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Stroke 1993, 24:1507-1512
Stroke is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 72514
 Print ISSN: 0039-2499. Online ISSN: 1524-4628

In Vivo Radiofrequency-Based Ultrasonic Tissue Characterization of the Atherosclerotic Plaque

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Background and Purpose: **The ultrasonic image can offer unique information on the composition of atherosclerotic plaque, ie, the relative content of lipids, fibrous tissue, and calcific deposits. To date, however, the echographic assessment of plaque structure is based on a subjective, qualitative evaluation of the bidimensional images. We evaluated the feasibility and accuracy of assessing, in vivo, the acoustic properties of arterial carotid plaques by means of a suitably modified echographic apparatus allowing direct access to the radiofrequency signal.**

Methods: **In 15 patients undergoing carotid thromboendarterectomy, the ultrasonic findings in 70 discrete sites (within the plaque, n=54; normal sites, n=11; or intraluminal thrombi, n=5) were correlated with the histological analysis (hematoxylin-eosin and Mallory trichrome stains) independently performed on the arterial samples. The pathological examination was carried out at a similar level of the insonation; the sites analyzed within the plaque were chosen because of their uniform echoic characteristics. In each ultrasonic region of interest selected from the echographic image, the integrated amplitude of the rectified radiofrequency signal was measured as the integrated backscatter index.**

Results: **The intimal-medial layer of normal carotid wall (n=11) exhibited values of −32.5±9.4 dB. The integrated backscatter index in fatty sites (n=11, −40.3±5.4 dB) differed from that of fibrous (n=12, −23.8±5.0 dB) and calcified (n=26, −11.5±5.2 dB, $P<.01$ for all intergroup differences) sites. Intraluminal thrombotic sites (n=5, −42±5.1 dB, $P<.01$) differed from fibrous and calcified subsets ($P<.01$) but overlapped (P=NS) with fatty sites. Histological sampling also showed two sites of intraplaque hemorrhage that exhibited very low backscatter values (−53 and −58 dB) and three fibrofatty sites showing backscatter values (−28, −28, and −32 dB) intermediate between the fibrous and the fatty subsets.**

Conclusions: **Quantitative analysis of integrated backscatter of the arterial wall is feasible in humans and provides an operator-independent assessment of plaque echoic structure. In particular, integrated backscatter is effective in distinguishing lipidic, fibrotic, and calcific components in human atherosclerotic plaques. (*Stroke*. 1993;24:1507-1512.)**

KEY WORDS • **atherosclerosis** • **carotid arteries** • **ultrasonics**

The ultrasonic image can offer unique information on the composition of atherosclerotic plaque, ie, the relative content of lipids, fibrous tissue, and calcific deposits.[1-4] To date, however, the echographic assessment of plaque structure is based on a subjective, qualitative evaluation of the B-mode images.

Ultrasonic tissue characterization consists of the identification and characterization of abnormalities in the physical state of biologic structures based on quantitatively analyzing interactions between ultrasound and tissue.[5,6] In particular, the measurement of the reflected ultrasonic energy ("backscatter") is a robust although technically demanding method to identify differences in echoic tissue characteristics[7,8] and appears ideally suited for noninvasive assessment of plaque structure, as demonstrated by in vitro studies.[9-15]

We performed ultrasonographic examinations of the carotid arteries and assessed the acoustic properties of atherosclerotic plaques by means of a suitably modified echographic apparatus allowing direct access to the radiofrequency signal in 15 patients who subsequently underwent endarterectomy. The ultrasonic findings were correlated with the histological analysis, independently performed on the arterial samples.

Subjects and Methods

In-hospital patients of the Neurosurgery Division, all with carotid stenotic (70% or greater lumen reduction) atherosclerotic plaques documented by conventional duplex scan and angiography and scheduled for thromboendarterectomy, were initially considered for ultrasonic tissue characterization study. All of them were symptomatic and affected by reversible ischemic attacks. Additional inclusion criteria were optimal ultrasonic imaging of the plaque (in 2 patients of the 17 initially considered, images were interpretable but of subopti-

Received September 16, 1992; final revision received May 20, 1993; accepted May 20, 1993.

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mal quality and therefore unsuitable for quantitative ultrasonic analysis) and consent to participate in a study. In 15 patients (8 men; 7 women; mean age, 66 years) a good quality ultrasonic study was completed, thromboendarterectomy performed, and histological evaluation carried out on the plaque specimens, which were excised in toto and carefully harvested to avoid plaque distortion and disintegration.

Serial longitudinal and transverse scanning of the carotid axis was performed with a modified two-dimensional mechanical sector scanner. For each patient, 7 to 10 transverse sections, spaced 0.5 to 0.8 cm, were obtained in caudocranial direction at the level of the plaque. At the time of thromboendarterectomy, the excised specimens were fixed in 10% formalin and sent to the pathologist. At least 5 transverse sections, spaced 0.8 to 1 cm, were obtained for each specimen. The carotid bifurcation was the anatomic reference point.

The pathological examination was performed at a level similar to that of the insonation; the sites analyzed within the plaque were chosen because of their uniform echoic characteristics. The carotid bifurcation and the arteriotomy sites were the reference points for the spatial orientation of the ultrasound and histology images. The previously acquired digitized ultrasonic images were projected side by side with the corresponding histology section. The off-line positioning of the region of interest for quantitative ultrasonic analysis was targeted to the sites within the plaque with uniform echoic characteristics. The pathological examination was performed at a level similar to that of the insonation on the image sites subjected to off-line quantitative analysis by a pathologist blinded to the ultrasonic data.

Ultrasonic Tissue Characterization

The quantitative analysis system was developed in our institution and is similar, from the conceptual as well as from the engineering point of view, to the one we adopted in previous cardiac studies.[16,17]

An ESAOTE Biomedica two-dimensional mechanical sector scanner echograph based on an electronically focused anular array transducer was used for spatial orientation of the ultrasound beam; quantitative analysis of ultrasonic reflectivity was performed in the regions of interest, always placed within the arterial wall. These regions were visualized in the short-axis view.

A 7.5-MHz nominal frequency transducer was used whose focal region extended from 0.7 to 4 cm, dynamically ensuring a uniform beam width all over the sampled tissue; this beam width was estimated as 0.8 mm at −3 dB. The acquisition was flashed at the time point of smallest artery size to minimize the sampling problems inherent to radial movement of the artery.

The "native" radiofrequency signal was sampled before the processing chain of the two-dimensional instrument (Fig 1). The radiofrequency signal underwent preamplification, bypassing the receiving circuits of the ultrasonic equipment. The analog signal was fed to an amplifier, and the gain sweep of the amplifier (2 to 60 dB) was accomplished in 30 steps. This allowed full utilization of the input dynamic range of the analog-to-digital converter. Sampling was performed by a flash converter with 8 bits of amplitude resolution, at a rate of 40 MHz. The digitized signal from the analog-to-digital converter was analyzed in real time by a hardware

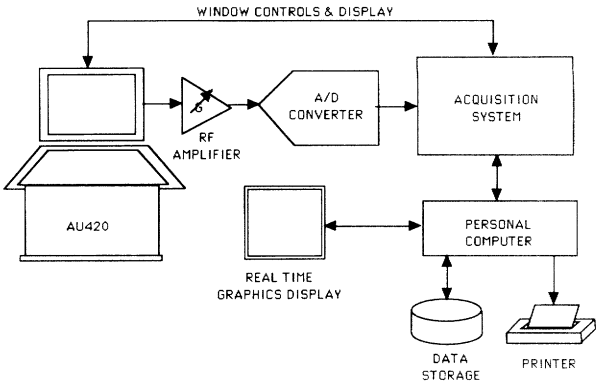

FIG 1. *Flow chart of ultrasonic data acquisition system. G indicates gain; RF, radiofrequency; and A/D, analog-to-digital.*

prototype developed in our electronics laboratory.[18] The two-dimensional acquisition gate was visualized on the two-dimensional image so as to ensure its proper positioning; it usually encompassed the whole cross-sectional two-dimensional image of the artery.

The hardware analysis involved the measurement of the integrated amplitude of the rectified radiofrequency signal corresponding to the two-dimensional area selected from the echographic image. The signal analysis was performed off-line on the previously digitized images. In normal walls, the region of interest was placed in the subintimal-medial layer. The adventitia and the lumen were excluded from the analysis. For analysis of the plaque, sampling the radiofrequency signal usually occurred in the subintimal-medial layers of the arterial walls, thus excluding intimal and adventitial specular reflections. The region of interest was of variable size (ranging from 1000 to 3000 pixels, the geometric dimension of the pixel being 18×250 μm) and shape, depending on the plaque dimension and geometry (Fig 2).

More analytically, the two-dimensional integrated backscatter index (2D-IB) was calculated over a tissue area, ie, corresponding to an (n-m) segment in depth and an (r-l) segment in lateral displacement, as follows:

$$2D\text{-}IB=\frac{1}{x_r-x_l}\sum_{j=r}^{l} IB(X_j)$$

where

$$IB\ (X_j)=\frac{1}{y_n-y_m}\sum_{i=n}^{m}|S(x_j, y_i)|$$

represents the processing over the depth of the interrogated tissue; $S(x_j, y_i)$ is the sequence of the digitized backscattered echoes over the selected two-dimensional area and is expressed in millivolts.

The system provides a simultaneous display of conventional information together with tissue characterization parameters (the 2D-IB alphanumeric index and the lateral displacement profile averaged over the selected depth). Alphanumeric 2D-IB data values were transferred on-line to a personal computer (AT model) for statistical analysis.

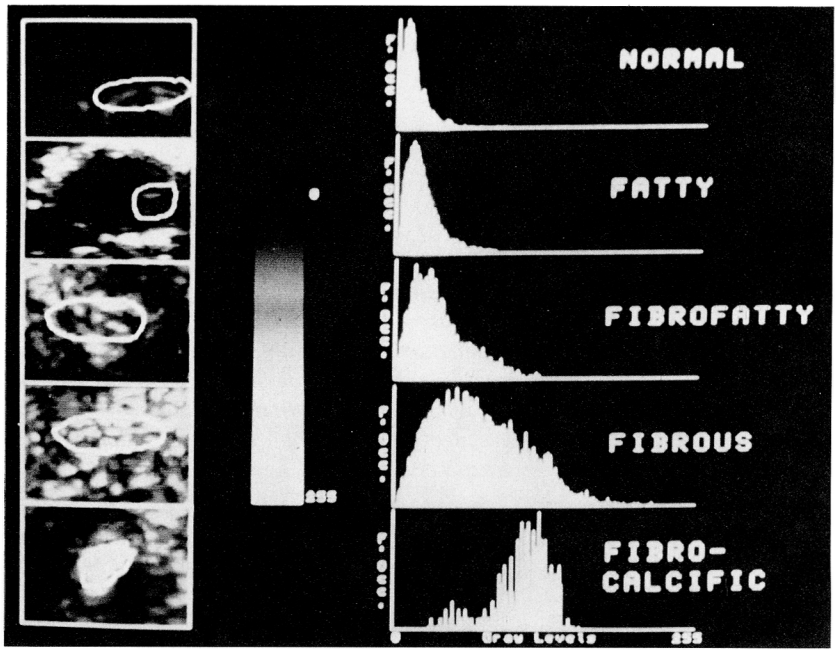

FIG 2. *Left, Two-dimensional cross-section of a carotid artery is shown; middle, corresponding gray-level scale; and right, gray-level histogram corresponding to the region of interest outlined on the B-mode image. Progressive increase in the fibrotic component shifts the frequency histogram to the right (toward brighter gray levels). Focal significant calcification in an otherwise fibrotic site (fibrocalcific) further increases the image brightness.*

Two-dimensional integrated backscatter index results were expressed as mean IB value and in decibels. To obtain the mean IB value, the global value of 2D-IB in the region of interest was divided by the number of pixels of the region of interest itself. The same values were also expressed in decibels (a relative measurement unit of sound energy, conventionally used), referred to an external specular reflection (previously acquired, and with very high reflectivity), and calculated as follows: 20 log V / Vr. V and Vr are both primary 2D-IB values and are expressed in millivolts. In particular, V is the value corresponding to the region of interest in the artery; Vr is the value corresponding to the reference external reflection.

Histological Analysis

Each arterial sample was studied histologically with hematoxylin-eosin and Mallory trichrome stains. In a side-by-side correlation with the ultrasonic image, each region of interest assessed by quantitative tissue characterization was also evaluated by an experienced pathologist (G.V.P.), who was blind to the echo results and allocated each region of interest according to generally accepted criteria[19] to one of the following groups: normal arterial wall; intraluminal thrombosis; intraplaque hemorrhage; fatty (characterized by accumulation of lipids in the intima); fibrofatty (usually characterized by a fibrous cap and a lipid core); fibrous (ie, wall thickening due to connective tissue, which occupied more than 50% of the sampled area); and calcified (presence of calcified atheromata within the arterial wall, with microscopic calcification being arbitrarily defined as significant when it covered 5% or more of the total sampled area).

Statistical Analysis

Data were reported as mean±SD. Intergroup differences were tested for significance using analysis of variance, with subgroup analysis by the Newman-Keuls test.[20] A value of $P<.05$ was considered to be statistically significant.

Results

A total of 70 regions of interests were analyzed by quantitative ultrasound; the corresponding histological data were also assessed.

Representative ultrasonic images of a normal wall and of a plaque belonging to different pathological subsets are shown in Fig 2 (with the conventional B-mode image and the frequency histogram of backscatter amplitude in the selected region of interest) and Fig 3 (with the time and frequency domain representation of the backscattered signal in the region of interest).

Of the 70 regions of interest, histology identified 11 normal regions (−32.5±9.4 dB), 5 sites of intraluminal thrombosis, and 54 atherosclerotic lesions: fatty in 11, fibrous in 12, calcified in 26, fibrofatty in 3, and intraplaque hemorrhage in 2. Fatty, fibrous, and calcified sites could be separated by quantitative ultrasound, showing a progressive increase in echoreflectivity (Table). The intimal-medial layer of a normal carotid wall was examined in 11 sites, showing a higher reflectivity (−32.5±9.4 dB) in comparison with 5 sites of intraluminal thrombosis (−42±5.1 dB, $P<.01$, P=NS versus fatty sites: −40.3±5.4 dB). Histological sampling also showed 2 sites of intraplaque hemorrhage, which exhibited very low backscatter values (−53 and −58 dB), and 3 fibrofatty sites showing backscatter values (−28, −28, and −32 dB) intermediate between the fibrous and the fatty subsets. Intraplaque hemorrhage and fibrofatty sites were not compared with the other subsets because of the very limited number of observations.

Discussion

Quantitative analysis of integrated backscatter of the arterial wall is feasible in humans, can be associated with conventional transcutaneous high-resolution B-scan systems, and provides an operator-independent assessment of plaque composition. In particular, inte-

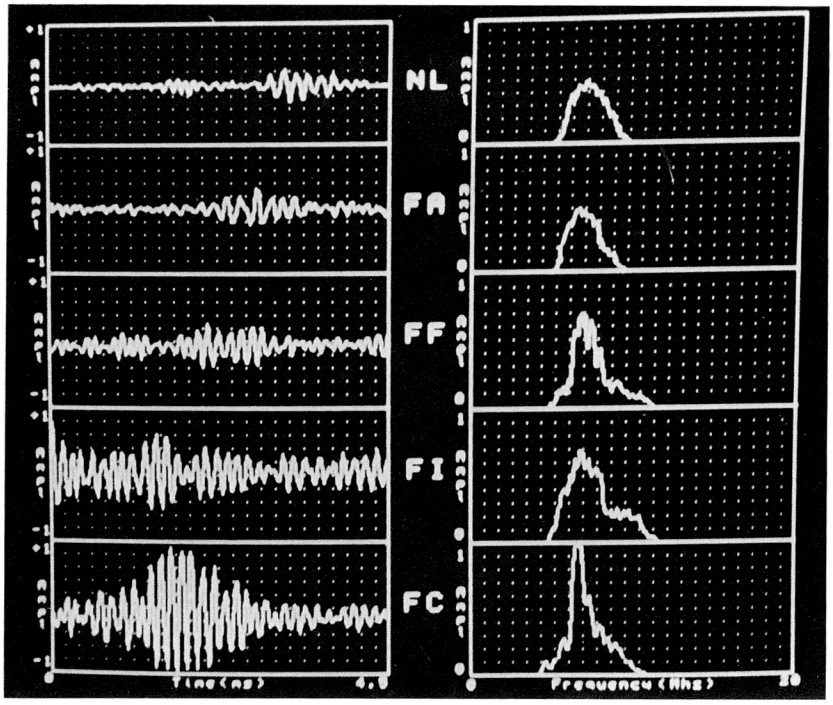

FIG 3. *Left, Time domain echo pattern; right, frequency domain pattern of five representative arterial sites: normal (NL), fatty (FA), fibrofatty (FF), fibrous (FI), and fibrous with significant calcification (FC). The signal amplitude of the time domain signal as well as the amplitude of the frequency spectrum tends to increase with the presence of the fibrotic and particularly of the calcific component.*

grated backscatter is effective in distinguishing fatty, fibrous, and calcified plaques.

Comparison With Previous Studies

Normal arterial walls and atherosclerotic plaques can be distinguished in vitro by a variety of acoustic parameters, based on ultrasonic attenuation[10] as well as on reflected signals.[9,11-15] In particular, the internal backscatter of the arterial wall rises markedly from lipidic to fibrofatty to calcific plaques. Such differentiation can be reliably achieved even with the "peak amplitude value" of the reflected signal, which, unlike the internal backscatter, is much simpler to obtain but also markedly phase sensitive.[9] In our study, we used an index related to the internal backscatter, which is derived through a frequency and spatial averaging method and can therefore be considered relatively phase insensitive, since fluctuations are expected to occur randomly and may fall within statistically specified limits.

We also elected to avoid the intimal interface in the acquisition of our regions of interest. In vitro studies document that if one measures only the first interface echo, there is a variable amplitude value for all plaque subsets except for lipidic plaque, showing a consistently low amplitude value.[13] Furthermore, the marked angle dependence of the intimal echo[14] makes it unsuitable for quantitative acoustic assessment in vivo, where regions of interest are frequently off axis. This is especially true in the setting of our study, since plaques could be located either in the common carotid artery, which typically is parallel to the skin surface and has interfaces that are relatively perpendicular to the angle of insonation, resulting in better imaging, or in the internal carotid artery/bulb area, which is frequently not parallel to the skin surface.

Furthermore, although the internal backscatter of the artery is partially angle dependent because of the architectural arrangement of arterial scatters, which can be physically identified in collagen bundles and calcium laminas, the difference in echoreflectivity between different plaque subsets is maintained over a large angular span.[11] Even at an angle of 14° off axis, calcified plaques reflect relatively more than fibrous sites, and fibrous sites reflect relatively more than fatty sites, although the absolute values are obviously decreased.[11]

The data of the present study confirm and extend the evidence obtained in vitro with B-mode and backscatter analysis and are also in full agreement with clinical

Values of Integrated Backscatter Index in Different Plaque Subtypes and Intraluminal Thrombi

Subset	No. of Observations	Integrated Backscatter, dB (mean±SD)	*P*			
			Thrombosis	Fatty	Fibrous	Calcified
Intraluminal thrombosis	5	−42±5.1	. . .	NS	<.01	<.01
Fatty	11	−40.3±5.4	. . .	. . .	<.01	<.01
Fibrous	12	−23.8±5.0	. . .	. . .	. . .	<.01
Calcified	26	−11.5±5.2	. . .	. . .	. . .	. . .

In the remaining five sites histology showed three fibrofatty sites (with values of −28, −28, and −32 dB) and two intraplaque hemorrhages (−53 and −58 dB). These two groups were not included in the statistical comparison because of the small sample size. NS indicates not significant.

experience with different imaging modalities (transcutaneous, epicardial, intravascular), which have confirmed that the ultrasonic image, even in the conventional video format, conveys information on the biochemical composition of the atherosclerotic plaque, ie, the relative content of lipids, fibrous tissue, and calcific deposits, since lipid-rich zones are demonstrated as echolucent areas, fibrosis appears bright, and regions of calcification generate hyperechoic signals with acoustic shadowing.[1-4]

Limitations of the Study

The correlation between the ultrasonic image obtained in vivo and the pathological section can be affected by several pitfalls because of plaque distortion and deformation taking place during endarterectomy and because of the heterogeneity of the plaque. However, we chose to correlate discrete sites of uniform echoic appearance within the plaque, and we positioned the region of interest (for off-line ultrasonic analysis) in a side-by-side comparison with the histological section.

The limited number of observations in some pathological subsets precluded an adequate characterization of acoustic properties of some clinically important groups, such as intraplaque hemorrhage or necrosis.

Subcutaneous and muscular tissue, of variable thickness and composition, is interposed between the transducer and the artery, giving rise to erratic diffraction and attenuation phenomena. We did not make any attempt toward a "rational gain compensation," which would have required a substantial increase in the complexity of the analysis.[21]

A relatively narrow frequency band (7.5 MHz) was used instead of a broad band frequency, allowing more intense backscatter phenomena and a reduction in phase cancellation artifacts with frequency averaging.[6]

Future technological implementations, as well as the extraction of more complex parameters derived from the ultrasonic signal,[22] will further increase the information yielded by ultrasound on plaque composition; at present it is impossible to distinguish thrombosis from mostly fatty plaques.

Clinical Implications

Increased understanding of the biology of the atherosclerotic plaque has suggested that "lipid lesions" are soft and weak and have a greater tendency to develop symptoms of thrombosis, embolization, and ulceration but are also more subject to regression.[23] The clinical noninvasive recognition of this lesion is certainly an attractive goal for the clinician.

To date, all studies have addressed the ultrasonic characterization of the plaque according to a visual, qualitative approach. The present method has several advantages over other approaches.

First, this method is based on the analysis of the native (raw) radiofrequency signal sampled before the processing chain of the conventional video presentation, which disrupts the linear relation between the received and the displayed signal.

Second, although in the present study the quantitative analysis was performed off-line to make the correlation with histological analysis easier, the digitized signal can be analyzed on-line as well with the display of the time domain radiofrequency signal, allowing a real-time assessment of the ultrasonic properties of the insonated structure.

Third, with the apparatus used in this study both conventional and quantitative (radiofrequency) imaging are built into the same basic hardware, allowing a simultaneous integrated representation of the insonated tissue in its variables of anatomy and structure.

Finally, the information generated by the analysis is not only discriminative but also immediately available to the physician, with the original radiofrequency signal in the time domain, captured along one line of the region of interest (somewhat similar to an M-mode line of view in a conventional B-mode image), side by side with a color-coded region of interest and with the fast-Fourier transform of the signal, in which the backscattered signal is proportional to the area under the curve.

Radiofrequency-based ultrasound imaging of the atherosclerotic plaque allows the quantitative assessment of plaque composition, thus integrating the conventional imaging information on plaque geometry in a short but diagnostically critical section of the carotid artery. This relatively simple, quantitatively displayed information can represent an important improvement for future studies on the biochemical remodeling of the atherosclerotic plaque after dietary, pharmacologic, or mechanical interventions.

Acknowledgment

We are grateful to Dr Antonio Caselli for reviewing the manuscript for English language usage and to Claudia Taddei for secretarial assistance. We thank technicians Vittorio Gattai and Luciana Pozzolini for histological processing.

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