Pixel distribution analysis of B-mode ultrasound scan images predicts histologic features of atherosclerotic carotid plaques

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Background: The correlation of B-mode ultrasonographic morphology with histologic characteristics of atherosclerotic carotid plaques remains ill-defined. The classification of plaques with recently reported measures of plaque echogenicity and heterogeneity has been unsatisfactory. We used computer-assisted duplex ultrasound (DU) scan image analysis to determine echogenicity of specific tissues in control subjects. This information was used to quantify each tissue in imaged carotid plaques with pixel distribution analysis (PDA). These objective observations then were quantitatively compared with plaque histology in symptomatic and asymptomatic patients.

Methods: We performed standardized DU scanning of healthy tissues in 10 volunteer subjects and of 20 carotid artery plaques (7 symptomatic and 13 asymptomatic) in 19 patients with carotid stenosis. The plaques underwent histologic analysis after carotid endarterectomy. The grayscale intensity ranges of blood, lipid, fibromuscular tissue, and calcium were calculated in the control subjects. We compared computer-assisted image analysis, B-mode images of plaques were linearly scaled to normalize data. Pixel distribution within the images then was analyzed. The grayscale ranges of known tissues obtained from control subjects helped define the amount of intraplaque hemorrhage, lipid, fibromuscular tissue, and calcium within carotid plaque images. This analysis was correlated with tissue composition measurements on histologic sections of excised plaques.

Results: The median grayscale intensity (range) in control subjects was 2 (0 to 4) for blood, 12 (8 to 26) for lipid, 53 (41 to 76) for muscle, 172 (112 to 196) for fibrous tissue, and 221 (211 to 255) for calcium. PDA-derived predictions for blood, lipid, fibromuscular tissue, and calcium within carotid plaques correlated significantly with the histologic estimates of each tissue respectively (blood: \( P = .012 \); lipid: \( P = .0006 \); fibromuscular: \( P = .035 \); and calcium: \( P = .0001 \)).

A significantly higher amount of blood and lipid was seen within symptomatic plaques compared with asymptomatic ones (\( P = .0048 \) and \( P = .026 \), respectively). Conversely, a larger amount of calcification was noted within asymptomatic plaques (\( P = .0002 \)).

Conclusion: Computer-assisted PDA of DU scan images accurately quantified intraplaque hemorrhage, fibromuscular tissue, calcium, and lipid. Symptomatic plaques had lower calcium content but larger amounts of intraplaque hemorrhage and lipid. Quantitative PDA may be used to determine carotid plaque tissue composition to assist in the identification of symptomatic and potentially unstable asymptomatic plaques. (J Vasc Surg 2002;35:1210–7.)

Randomized controlled trial data1-3 have demonstrated the benefit of carotid endarterectomy (CEA) in the prevention of stroke. In these studies, the degree of internal carotid artery stenosis was the only criterion for selection of patients at high risk for stroke. A subsequent report4 indicated that plaque ulceration at each level of stenosis resulted in higher stroke rates than those associated with lesions without ulceration. These trials also noted that most patients with high-grade stenoses remained stroke free even with medical therapy alone.5 Therefore, it has been proposed that factors in addition to the degree of stenosis may be responsible for the determination of stroke risk.6-9

Carotid plaque disruption and distal embolization of atheromatous debris is the most common pathogenic mechanism for cerebral ischemia from carotid stenosis.10 Histologic studies have shown that large lipid/necrotic cores11-13 located close to the flow lumen,14 fibrin cap disruption,15 intraplaque hemorrhage,16 and surface ulceration17 occur more frequently in symptomatic plaques. Quantitative characterization of these histologic changes with a noninvasive method would allow CEA to be offered selectively to patients with these high-risk plaques.

Despite advances in duplex ultrasound (DU) scan technology, the process of acquiring, analyzing, and interpreting B-mode images has remained observer dependent. Initial attempts at objectivity were focused on visual quantification of bright (hyperechoic) and dark (hypoechoic) areas of the plaque. This classification was found to have
errors that arose from variable DU scan settings and from misinterpretation of bright and dark areas on the plaque.

It is now possible to digitize B-mode images and accurately count their brightness with image-analysis software. Digitized grayscale images are stored on a computer as a collection of individual spots or pixels of light. A number that ranges from 0 for black to 255 for white is used to represent the brightness of each pixel. The same software can be used to select an area of interest in an image and count its median pixel brightness or intensity. Some investigators have contended that a median of the pixel intensities of the entire plaque (grayscale median [GSM]) of less than 32 corresponds to a high-risk plaque. Of note, however, the GSM is a global measure of total plaque brightness and does not reflect regional variability within the plaque. On the basis of histologic analyses of carotid plaques, Bassiouny et al and other investigators contended that quantification of regional plaque variability and its individual constituents may be critical to stroke risk assessment.

A dark (hyperechoic) area in a plaque is generally believed to indicate hemorrhage or lipid. However, it is not known whether different shades of gray indicate differences in tissue composition. In this report, we show that standardized B-mode DU scan imaging coupled with advanced image analysis may be used to obtain useful information about the tissue composition of carotid plaques. We performed preoperative DU scanning on patients who underwent CEA. With computer-assisted image analysis, B-mode images were linearly scaled to normalize data, and the distribution of pixel intensities within the plaque images was analyzed. The quantity and location of observed lipid and of intraplaque hemorrhage, fibrous tissue, and calcium were determined with a count of the percent of pixels in the grayscale ranges determined to represent these tissue components in volunteer subjects. This analysis then was correlated with tissue composition measurements on histologic sections of excised plaques.

**METHODS**

**Duplex ultrasound scan of control tissues.** After appropriate consent, 10 healthy subjects (five men and five women) who had no known illnesses underwent scanning with an Acuson 128 XP/10 ultrasound scan machine (Siemens, Mountain View, Calif) with a 7-MHz linear array transducer. Several variables on the scanner were standardized to optimize image quality and to avoid variability in B-mode image acquisition between subjects. The aim was to allow acquisition of maximal data with least image modification by the scanner or operator. Briefly, the log was set at 60 dB, preprocessing at 0, persistence at 2, postprocessing at 0, and the gain at −5 dB, and the depth gain compensation was maintained as flat. The probe was positioned directly over the area of interest. The following anatomic areas were examined: subcutaneous fat (abdomen; Fig 1), muscle (biceps), fibrous tissue (iliotibial tract), and calcified structure (tibia [Fig 1] and skull). Care was taken to image a blood vessel in the same frame to allow image normalization. The images were recorded live on super VHS tape. These analog images were digitized to a Gateway computer (PII 500 MHz 256 RAM, Gateway Inc, San Diego, Calif) with commercially available video-grabbing software, Dazzle DVC (Dazzle Multimedia, Fremont, Calif). The digitized images then were opened in an image-editing program, Adobe Photoshop 6.0 (Adobe Systems, Inc, San Jose, Calif), used to outline the tissue of interest and determine the median grayscale value of pixel intensity representing blood, fat, fibrous tissue, muscle, and calcium.

**Duplex ultrasound scan of carotid arteries.** After appropriate consent, 19 patients with 20 carotid stenoses that needed CEA with North American Symptomatic Carotid Endarterectomy Trial2,3 (50% stenosis, symptomatic)
or Asymptomatic Carotid Atherosclerosis Study (ACAS; 60% stenosis, asymptomatic) criteria underwent preoperative DU scan examination. The same Acuson 128 XP/10 scanner with a 7-MHz transducer was used. Degree of stenosis was determined on the basis of the peak systolic and end diastolic velocities. B-mode images were acquired with the same settings used for the control subjects detailed previously. The carotid bifurcation was identified, and the plaque was magnified to visualize the entire lesion and both the far and the near walls of the artery in the same frame. The best longitudinal view of the bifurcation was then recorded live on super VHS tape.

Image normalization. The images were digitized in the same manner as the control tissues. The longitudinal grayscale image file was opened in an image-editing program, Adobe Photoshop 6.0. With the histogram facility, the pixel values of two reference areas in the image were measured: an echo-free area in the lumen (blood) and an echogenic area in the normal arterial wall (adventitia). The grayscale value of blood was adjusted to 0 and that of adventitia to 190. The grayscale values of all pixels in the image shifted according to the new standard linear scale, producing a normalized image. All images were scaled similarly, and this ensured that accurate comparisons were made between patients. Color-flow images were used to assist in the definition of the edges of hypoechoic plaques.

Grayscale median and Gray-Weale classification of carotid plaques. The GSM and Gray-Weale classification were obtained as described by previous authors. With the normalized image, the plaque was outlined and the median value of the pixel intensity generated by the entire plaque was determined (GSM). The Gray-Weale classification was determined by obtaining the percent of pixels with intensity less than 40. This percentage was used to stratify the plaque according to the modified Gray-Weale classification: type I: uniformly hypoechoic; type II: predominantly hypoechoic (>50% hypoechoic); type III: predominantly hyperechoic (<50% hypoechoic); type IV: uniformly hyperechoic; type V: plaques that cannot be classified because of heavy calcification causing acoustic shadowing.

Pixel distribution analysis of carotid plaques. The postprocessed, normalized longitudinal image of the plaque was transferred to another imaging program, Image Pro-plus 3.0.1 (Media Cybernetics, Silver Spring, Md). With the same region of interest, the grayscale ranges represented by blood, fat, fibrous tissue, muscle, and calcium, as determined by results from the control subjects, were assigned different colors. The mean grayscale value (range) for blood was 2 (0 to 4), for fat was 12 (8 to 26), for muscle was 53 (41 to 76), for fibrous tissue was 172 (112 to 196), and for calcium was 221 (211 to 255; Fig 1). These ranges were identified within the carotid plaque images and mapped onto them. The surface area occupied by each grayscale range then was calculated as a percent of the total area of the plaque in longitudinal view (Figs 2 and 3).

Histologic processing of carotid plaques. Twenty explanted carotid plaques from 19 patients were analyzed.
for plaque composition to compare with preoperative pixel distribution analysis (PDA). The plaques were excised with a modified open technique to minimize iatrogenic histopathologic artifacts. Briefly, the depth of the arteriotomy was limited to the outer media to minimize disruption of the luminal surface. The harvested specimen included plaque involving the distal common carotid artery in continuity with the bifurcation and its extension into the internal and external carotid arteries. The fresh plaque was rinsed, inspected for ulceration, and sequentially cut in cross section at 3-mm intervals. The specimens were moved to a tissue processor (Auto-Technicon tissue processor model 2A, The Technicon Company, Tarrytown, NY) for overnight dehydration in 70%, 80%, 95%, and 100% graded ethyl alcohol solutions followed by paraffin embedding. Eight 5-μm sections were obtained on a rotary microtome (Olympus 4060-Olympus America Inc, Melville, NY) from each ring, half of which were stained with hematoxylin and eosin and the remaining half stained with Gomori Trichrome (Fig 4).

Image analysis of histologic specimens. Each section was photographed with videomicroscopy (Nikon Optiphot 2 microscope, Garden City, NY) with methods previously described by our group. Briefly, the Image Pro Plus software was calibrated with a fixed micrometer for a length of 0.01 mm. Four sections from each ring were analyzed, and the mean ± the standard error of the mean (SEM) of the results was reported. An investigator blinded to each patient’s identity evaluated the sections. Histology images were digitized, each histologic component (calcium, fibromuscular tissue, lipid/cholesterol core, and thrombosis/hemorrhage) was outlined, and the surface area was measured (Fig 4). The volume of individual histologic components was calculated as the sum of the product of the individual areas and the thickness of each ring (3 mm). The volume of each tissue component was calculated as a percentage of the total plaque.

Statistics. All results are reported as the mean ± the SEM. Comparisons have been drawn between DU scan-derived plaque composition measurements and histologic analyses of plaques with Spearman coefficient of correlation. Differences between symptomatic and asymptomatic plaques on the basis of DU scan results were determined with a two-tailed t test.

RESULTS

Patient population. Twenty carotid plaques from 19 patients were included in the study. Of the 19 patients, 16 were men and three were women. The mean age was 66.5 years (range, 52 to 77 years). The degree of stenoses ranged from 70% to 99%. Seven plaques were obtained from symptomatic patients (five strokes and two transient ischemic attacks), and the remaining 13 were from asymptomatic patients. The mean interval between symptoms and CEA in the symptomatic patients was 13 weeks. The control group comprised five male and five female volunteers, with a mean age of 42 years.

Duplex ultrasound scan examination of control tissues. Five images from each tissue study from each healthy volunteer were normalized, and the grayscale intensity was
calculated. Two hundred and fifty images were evaluated to determine the grayscale range of blood, lipid, fibromuscular tissue, and calcium in 10 subjects. The study results showed that the median grayscale value (range) for blood was 2 (0 to 4), for fat was 12 (8 to 26), for muscle was 53 (41 to 76), for fibrous tissue was 172 (112 to 196), and for calcium was 221 (211 to 255; Fig 1).

Grayscale median and Gray-Weale classification of plaques. The mean GSM (mean ± SEM) for symptomatic plaques was 32 ± 7.5 and for asymptomatic plaques was 49.3 ± 6.7. The GSM was not significantly different between the symptomatic and the asymptomatic carotid plaques (P = .07). Four of seven symptomatic plaques (57.1%) were Gray-Weale type II, and three were type III. Of the asymptomatic plaques, nine of 13 (69.2%) were type II, three were type III, and one was type IV. Therefore, the Gray-Weale classification did not differentiate between symptomatic and asymptomatic plaques.

Pixel distribution analysis of plaques. In the seven symptomatic plaques, PDA results showed the following percent distribution (mean ± SEM) of tissue components: blood, 11.22 ± 3.16; lipid, 29.38 ± 5.96; fibromuscular tissue, 36.50 ± 3.34; and calcium, 1.89 ± 0.70 (Fig 2). In the 13 asymptomatic plaques, the following tissue composition was seen: blood, 2.05 ± 1.48; lipid, 13.34 ± 7.24; fibromuscular tissue, 42.77 ± 5.93; and calcium, 11.13 ± 1.29 (Fig 3). There was a significantly higher amount of blood and lipid within symptomatic plaques compared with asymptomatic ones (P = .0048 and P = .026, respectively). Conversely, there was a larger amount of calcification within asymptomatic plaques (P = .0002), and the amount of fibromuscular tissue tended towards more abundance in asymptomatic plaques (P = .256).
accumulate a lipid core. The fatty streak becomes a fibroatheroma as fibrous tissue accumulates over the core and forms a fibrous cap. Through unknown mechanisms, some plaques become unstable, resulting in enlargement of the lipid core, fibrous cap rupture, ulceration, intraplaque hemorrhage, and subsequent embolization of plaque material or luminal thrombosis. These histomorphologic features have been associated with plaques that produce atheroemboli and neurologic symptoms. If these features could be identified noninvasively, before the development of symptoms, CEA could be offered selectively to these patients at high risk for strokes. DU scan is currently an integral part of the work-up of patients with carotid occlusive disease because it can accurately determine the degree of stenosis. B-mode images can be easily and economically obtained during this study, and their analysis for plaque composition may have the potential to identify such high-risk patients.

Reilly et al first suspected that echo patterns in the B-mode image of a carotid plaque could be related to the composition of the plaque. Plaque echogenicity was qualitatively expressed as the degree of acoustic brightness of a plaque when interrogated with DU scan. Gray-Weale and colleagues further classified plaques into the following four major subtypes: echolucent (type 1), echoluent with small echogenic areas (type 2), echogenic with small echolucent areas (type 3), and echogenic (type 4). Symptomatic patients were noted to have predominantly type 1 and 2 lesions, whereas the asymptomatic patients had predominantly type 3 and 4 lesions. However, the acquisition of B-mode images was not standardized, and their assessment was entirely on the basis of visual perception, which resulted in variable results.

With the introduction of computerized digital image processing, the pixel intensity (brightness) of DU scan-derived images could be objectively expressed with a grayscale range from 0 (black) to 255 (white). El Barghouty et al analyzed B-mode images with this technique and quantified the median value of the grayscale intensity of the entire plaque (GSM). They have subsequently reported that low GSM values may be associated with a higher incidence of ipsilateral hemispheric symptoms. However, GSM only measures the median brightness of the entire plaque; segmental areas of plaque instability may exist within a plaque with a mid-range GSM value. This would explain why different reports have found different GSM values that differentiate symptomatic from asymptomatic plaques, ranging from 32 to 50. In addition, different DU scan machines with different operators may not have been sufficiently standardized or normalized. In our study, the mean ± SEM GSM of symptomatic plaques was 32 ± 7.5 and of asymptomatic plaques was 49.3 ± 6.7. Therefore, GSM alone could not differentiate symptomatic from asymptomatic plaques (P = .07).

Geroulakos, Hobson, and Nicolaides modified the Gray-Weale classification by separating plaques on the basis of the percentage of low intensity pixels within them. They suggested that type II (heterogeneous) plaques contained the largest amount of intraplaque hemorrhage and risk of neurologic complications. Although this is an attempt to improve assessment of regional variations in the plaque, it still fails to directly measure individual tissue components of the plaque. Our study found four of seven symptomatic plaques (57.1%) to be Gray-Weale type II and three were type III. Of the asymptomatic plaques, nine of 13 (69.2%) were type II, three were type III, and one was type IV. This underscores the need for a more accurate measurement of regional variability within the plaque or a method to identify its exact tissue composition.

The histologic features that have been shown to characterize symptomatic carotid plaques are large lipid/necrotic cores, intraplaque hemorrhage, and fibrous cap dis-
ruption with ulceration.11-17 Bassiouny et al14 and other investigators9,17 have contended that quantification of these specific histologic components may be critical in the identification of unstable plaques and in assessment of stroke risk. However, none of these features have been accurately quantified with preoperative noninvasive imaging. In addition to allowing identification of patients at high risk, such noninvasive quantitative imaging would enable serial monitoring of progression of atherosclerosis.

Our study incorporates the combined use of standardized DU scan image acquisition, digital image normalization, and image PDA to obtain quantitative information regarding tissue composition of carotid plaques. Standardization and image normalization allow reliable patient-to-patient comparisons. Further, our results for grayscale intensities of blood, lipid, fibrous tissue, muscle, and calcium in healthy control subjects provide the basis for identification of these tissues in atherosclerotic carotid plaques. Of note, the grayscale ranges for these tissues are discrete with grayscale voids between them. Carotid plaques are comprised of several additional tissues, but we focused on elements identified in the literature as risk factors for athrorembozation.11-17 Tissues excluded from analysis (gray-scale voids) include inflammatory cells, vascular tissue, mixed connective tissue, and amorphous ground substance. Previous studies have not been able to differentiate hemorrhage from lipid on B-mode images. This is because both tissues generate few echoes and appear as more or less dark areas within the plaque on visual examination of the images. We used image analysis programs to normalize data and to discriminate minor but significant differences in the pixel intensity of these two tissues. Differences in pixel intensity within the plaque image represent different echogenicities of individual tissues. Pixels with the same grayscale intensities as individual tissues in control subjects were identified on the plaque images and mapped with a different color (red, blood; yellow, lipid; green, fibromuscular; and blue, calcium). There was good correlation between the percent of each tissue predicted with PDA and the percent of the same tissue subsequently measured with histology. The study did note that there were differences in correlation for the different tissue types (Spearman correlation: blood, r = 0.61, P = .012; fat, r = 0.77, P = .0006; fibromuscular, r = 0.53, P = .035; and calcium, r = 0.85, P = .0001). A possible explanation could be the nature of individual tissues, their histologic arrangement, and subsequent echogenicity. Our data regarding the tissue grayscale ranges show a much larger spread for fibrous or muscular tissue than for calcium (muscle, 41 to 76, and fibrous tissue, 112 to 196, versus calcium, 211 to 255).

Several studies have noted that enlargement of the lipid core, intraplaque hemorrhage, and subsequent plaque rupture may be the mechanism for athrorembozation. Other investigators have emphasized the thickness of the fibrous cap as an additional marker for risk of neurologic complications. Presumably, as the lipid core enlarges, the fibrous cap thins and ultimately ruptures. Noninvasive identification of the fibrous cap thickness or lipid core may therefore be useful predictors of risk for stroke. Hatsuiki et al20 have used modified magnetic resonance imaging protocols with specialized coils placed on the neck to measure fibrous cap thickness. Despite limitations of high cost, artifacts generated with arterial pulsation, swallowing and breathing, and the need for special coils, it is a promising technique for this measurement.

Of note, PDA results of the 20 carotid plaques in our study showed a statistically significant difference in the amount of lipid, intraplaque hemorrhage, and calcium between symptomatic and asymptomatic groups. This pilot study shows a high level of agreement between computer-assisted PDA of DU scan images and histologic measurements of intraplaque hemorrhage, lipid, fibromuscular tissue, and calcium content of advanced carotid artery atherosclerosis. Although it is currently an investigational method, PDA has the potential of being automated and integrated into the routine B-mode imaging of DU scan, providing an extremely inexpensive but quick assessment of plaque composition. Larger multiinstitutional studies will be necessary to assess the accuracy and applicability of PDA in discriminating symptomatic from asymptomatic plaques or in identifying asymptomatic high-risk plaques.

REFERENCES


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