Noninvasive Quantitative Tissue Characterization and Two-Dimensional Color-Coded Map of Human Atherosclerotic Lesions Using Ultrasound Integrated Backscatter

Comparison Between Histology and Integrated Backscatter Images

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OBJECTIVES

The purpose of the present study was to define clinicopathologically whether integrated backscatter (IB) combined with conventional two-dimensional echo (2DE) can differentiate the tissue characteristics of calcification (CL), fibrosis (FI), lipid pool (LP) with fibrous cap, intimal hyperplasia (IH) and thrombus (TH) and can construct two-dimensional tissue plaque structure in vivo.

BACKGROUND

It is difficult to characterize the components of plaque using conventional 2DE techniques.

METHODS

Integrated backscatter values of plaques were measured in the right common carotid and femoral arteries (total 24 segments) both during life and after autopsy in 12 patients (age 68 to 84 years, 10 men and two women). Integrated backscatter values were determined using a 5–12 MHz multifrequency transducer, setting the region of interests (ROIs) (11 × 11 pixels) on the echo tomography of the entire arterial wall (55 × 10 ROI/segment) and comparing it with histologic features in the autopsied arterial specimens.

RESULTS

Corrected IB values obtained before death and at autopsy were significantly correlated (r = 0.93, p < 0.01). Corresponding to the histologic features, corrected IB values on the rectangle ROIs obtained during life were divided into five categories: category 1 (TH) 4 < IB ≤ 6; category 2 (media and IH or LP in the intima) 7 < IB ≤ 13; category 3 (FI) 13 < IB ≤ 18, category 4 (mixed lesion) 18 < IB ≤ 27 and category 5 (CL) 28 < IB ≤ 33. In category 2, media and intima were differentiated using conventional 2DE. Under the above procedures, color-coded maps constructed with IB-2DE obtained during life precisely reflected the histologic features of media and intima.

CONCLUSIONS

Integrated backscatter with 2DE represents a useful noninvasive tool for evaluating the tissue structure of human plaque. (J Am Coll Cardiol 2001;38:486–92) © 2001 by the American College of Cardiology

Generally, plaque compositions include calcification (CL), fibrosis (FI), lipid pool (LP) with fibrous cap, intimal hyperplasia (IH) and thrombus (TH). Plaque is also classified as stable or unstable (1). The former consists of fibrous tissue or small LP with thick fibrous cap. The latter consists of large LP with thin fibrous cap or IH. Large LP with thin fibrous cap is considered especially dangerous because it may cause plaque rupture with thrombi followed by sudden and severe stenosis of the lumen. Therefore, it is clinically important to develop noninvasive techniques for differentiating plaque compositions and determining their extent.

A new technique has recently been developed, ultrasonic tissue characterization of the myocardium with an integrated backscatter (IB) analysis, that is capable of providing both conventional two-dimensional echographic (2DE) images and IB images. In studies of the myocardium, calibrated myocardial IB was significantly correlated with the volume fraction of interstitial FI (2,3). In preliminary in vitro studies, IB values reflected the structural and biochemical composition of atherosclerotic lesions and could differentiate fibrofatty lesions, fatty lesions and CL of arterial walls (4–6). It has also been reported that anisotropy of the direction and backscatter power is related to plaque type (7). These studies were done ex vivo, and only a few local lesions were measured from different plaque types. Recently, Takiuchi et al. (8) reported that quantitative ultrasonic tissue characterization could identify LP and FI in human carotid and/or femoral arteries. However, this technique was not precise, because IB values of LP and IH were similar. Discrimination of IH, fibrous cap and TH, and sensitivity and specificity of these measurements, were not
studied in these reports. Furthermore, extent evaluation of each composition (i.e., two-dimensional [2D] tissue structure) in the entire plaque has not been examined.

To define whether IB combined with 2DE is useful for tissue detection and construction of each tissue composition in entire plaques in vivo, conventional echo images and IB values in carotid and femoral arteries were measured in patients before and immediately after death, and these IB values were compared with their histopathologic features. Subsequently, 2D color-coded maps of arteries with plaque were constructed to assess visually the arterial tissue characteristics.

**MATERIALS AND METHODS**

**Subjects.** Twelve carotid arteries and 12 femoral arteries from 12 patients (age 68 to 84 years, 10 men and two women) were used in the present study. In all of these patients, in vivo and postmortem ex vivo ultrasound studies and pathologic examinations were available. The causes of death were pulmonary emphysema; pneumonia; old myocardial infarction; hypertrophic cardiomyopathy; and cancer of the lung, tongue, larynx or pancreas. One patient had a history of myocardial infarction, and another patient was diagnosed with hypertrophic cardiomyopathy. Informed consent was obtained from patients or their relatives in all cases.

**Study protocol.** Initially, IB imaging was performed in the carotid and femoral arteries within one month before patient death (Fig. 1A). Conventional and IB echo measurements of carotid and femoral arteries were performed in patients during the terminal stage of various diseases. Only those patients who died within one month after the above

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**Abbreviations and Acronyms**

- **CL** = calcification
- **dB** = decibels
- **2D** = two-dimensional
- **2DE** = two-dimensional echo
- **FI** = fibrosis
- **IB** = integrated backscatter
- **IH** = intimal hyperplasia
- **LP** = lipid pool
- **ML** = mixed lesion
- **ROI** = region of interest
- **TH** = thrombus

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![Figure 1](image-url)

**Figure 1.** Conventional ultrasound image and integrated backscatter (IB) image from the carotid artery at the same lesion. (A) Note that the intima and media are anatomically separated using conventional ultrasound imaging, as shown by the arrow. (B) Integrated backscatter values are measured at the region of interest placed in the plaque, as shown by a square, using the IB image. (C) Anterior arterial wall with an angle span of 120° between −60° and +60° were excluded from the IB evaluation because of the erratic diffraction phenomena and the influence of angle dependency. That is, the evaluation of IB values of the arterial wall was performed in the posterior wall with the exception of the anterior wall. Also, IB values of arterial lumen were measured just above posterior wall as shown by *.

2DE = two-dimensional echo.
conventional and IB echo measurements, and on whom autopsies were performed within 6 h after death, were included in the present study. At autopsy within 6 h after death, these arteries were dissected and subjected to ex vivo IB imaging in 0.9% saline. Subsequently, similar imaging procedures were repeated after the fixation. Integrated backscatter values were determined in almost identical areas of these three images to compare each of the measurements. Regions of interest (ROIs) were established in latticed arrangements. Next, the IB values of ex vivo IB images were compared with the histology.

**Integrated backscatter system presets and data acquisition.** For the in vivo studies, the subjects were kept supine with the head slightly extended. Transverse scans were performed between the middle of the right common carotid arteries and bifurcation of the external carotid artery and internal carotid artery. To identify the target regions for studies ex vivo and in vivo, long-axis scans and transverse scans were performed to measure the distance between the target region and the bifurcation (the bifurcation of internal and external carotid arteries in the measurement of carotid arteries and the bifurcation of femoral artery and deep femoral artery in the measurement of femoral arteries). The distance was used as a reference point to determine the same region in the ex vivo and in vivo studies. Conventional echo images and IB images were acquired with a commercially available 2D ultrasonic imaging system (Hewlett-Packard Sonos 5500, Andover, Massachusetts). The system characterized arterial tissue at the bedside using a 5 to 12 MHz multifrequency transducer for all studies. This software enabled the acquisition, storing and retrieving of a sequence of continuous 2D conventional and IB images, forming a continuous loop digital recording of 2 s (60 frames in 2 s). Offline analysis of the 2D IB images was performed by retrieving the previously stored data from the built-in optical disc drive in the system. Each data set was acquired by averaging the IB value for 2 s at the same region to minimize the sampling problems inherent with radial movement of the artery. Integrated backscatter was calculated as the average power of the ultrasound backscattered signal from a small volume of tissue using the following formula measured in decibels (dB):

\[
\text{IBS} = 20 \log \left\{ \frac{1}{T} \int_{0}^{T} V^2 \mathrm{d}t \right\} \left/ \frac{1}{T} \int_{0}^{T} V_0^2 \mathrm{d}t \right\}
\]

where \( V \) is the signal voltage from ROI, \( V_0 \) is the smallest signal voltage that the system can detect, and \( T \) is the integration interval. In the present study, we used an \( 11 \times 11 \) pixels (0.6 mm \( \times \) 0.6 mm) rectangle-shaped ROI and set the time gain compensation at 0 dB and the lateral gain compensation at 50 dB at every measurement in both ex vivo and in vivo studies. At this setting, IB values of stainless steel at a distance of 1 to 2 cm from the transducer were 50 dB, which was within the dynamic range of the system. When the frequency of a transducer was 5 to 12 MHz and the speed of sound in a tissue was approximately 1,540 m/s, the resolution was calculated as 128 to 308 \( \mu \)m.

**Ex vivo study.** To know the rotational position of the excluded segment, the following procedures were performed. Before excision of the arteries at autopsy, stainless steel pins were carefully inserted to mark the anterior wall of the arteries to be used as a reference point in the ex vivo studies. The distance between the target region and the bifurcation was used as a reference of the ex vivo and in vivo studies. In the ex vivo measurements, the specimen was immediately placed 1 to 2 cm from the transducer in a 0.9% saline solution after excision at autopsy. Integrated backscatter and conventional 2DE images were obtained. Then each specimen was fixed with neutral 10% buffered formalin. Integrated backscatter images of the specimens after fixation with 10% buffered formalin were obtained in the same setting used in the measurements before fixation.

**Histologic study.** Carotid and femoral arteries were excised at autopsy and were fixed with 10% neutral buffered formalin. Ringlike arterial specimens obtained at a similar level to the ultrasound study were decalcified in a standard K–CX solution for 5 h and were embedded with paraffin and cut into 4–\( \mu \)-thick transverse sections perpendicular to the longitudinal axis of the artery. They were stained with hematoxylin–eosin, elastic van Gieson and Masson's trichrome. In addition, immunohistochemical analysis using anti-actin antibody was performed for detection of smooth muscle cells. According to generally accepted criteria (9), seven pathologic subsets were identified in each ROI: TH (collections of erythrocytes embedded in a net of platelets), LP (characterized by accumulation of lipids in the intima), IH consisting of smooth muscle cells that occupied >50% of the sample area, FI consisting of fibrous tissues that occupied >50% of the sample area, mixed lesion (ML) (CL, FI and lipid were mixed), CL and media. We used decalcified tissue specimens for histology because of the necessity of cutting specimens 4 \( \mu \)m thick. Because calcium remained microscopic despite decalcification, microscopic analysis was possible in the present study. These histologic determinations were based on the concordance of two specialists who were ignorant of the ultrasound echo study.

**ROI setting and correction of IB values.** Integrated backscatter values in the anterior wall of the arteries (an angle span of 120° between −60° and +60°) were excluded from the analysis because of the erratic diffraction phenomena and the influence of angle dependency (Fig. 1C). That is, the IB evaluation was performed using the posterior arterial wall excluding the anterior arterial wall. The IB values of the posterior arterial wall were corrected (corrected IB) by subtracting the IB values of the vessel wall (see technical considerations in Discussion). Regions of interest were set over the intima and media in the ringlike lumen and on whom the anatomic information from the connectional 2DE images. Offline analysis after retrieving the IB images allowed us to set the ROIs one by one by referring to the pathologic characteristics using pathologic
photographs and 2DE images. An average of 55 ± 10 ROIs per artery were set in each of the 24 arteries.

**Statistical analyses.** Values were reported as the mean ± standard deviation. The significance of difference of IB values among tissue characteristics of arterial wall was tested using analysis of variance followed by Fisher exact test, which was used for the post-hoc test. Correlation among the IB values during life, before and after fixation was tested for significance using the Pearson's correlation coefficient. A p < 0.05 was considered statistically significant.

**RESULTS**

**Comparison between IB values measured in vivo and ex vivo.** Figure 1 shows the images of the carotid arteries obtained using the conventional 2DE and IB methods. In the conventional 2DE images, the medial layer was easily differentiated as an echo-free zone surrounded by high-intensity signals. Referring to this anatomical information from the 2DE image, the 11 × 11 pixels were set over media and thickened intima of the IB image. The corrected IB values obtained in vivo during life (X) and the corrected IB values obtained at autopsy (Y1) or after fixation (Y2) were compared with each other. The correlation was satisfactory (r = 0.87, r = 0.93, p < 0.01), confirming the feasibility of using the IB measurement in vivo. The corrected IB values in the in vivo study are somewhat lower than those in the ex vivo studies (Y1 = 1.0 X + 3.1, Y2 = 1.1 X + 2.7). The ex vivo corrected IB values before and after fixation were almost the same (the difference: 0.9 ± 0.3 dB).

**Comparison between histology and ex vivo or in vivo corrected IB values.** To compare the corrected IB values and their pathology, a total of 121 sampling sites with typical histology were examined in the 24 arteries (Fig. 2). Histology of these sampling sites was divided into TH (n = 5), LP (n = 31), IH (n = 7), FI (n = 25), ML (n = 12) and CL (n = 17) in the intima, and the media (n = 24). Each corrected IB value of these tissues after fixation at autopsy was 7.3 ± 1.5, 13.0 ± 3.2, 10.9 ± 1.0, 19.3 ± 2.4, 28.2 ± 3.3 and 39.3 ± 3.6 dB in the intima, respectively, and 11.3 ± 1.9 dB in the media. Each corrected IB value during life was 4.9 ± 1.0, 10.0 ± 2.4, 8.0 ± 0.8, 16.0 ± 2.0, 23.5 ± 3.4 and 30.5 ± 2.5 dB in the intima, respectively, and 8.4 ± 1.8 dB in the media. In each of the ex vivo and in vivo studies, the corrected IB values were highest in calcified plaque and lowest in TH (Fig. 3). The differences among TH, FI, ML, CL and LP, IH and media were statistically significant. However, LP, IH and media had similar IB values.

**Construction of 2D color-coded maps using in vivo IB values and conventional 2DE.** To construct IB color-coded maps in the arterial wall, IB values on the ROI were divided into five categories based on the mean corrected IB values ± one standard deviation in each of the ex vivo and in vivo studies as shown in Table 1. Compared with the histology, the sensitivity for detecting each category of each ROI in the in vivo study was 80%, 84%, 80%, 85% and 89%, and the specificity was 91%, 78%, 85%, 87% and 91%, respectively.

In category 2, the media and intima were differentiated using conventional 2DE. Generally, the LP (category 2) is anatomically located under a fibrous cap consisting of FI (category 3). Therefore, the presence of ROIs with category 2 under a layer of ROIs with category 3 was defined as the LP, but not as IH. A total of nine segments with pathologic LPs were observed. Seven specimens showed echographic LPs. Two additional pathologic LPs, which were small in size (1 mm × 0.2 mm, 2 mm × 0.3 mm) and surrounded by diffuse FI, were overlooked and echographically counted as FI. In contrast, seven LPs were found using 2DE and IB. Each was observed pathologically as an LP. A total of eight segments with IH were observed by pathology. Five were >0.6 mm in width and showed echographic IH using 2DE and IB. The remaining three pathologic IH were thin (0.15 mm, 0.2 mm and 0.3 mm) and were counted as media, but not intima, using 2DE and IB. The color-coded maps of tissue characterizations reflected well the pathology of the arterial wall, except for small LPs and thin IH.

Based on the above definitions using in vivo 2DE and IB, 2D color-coded maps of tissue characterizations were constructed in vivo images of 24 plaques (Fig. 3). These also reflected the pathology well.

**DISCUSSION**

The present study showed that IB combined with conventional 2DE can differentiate CL, mixed type, FI, LP with fibrous cap, IH and TH and construct a 2D color-coded map of entire plaque.

There have been several approaches to the characterization arterial tissues, such as TH, CL, LP or FI (10–12). Magnetic resonance imaging may be a promising method to discriminate them in human atheromatous plaques in vivo. However, this technique requires expensive and large-scale equipment and therefore is far from convenient for screening plaque. Angiography, an invasive procedure, can demonstrate percent diameter stenosis, but not the tissue character. Also, currently available conventional ultrasound technologies do not discriminate LPs from fibrous tissue even if the intravascular approach is undertaken. Recently, ex vivo analyses of arterial tissue characterization have been reported using B-mode images of IB (13,14). In these studies, however, the constructed B-mode image was too small to characterize the entire arteries. Meanwhile, a commercially available 2D ultrasonic imaging system (Hewlett-Packard Sonos 5500) allowed the setting of ROIs at any time and the quantitative analysis of the image data after retrieving and storing the IB image to an optical disk without any reconstruction for videotape recorder images. This offline analysis allowed in vivo IB values to be determined from ROIs at almost identical areas of interest on the pathologic photographs taken after the autopsy.
Thus, the 2D structures of arterial walls based on the IB values were visualized.

**Technical considerations.** Ultrasonic backscatter is angle-dependent, and this may limit quantitative ultrasonic diagnosis (15). In the IB measurements of the anterior arterial walls, subcutaneous tissues between a transducer and the arterial wall may have caused erratic diffraction (5), in addition to the influence of the angle dependency in the anterior arterial wall, when the subcutaneous tissues were considerably thick. Therefore, the anterior arterial wall (an angle span of 120° between -60° and +60°) of the arteries was excluded from the analysis in this study (Fig. 1C), and the IB evaluation was performed using the posterior arterial wall except for the above tissue area.

Because the reverberation phenomena may have had an effect on the evaluation of IB values of the posterior arterial wall in the present ex vivo and in vivo studies, the reverberation phenomena should be excluded for precise comparison of the IB values. Therefore, we corrected the IB values of the posterior arterial wall by subtracting the IB values of the lumens with saline in the ex vivo study and with flowing blood in the in vivo study, because the same method has been performed in myocardial tissue characterization using IB (3). In the present ex vivo study, the lumen IB values

**Figure 2.** Corrected integrated backscatter (IB) values of various tissue types. In each of the ex vivo and in vivo studies, the corrected IB values from calcification (CL), mixed lesion (ML), fibrosis (FI), thrombus (TH) and lipid pool (LP), intimal hyperplasia (IH) or media show significant differences from each other. However, there are no significant differences among LP, IH and media. dB = decibels.
included the reverberation phenomena originating from the anterior arterial wall. In the in vivo study, however, the lumen IB values also included the IB values due to the reverberation phenomena originating in skin and/or subcutaneous tissue and the IB values of flowing blood. Therefore, the corrected IB in the in vivo studies should theoretically be somewhat lower than that of the ex vivo studies. There is a very serious technical issue concerning the calibration of the integrated backscatter measurements. As implemented on the Sonos 5500 platform, IB is a very reliable relative measure of backscatter energy. However, IB is not calibrated absolutely. This is a limitation of the available instrumentation.

According to the in vitro study by Picano et al. (15), angular scattering behavior is large in calcified tissues, whereas it is slight to null in normal and fatty plaque. This angle dependency might cause, in part, the variation of corrected IB values obtained from CL in the posterior

### Table 1. Defined Ranges of IB Values for the Color-Coded Maps in Each Histologic Category

<table>
<thead>
<tr>
<th>Histology</th>
<th>Calibrated IB (dB) Mean ± 1 SD</th>
<th>Definition (dB)</th>
<th>Ex Vivo/In Vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ex Vivo/In Vivo</td>
<td>Ex Vivo/In Vivo</td>
<td></td>
</tr>
<tr>
<td>Intima (n = 17)</td>
<td>36.3 ± 3.6/30.5 ± 2.5</td>
<td>32 &lt; IB ≤ 45/28 &lt; IB ≤ 33</td>
<td></td>
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<tr>
<td>Mixed lesion* (n = 12)</td>
<td>28.2 ± 3.3/33.5 ± 3.4</td>
<td>23 &lt; IB ≤ 32/18 &lt; IB ≤ 27</td>
<td></td>
</tr>
<tr>
<td>Fibrosis (n = 25)</td>
<td>19.3 ± 2.4/16.0 ± 2.0</td>
<td>16 &lt; IB ≤ 23/13 &lt; IB ≤ 18</td>
<td></td>
</tr>
<tr>
<td>Lipid pool (n = 31)</td>
<td>13.0 ± 3.2/10.0 ± 2.4</td>
<td>9 &lt; IB ≤ 16/7 &lt; IB ≤ 13</td>
<td></td>
</tr>
<tr>
<td>Intimal hyperplasia (n = 7)</td>
<td>10.9 ± 1.0/8.0 ± 0.8</td>
<td>9 &lt; IB ≤ 12/7 &lt; IB ≤ 9</td>
<td></td>
</tr>
<tr>
<td>Thrombus (n = 5)</td>
<td>7.3 ± 1.5/4.9 ± 1.0</td>
<td>6 &lt; IB ≤ 9/4 &lt; IB ≤ 6</td>
<td></td>
</tr>
<tr>
<td>Media (n = 24)</td>
<td>11.3 ± 1.9/8.4 ± 1.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Region in which calcification and fibrosis were mixed.

dB = decibels; IB = integrated backscatter; SD = standard deviation.
arterial wall. In the present study, the variation was marked in CL. However, each IB value in CL was higher than in other categories. Therefore, the differentiation between category 5 and other categories was easy despite the relatively large variation in IB values in CL.

At present, it is controversial whether a fixation of arterial tissue with formalin influences the echographic characteristics (16,17). The present studies, however, showed that the IB values recorded in vivo were precisely correlated with the IB values obtained just after the excision at autopsy or the IB values after fixation. The ex vivo IB values before and after fixation were almost the same. These findings are compatible with the previous findings of Lockwood et al. effect (17) that fixation has no significant.

Correlation of in vivo IB values with histopathology. In the present study, plaque tissues were histopathologically classified into six lesions: TH, LP with fibrous cap, IH, FI, an ML of CL and FI, and CL. According to this classification, it was found that the IB spectra can be divided into five categories: category 1, TH; category 2, LP or IH; category 3, FI; category 4, ML; category 5, CL. Because it was difficult to differentiate LP from IH using IB values, the anatomical features were used for this purpose. As LPs (category 2), are generally located under a fibrous cap (category 3), the presence of ROIs of category 2 under a layer of ROIs with category 3 was defined as an LP. Intimal hyperplasia was diagnosed when the ROIs with category 2 were identified without a fibrous cap (category 3). Most of these two lesion types were differentiated using this method. However, there was a limitation because of the difficulty in identifying a fibrous cap of $<0.3$ mm. In addition, an LP was undetectable when it was smaller than 0.6–0.6 mm. However, it was a great advance to differentiate the above six lesions in the arterial wall in vivo.

Two-dimensional color-coded map and clinical implications. Two-dimensional color-coded maps were constructed to evaluate tissue characterization of plaque consisting of TH, LP with fibrous cap, IH, FI, ML and CL. With this technique, compositions of the intimal lesion were easily detected visually. As this method is noninvasive, long-term prospective study on prognosis of plaques with various tissue characterizations is possible. Stable and unstable plaques would be clinically evaluated.

Conclusion. Integrated backscatter values measured in vivo in human large arteries correlated well with postmortem histologic classification. This new noninvasive technique using IB and conventional 2DE is useful in characterizing the 2D structures in arterial intimal plaques in vivo.

REFERENCES