images at each interval were captured and saved to a digital file on a magneto-optical disk (MOD). An average of 12 images (range 8-20) was obtained from each plaque for further quantitative echodensity analysis (Fig 2B).

Computer-assisted analysis of plaque echodensity. Plaque echodensity was measured in each plaque image and then averaged to calculate the mean plaque echodensity. Using commercially available software (Adobe Photoshop
3.0), the GSM in the noncalcific regions of the plaque was used as an objective measure of echodensity, with lower values corresponding to echolucency and higher values corresponding to echodensity. The following protocol for image standardization was used:

1. Color information was discarded.
2. GSM measurement of the plaque lumen and echodense area of plaque was performed.
3. GSM measurement of the entire plaque using a temporary logarithmic scaling was done.
4. Image standardization using algebraic scaling of the whole image using the “curve” facility of the software was performed. The gray scale was adjusted so that the image of the plaque lumen would be in the 0-5 range and the echodense region would be in the 185-195 range. Thus, the gray values of all pixels would change according to the new linear scale defined by the reference values for plaque lumen and echodense region.
5. Measurement of GSM of the noncalcific regions of the plaque using the standardized scale (Fig 3).

Histopathologic/histomorphometric analysis. Plaques were fixed and sectioned transversely into 3-mm blocks (4-7 blocks/plaque). The blocks were embedded in paraffin and sectioned at 5-μm intervals. Sections from each block were stained with hematoxylin and eosin (H & E), and the presence of atheroma, necrotic lipid core, calcification, fibrous cap integrity, and ulceration was determined in each section. Computer-assisted morphometric techniques were used to quantify the percentage of area of calcification, percentage of area of necrotic core, and shortest distance of the necrotic core to the lumen for each section (Fig 4).

Statistical analysis. For continuous demographic variables, results were expressed as mean ± SD, and t test were used for group comparison. For categorical demographic variables, χ² tests were used for group comparison. When multiple measurements were made of a particular parameter in a given plaque, the mean value of those

Fig 2. Intact endarterectomized plaques were embedded in gelatin with a 5-mm echogenic scale (A). The ex vivo plaques were then subjected to B-mode ultrasonography. Serial cross-sectional views were obtained at 2- to 3-mm intervals, with an average of 12 sections/plaque (range 8-20). Images were then stored digitally on a magneto-optical disk (B).
measurements was used in the statistical analysis. Differences were considered significant at the $P < .05$ level.

To classify asymptomatic and symptomatic patients on the basis of the percentage of area necrotic core and GSM, the sensitivity and specificity were calculated for a range of cutoff points. Receiver operating characteristic (ROC) curves were constructed to determine the optimal threshold values. Linear regression was used to correlate GSM with the percentage of area of necrotic core.

**RESULTS**

**Demographic characteristics.** The patients’ mean age ($\pm$ SD) and mean carotid percentage of stenosis were $66 \pm 8$ years and $76 \pm 16\%$ in the symptomatic and $72 \pm 7$ years and $82 \pm 11\%$ in the asymptomatic ($P > .05$). Forty percent patients in the symptomatic group (10/25) and 43% of patients in the asymptomatic (10/23) were females ($\chi^2 = 0.06, P > .05$). Clinical risk factors for atherosclerosis, including coronary artery disease, diabetes mellitus, hypertension, and smoking were similar for all patients. There was a higher incidence of hypercholesterolemia in symptomatic patients (38%, 10/26) than in asymptomatic patients (25%, 6/24), $P < .05$.

Preoperative CDU estimation of ICA stenosis using CDU spectral waveform analysis and velocity profile showed that 54% (14/26) of symptomatic patients had 50%-79% stenosis and 46% (12/26) had 80%-99% stenosis. In asymptomatic patients 42% (10/24) had 50%-79% stenosis and 58% (14/24) had 80%-99% stenosis. Differences were not significant. The mean percentage of stenosis measured by B-mode US was 76% for symptomatic patients and 82% for asymptomatic patients (Table I).

Table I. Degree of carotid stenosis using preoperative B-mode ultrasonography and Doppler spectral analysis with velocity profile

<table>
<thead>
<tr>
<th>Doppler spectral analysis velocity profile</th>
<th>B-mode ultrasonography</th>
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<tr>
<td>50%-79%</td>
<td>80%-99%</td>
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<tr>
<td>Symptomatic ($n = 26$)</td>
<td>$54%$ (14/26)</td>
</tr>
<tr>
<td>Asymptomatic ($n = 24$)</td>
<td>$42%$ (10/24)</td>
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Strandness criteria were used to classify stenosis based on velocity criteria.

**Ex vivo B-mode US and computer-assisted analysis of plaque echodensity.** Ex vivo B-mode US images (mean $= 12$) for each plaque were captured and digitized for quantitative computer-assisted echodensity measurements. Plaques from symptomatic patients ($n = 26$) were more echolucent, with a mean GSM of $41 \pm 19$, when compared to plaques from asymptomatic patients ($n = 24$), whose GSM mean was $60 \pm 13$ ($P = .03$). The mean GSM of plaques that were identified by preoperative CDU as echolucent was $33.8 \pm 11.5$, and the mean GSM of echodense plaques was $53.6 \pm 10$ ($P < .001$).
Histopathologic/histomorphometric analysis. The presence of necrotic core (foam cells, cholesterol clefts, myxomatous debris) was more marked in symptomatic plaques than asymptomatic plaques. The mean percentage of plaque necrotic lipid core was 51% ± 18% for symptomatic plaques and 23% ± 20% for asymptomatic plaques. The necrotic core was twice as close to the lumen in symptomatic plaques compared to asymptomatic plaques (0.29 ± 0.2 vs 0.53 ± 0.4, P < .05). Figure 5 summarizes histopathologic characteristics of symptomatic and asymptomatic plaques.

The mean percentage of calcification area was 66% and 27%, respectively, in plaques with and without acoustic shadowing (P < .05).

Correlation between symptomatology, percentage of necrotic core area, and GSM with ROC analysis. A strong inverse correlation (R = 0.9, P < .001) was found between the GSM scores and histomorphometric measures of necrotic core burden. The lower the GSM was, ie, greater plaque echolucency, the larger the percentage of necrotic core area/plaque (Fig 6). ROC analysis was performed that demonstrated that a GSM 47 could predict symptomatic plaques with a sensitivity of 73% and a specificity of 83% (Fig 7). ROC analysis of the percent area

![Fig 5. Bar graph of the histomorphometric quantitative characterization of symptomatic and asymptomatic carotid plaques.](image1)

![Fig 6. Correlation between gray-scale median (GSM) analysis and percentage of area of necrotic core was −0.9 (P < .001), indicative of the strong association between echolucency (low GSM score) and percentage of necrotic core area.](image2)

![Fig 7. Receiver operating characteristic curve for differentiating between asymptomatic and symptomatic groups based on gray-scale median (GSM). A cutoff point of 47% yielded the highest sensitivity and specificity.](image3)

![Fig 8. Receiver operating characteristic curve for differentiating between asymptomatic and symptomatic groups based on the percentage of necrotic core area. A cutoff point of 29% yielded the highest sensitivity and specificity.](image4)
necrotic core (histology) and symptomatic plaques was performed which showed that a percent area necrotic core of 29% could predict symptomatic plaques with a sensitivity of 100% and a specificity of 67% (Fig 8).

**DISCUSSION**

In this study, preoperative subjective evaluation of carotid plaque echolucency revealed that 58% of symptomatic plaques were predominantly echolucent compared to only 18% of asymptomatic plaques. These findings are supported by several studies relating plaque echolucency to neurologic events. Langfield et al\(^{29}\) and Belcaro et al\(^{30}\) demonstrated that echolucent or heterogeneous plaques are at a significantly increased risk of becoming symptomatic when compared to dense, echo-rich plaques. A study by O’Holleran et al\(^{31}\) followed 293 patients over a 4-year period, and all 42 patients with a soft lesion (lipid and intraplaque hemorrhage) with >75% stenosis became symptomatic. Confirming these results, Bock et al\(^{19}\) showed that echolucent plaques were associated with a 5.7% annual incidence of transient ischemic attack and stroke compared to only 2.4% for echogenic plaques. In a cross-sectional study, Geroulakos et al\(^{32}\) found echolucent plaques to be associated with a 37% incidence of brain infarcts on CT scans compared to a combined incidence of 18% in plaques that were predominantly echolucent (type II), predominantly echogenic (type III), and echogenic (type IV). In contrast to these findings, Holdsworth et al\(^{33}\) in their study of 4258 patients only found amaurosis fugax to be associated with echolucent and heterogeneous plaques, whereas the degree of stenosis overall seemed more predictive of events.

To confirm the preoperative subjective B-mode evaluation of carotid plaque echolucency, we quantitated the echolucency of the ex vivo plaques following CEA using GSM analysis of multiple cross-sectional views. When the preoperative subjective assessments of echogenicity were compared to the ex vivo GSM values, subjectively echolucent plaques had significantly lower GSM scores (mean GSM = 34) compared to subjectively echodense plaques (mean GSM = 53). This observation agrees with a previous study by Mayor et al\(^{34}\) who found a correlation between visual evaluation of plaque echogenicity and computerized measurements of GSM. Our results indicate that symptomatic plaques were more echoluent than asymptomatic plaques having a significantly lower GSM score (GSM scores 41 ± 19 vs 60 ± 13, respectively). The threshold GSM value that would best separate symptomatic and asymptomatic plaques was a GSM of 47 (Fig 7). This threshold has been observed in other studies.\(^{23,35-37}\) Elatrozy et al\(^{38}\) studied 91 plaques from 77 patients and demonstrated that the GSM score of symptomatic plaques (mean = 21.5 ± 16) was significantly lower than that of asymptomatic plaques (mean = 37.6 ± 26). El Barghouty et al\(^{39}\) noted that plaques with a GSM >32 (echogenic) were associated with an incidence of 11% CT infarction compared to a 55% incidence of CT infarction for echolucent (GSM ≤32) plaques. More recently, in a prospective study, Gronhodt et al\(^{40}\) found that plaque echolucency could predict future stroke in symptomatic patients with 50%-99% stenosis but not in asymptomatic patients with comparable degrees of stenosis. In that study, echolucency was defined by the mean of the standardized GSM values, with GSM ≤74 equaling an echolucent plaque and a GSM >74 equaling an echo-rich plaque. These studies involved the in vivo determination of the GSM score in contrast to this report in which the GSM was calculated ex vivo. Plaque ex vivo fixation may have resulted in an overall increase in plaque echogenicity and an upward drift of the GSM score range.

Most previous studies have relied on a single longitudinal image for data acquisition and GSM score. In this study, however, the GSM score was determined from transverse as well as longitudinal images offering a more detailed representation of echogenicity of the entire plaque. Wijeyaratne et al\(^{40}\) found that multiple cross-sectional views in vivo (five to eight per plaque) increased GSM analysis sensitivity in detecting differences between symptomatic and asymptomatic patient populations. They also emphasized that a single longitudinal view was limited in accurately representing plaque echolucency.

Finally, histomorphometric analysis of the scanned plaques was undertaken to develop a correlative analysis between the GSM scores and the percentage of necrotic core area (Fig 6). The area of necrotic core in symptomatic plaques was twofold greater than in asymptomatic plaques. Additionally, the distance of the necrotic core to the lumen was doubled in asymptomatic patients compared to symptomatic patients. We found a strikingly significant inverse relationship between the GSM scores and the percentage of necrotic core area (\(R = -0.9, P < .001\)). This is in agreement with previous studies which have shown that pixel evaluation can be used to differentiate between tissue types.\(^{40,41}\)

The presence of carotid plaque calcification as evidenced by significant acoustic shadowing was more commonly found in asymptomatic plaques on preoperative US imaging (41% vs 7%, \(P < .01\)) than symptomatic plaques. This concurs with our previous report using ex vivo spiral CT to assess plaque calcification area in symptomatic and asymptomatic plaques.\(^{10}\) In the previous study, asymptomatic plaques exhibited more extensive calcification in contrast to symptomatic plaques.

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**Table II.** B-mode ultrasonographic characterization of carotid atherosclerotic plaques, including preoperative subjective evaluation and ex vivo gray-scale median analysis

<table>
<thead>
<tr>
<th>Group</th>
<th>Symptomatic (n = 26)</th>
<th>Asymptomatic (n = 24)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Echolucent plaque</td>
<td>58% (15/26)</td>
<td>18% (4/24)</td>
<td>&lt;.03</td>
</tr>
<tr>
<td>Calcified plaque</td>
<td>7% (2/26)</td>
<td>41% (10/24)</td>
<td>&lt;.03</td>
</tr>
<tr>
<td>GSM (Ex-vivo B-mode ultrasound)</td>
<td>41 ± 19</td>
<td>60 ± 13</td>
<td>&lt;.03</td>
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</tbody>
</table>
From the data presented herein and the above discussion, it is evident that the B-mode US findings of ICA plaque echolucency and minimal calcification are associated with plaque necrosis, potential structural instability, and potentially a higher risk of ischemic thromboembolic events. Future refinements in the in vivo three-dimensional quantitative assessment of plaque echolucency and burden by B-mode US will further advance our understanding of the natural history of significant asymptomatic ICA stenoses and assist in better selection of patients for carotid intervention.

REFERENCES