Quantitative Ultrasonic Tissue Characterization Can Identify High-Risk Atherosclerotic Alteration in Human Carotid Arteries

Shin Takiuchi, MD; Hiromi Rakugi, MD; Katsuya Honda, MD; Tohru Masuyama, MD; Nobuaki Hirata, MD; Hiroshi Ito, MD; Ken Sugimoto, MD; Yoshihiro Yanagitani, MD; Koichi Moriguchi, MD; Atsunori Okamura, MD; Jitsuo Higaki, MD; Toshio Ogihara, MD

Background—Recently, ultrasonic tissue characterization of the composition of plaques has been performed in a quantitative fashion on the basis of integrated backscatter (IBS) analysis, but most of those studies have used high-frequency ultrasound to obtain microscopic images.

Methods and Results—We performed B-mode measurement and IBS signal analysis with acoustic densitometry with a 7.5-MHz linear-array transducer in freshly excised human aortas (n=58) (normal, atheromatous, and fibrous tissue) obtained at autopsy. Atheromatous and fibrous tissue had a similar intima-media thickness (IMT), but the IBS value in atheromatous specimens was lower than that in fibrous specimens. We further applied this method to human carotid ultrasonography. The subjects were young (80 regions), middle aged with 1 or no coronary risk factors (low risk) (120 regions), middle aged with ≥2 coronary risk factors (high risk) (240 regions), or elderly (80 regions) or were patients with myocardial infarction (MI) with multivessel disease (90 regions). The IMT was similar in middle-aged, elderly, and MI subjects. In contrast, the IBS value was significantly higher in elderly subjects and lower in high-risk middle-aged and MI subjects compared with that in low-risk middle-aged subjects. The percent of regions diagnosed as atheromatous (IBS less than mean minus 2-SD value of IBS in young subjects) was 11% in low-risk middle-aged subjects, 29% in high-risk middle-aged subjects, and 63% in the MI group.

Conclusions—In conjunction with conventional B-mode imaging, IBS analysis with carotid ultrasonography appeared to provide prognostic information to identify a high-risk group with systemic atherosclerosis, which could lead to coronary heart disease in individuals with early-stage disease. (Circulation. 2000;102:766-770.)

Key Words: atherosclerosis ■ ultrasonics ■ plaque ■ arteries ■ myocardial infarction

Diagnostic ultrasonography is increasingly used as a noninvasive method to study atherosclerosis in the carotid and femoral arteries. The thickness of the carotid intima-media complex has been shown to be related to cardiovascular risk factors in cross-sectional studies 1 and to the occurrence of coronary artery disease. 2,3 The ulcerative formation of atherosclerotic lesions is thought to be related to the incidence of cerebrovascular disease. 4 Thus, a conventional diagnostic ultrasound system that includes B-mode and color and pulsed Doppler imaging provides excellent clinical information about the vessel wall in terms of characterization of the dimension and flow pattern of the arteries. However, structural alterations of the vessel wall, such as fibrosis, calcification, or deposition of lipids, cannot be accurately identified with these methods. The development of occlusive or mural thrombi, which are associated with fissured atherosclerotic plaques, is the most common cause of acute cerebral and myocardial infarction, and plaque rupture is dependent on the content of lipid in the atheroma and the thickness of the fibrous cap. 5,6

One approach to quantitative evaluation of the composition of plaque is to define its acoustic propagation properties through the estimation of native radiofrequency signals from the tissue. The measurement of integrated backscatter (IBS) is based on an analysis of unprocessed radiofrequency signals to derive quantitative ultrasonic indexes with which to differentiate normal from pathological myocardial structures. 7,8 Quantitative ultrasonic tissue characterization of the biophysical composition of plaque and the vessel wall has been performed with high-frequency, high-resolution acoustic microscopy in vitro 9-12 and with a single focused transducer. 13,14 However, these tools are available only in an experimental

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setting and cannot be used to clinically detect vascular signals in humans.

Acoustic densitometry is a clinically applicable ultrasonic backscatter imaging technology that provides an integrated on-line capability to measure, display, and analyze the average acoustic image intensity within a region of interest (ROI). In the present study, we examined the use of this technology in the clinical setting by comparing its results with pathological findings. First, we compared ultrasonic tissue characterization data of excised specimens of human aorta with histological findings. Then, we carried out ultrasonic tissue characterization of the human carotid arterial wall in the clinical setting to compare IBS among different age groups and to investigate the effect of coronary risk factors and the existence of coronary artery disease on IBS.

Methods

Experimental Specimens
Fifty-eight fresh samples of arterial wall were obtained from human aortas at autopsy. The average time from autopsy to ultrasonic examination was ~6 hours. Sections of thoracic and abdominal aorta were opened longitudinally, and full-thickness samples (~1×3 cm) were excised. The samples were mounted flat with the endothelial side up between 2 echo pads (Aquaplex; Parker Laboratories, Inc) that adhered to each other, placed on a steel plate, and positioned at the focus of the transducer. The thickness of the echo pad was ~1.5 cm. The institutional ethics committee approved the study protocol, and informed consent was obtained from the relatives of each patient.

Subjects for Carotid Ultrasonography
Eighty-eight fresh samples of carotid wall were obtained from human aortas during autopsy. The average time from autopsy to ultrasonic examination was ~6 hours. Sections of thoracic and abdominal aorta were opened longitudinally, and full-thickness samples (~1×3 cm) were excised. The samples were mounted flat with the endothelial side up between 2 echo pads (Aquaplex; Parker Laboratories, Inc) that adhered to each other, placed on a steel plate, and positioned at the focus of the transducer. The thickness of the echo pad was ~1.5 cm. The institutional ethics committee approved the study protocol, and informed consent was obtained from the relatives of each patient.

Table 1. Clinical Characteristics of Subjects in Five Groups

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<tr>
<th>Characteristic</th>
<th>Young</th>
<th>Middle-Aged Low Risk</th>
<th>Middle-Aged High Risk</th>
<th>MI</th>
<th>Elderly</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>12</td>
<td>24</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Age, y</td>
<td>28 ± 1</td>
<td>48 ± 2</td>
<td>43 ± 1</td>
<td>63 ± 3</td>
<td>79 ± 2</td>
</tr>
<tr>
<td>Male, %</td>
<td>75</td>
<td>75</td>
<td>71</td>
<td>67</td>
<td>50</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>0</td>
<td>25</td>
<td>75</td>
<td>78</td>
<td>50</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>0</td>
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<td>Dyslipidemia, %</td>
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<td>75</td>
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<td>25</td>
</tr>
<tr>
<td>Smoker, %</td>
<td>25</td>
<td>17</td>
<td>67</td>
<td>56</td>
<td>13</td>
</tr>
</tbody>
</table>

Low risk indicates subjects with 1 or no coronary risk factor (hypertension, diabetes mellitus, dyslipidemia, and smoking habit); high risk, subjects with ≥2 coronary risk factors; MI, patients who had a multivessel MI within the past 3 months.

Values expressed as percent incidence could not be statistically compared because the minimum expected value on a chi² test was <5.

Acquisition and Analysis of IBS Data in the Experimental Setting
For each specimen, conventional B-mode images of optimal image quality were first obtained with the 7.5-MHz linear-array probe, and then 60 two-dimensional IBS images were acquired into cine-loop memory and stored on optical disk. The image preprocessing, transmit power, focus, and time-gain compensation settings were adjusted to yield optimal images from the specimens, and the system controls were unchanged for measurements of all specimens. For analysis of the image data, the backscatter images were first retrieved from disk into the system memory. In this study, we used 11×11 or 21×21 pixel rectangular-shaped ROIs and placed them in the intima-media complex of the vessels. The average power of the IBS signal contained within the ROI was measured and displayed in dB for a total of 60 time frames. The system automatically calculated the average value of the IBS signal, which was also displayed in dB.

In advanced plaque specimens, IBS data were sampled from the ROI, with care taken to exclude calcified regions, which were more readily identified with conventional B-mode ultrasound. The relative IBS values of the adventitia of pathologically different samples were almost the same within ±2 risk factors. All elderly patients had ≥2 coronary risk factors. Patients with MI had ≥2 risk factors. The average duration between the examination and the onset of MI was 1.5 ± 0.2 months. Patient characteristics for each group are shown in Table 1. An excellent image of the bilateral common carotid arteries was obtained in all patients, and all showed no obvious atherosclerotic plaques or calcified lesions. The hospital ethics committee approved the study protocol, and informed consent was obtained from each patient.

Fundamentals of Acoustic Densitometry
We used a special software package, acoustic densitometry, that is available as an option with the Hewlett-Packard SONOS 5500 equipped with a 7.5-MHz linear-array transducer (axial resolution 0.1 mm). This system is capable of providing either conventional 2-dimensional envelope-detected echocardiographic images or IBS images in which the gray level is displayed proportional to the integrated backscattered power. A maximum of 60 frames displayed at a real-time frame rate of 30 Hz (30 frames/s) are captured into cine-loop memory and subsequently stored on optical disk in a digital format with the same resolution as the scan converter memory (512×512, 8 bits). The return echoes that impinge on the individual elements of the phased-array transducer are amplified, mixed to the intermediate frequency, and summed with appropriate time delays to obtain a focused beam. This intermediate frequency signal, which is uniquely related to the radiofrequency signal, is sent to either a standard ultrasound video processing chain or a special IBS processor before scan conversion. With the use of specialized hardware, the IBS processor computes the IBS power along each scan line before scan conversion and real-time display on the ultrasound monitor. The resulting image is relatively free of random acoustic speckle (noise) because of the local averaging or integration of the backscatter signal power along each scan line. The IBS image is internally calibrated in dB and has a dynamic range of ~64 dB in the SONOS 5500 system. This system has a unique feature in which the transmit power, log compression, and time-gain compensation values are displayed on a screen (and can be stored with the images), which allows an operator to adjust the system to the same values at every examination.

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Values expressed as percent incidence could not be statistically compared because the minimum expected value on a chi² test was <5.
from the IBS value obtained from a reference ROI placed within the adventitia (calibrated IBS value [dB]).

Acquisition and Analysis of IBS Data in Clinical Setting

We obtained data of intima-medial thickness (IMT) in 5 consecutive regions every 5 mm from the bifurcation of the common carotid artery with use of the 7.5-MHz linear-array probe. A total of 10 regions from the bilateral carotid arteries were examined in each person. IBS data were also sampled in the same regions in which IMT was measured. We also used 11×11 or 21×21 pixel rectangular-shaped ROIs and placed them in the intima-media complex of the vessels. Data analysis was performed with Acoustic Densitometry in the same manner. In the present study, the same relative TGC profile was used for all subjects. We also expressed the relative IBS value of the intima-media complex as the difference from the IBS value obtained from a reference ROI placed within the adventitia (calibrated IBS value [dB]). Absolute IBS values of the adventitia were between 52 and 55 dB, which were within the dynamic range of the SONOS 5500 system.

Reproducibility of Data
We determined the intraobserver and interobserver variabilities of IBS value of the tissue in 10 randomly selected recordings twice by the same observer and once each by 2 independent observers. Intraobserver and interobserver variabilities of IBS value were 2.3±0.3% and 2.5±0.2% in the experimental study and 2.3±0.1% and 2.0±0.2% in the clinical study, respectively.

Histological Analysis
Representative tissue specimens were fixed in formalin and sectioned through the midpoints of lesions where radiofrequency data were recorded. The tissue was embedded in paraffin, and 5-μm sections of each sample were studied histologically with hematoxylin and eosiin, and Masson’s trichrome stains. The structural composition of the aortic wall at each site of ultrasonic interrogation was determined through sequential examination of the subserial sections of each specimen. Two experienced pathologists who were blinded to the echographic results evaluated each tissue sample of arterial wall and characterized them histologically into 1 of 3 categories: (1) normal tissue specimen, (2) fibrous tissue specimen (wall thickened by connective tissue), and (3) atheromatous tissue specimen (characterized by accumulation of lipid in the intima or by a fibrous cap and a lipid core). Moreover, we measured the IMT with the photographs of each specimen. Specimens with significant necrosis and hemorrhage were not considered. Thickness was measured at 5 points in the center of each specimen to obtain the average value. With a thin steel wire as a spatial marker, the transducer was oriented toward the center of the specimen. Thus, only the central value. With a thin steel wire as a spatial marker, the transducer was measured at 5 points in the center of each specimen to obtain the average value.

Statistical Analysis
Data were expressed as mean±SEM. For all statistical analyses, we used a computer software application, StatView (Abacus Concepts Inc). Values expressed as percent incidence were compared by χ² test when the minimum expected value was >5. Values expressed as mean±SEM were compared by 1-way ANOVA, followed by Fisher’s protected least significant difference test. Values of P<0.05 were considered statistically significant.

Results

Experimental Study

IBS Analysis of Excised Aortas and Comparison With Histological Findings
Histological examination showed that 58 samples consisted of 16 normal regions, 18 atheromatous lesions, and 24 fibrous lesions. The mean values of IBS and IMT for the 3 histological categories are shown in Table 2. The mean IMT was significantly thinner in normal regions than in atheromatous and fibrous lesions (P<0.01). The mean IBS of the fibrous and atheromatous lesions was the same. The mean value of calibrated IBS in normal regions was significantly lower than that in fibrous lesions (P<0.01). The mean value of calibrated IBS in atheromatous lesions was significantly lower than that in fibrous lesions (P<0.001) and normal regions (P<0.01) (Table 2). IBS in atheromatous lesions differed from that in fibrous lesions, although IMT values were identical. Figure 1 shows the data for calibrated IBS values and IMT in each specimen in the 3 histological groups. We classified most specimens as 1 of the 3 categories of tissue according to ultrasonic tissue characterization in conjunction with thickness measurement.

In Vivo Study

Carotid IMT and IBS Data In Vivo
Mean IMT and calibrated IBS values are depicted in Figure 2. IMT gradually thickened with age, and IMT in the MI group was significantly thicker than that in young and middle-aged subjects. In the middle-aged group, IMT in patients with fewer risk factors was similar to that in patients with more risk factors. Of interest, the calibrated IBS values in the high-risk middle-aged group and MI groups were significantly lower than those in the young and low-risk middle-aged groups. In contrast, the calibrated IBS value in elderly patients was significantly higher than that in any other group.

To investigate the distribution of calibrated IBS values in each group, we defined the normal range of calibrated IBS according to the IBS value in young healthy subjects. The values for mean±2 SDs and mean±1.96 SDs of IBS in young healthy subjects were −14.3 and −9.0 dB, respectively. As presented earlier with the histological comparison, it is likely that relatively fatty tissue shows a lower IBS value than

### Table 2. IMT and Calibrated IBS for Atherosclerotic Lesions

<table>
<thead>
<tr>
<th>Type of Plaque</th>
<th>IMT, mm</th>
<th>Value of Calibrated IBS, dB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n=16)</td>
<td>0.40±0.02</td>
<td>−11.9±0.6</td>
</tr>
<tr>
<td>Atheromatous lesion (n=18)</td>
<td>0.75±0.05*</td>
<td>−14.5±1.0*</td>
</tr>
<tr>
<td>Fibrous lesion (n=24)</td>
<td>0.63±0.04*</td>
<td>−8.1±3.0†</td>
</tr>
</tbody>
</table>

*P<0.01 vs normal segment.
†P<0.001 vs atheromatous lesion.
Values are mean±SEM.

![Figure 1. Plots of IMT (horizontal axis) and calibrated IBS value (C-IBS; vertical axis) for each sample. Normal segment. Atheromatous segment. Fibrous segment.](image)
Mean 1.2 SDs of IBS in young healthy subjects were compared with normal range in young subjects. Mean 2 SDs and 2 SDs of IBS in young healthy subjects were –14.3 dB and that fibrous tissue shows a higher IBS value than –9.0 dB. Figure 3 shows the distribution of calibrated IBS values in each group. Lesions with a higher IBS value were more frequent and lesions with a lower IBS value were less frequent in elderly patients than in middle-aged patients. In the high-risk middle-aged patients and MI patients, there were more lower-IBS lesions than in the low-risk middle-aged patients. MI patients showed more frequent lower-IBS lesions than did high-risk middle-aged patients.

Discussion

The results of the present study confirm that IBS analysis is indeed an effective ultrasonic technique with which to distinguish atheromatous (fibrofatty) tissue from fibrous tissue in experimental settings and further demonstrated that acoustic densitometry with a 7.5-MHz linear-array transducer can quantitatively identify high-risk patients with unstable atherosclerosis in a clinical setting.

According to the ultrasonic diagnosis of vascular composition, conventional B-mode analysis was applied first. Grønholdt et al 17 demonstrated that triglyceride-rich lipoprotein level in the fasting, postprandial state can be used to predict carotid plaque echolucency and that echolucency was associated with a high lipid content of plaque. Beletsky et al 18 performed densitometric analysis of the B-mode images of carotid plaques in experimental settings and further demonstrated that acoustic densitometry appeared to provide reliable information about plaque composition in the clinical setting.

Figure 3 shows the distribution of calibrated IBS data (especially lower values) in patients who underwent endarterectomy. Digital densitometric evaluation in both studies allowed differentiation of the various possible components of carotid plaques, but densitometric analysis was subject to inherent nonlinear transformations in the ultrasound imaging chain that resulted from log compression and postprocessing functions.

There are several reports on the usefulness of IBS in the determination of tissue composition of atherosclerotic plaques through the use of experimental methods. 9, 10 Wickline et al 10 reported that the IBS of intimal fatty plaques was >10-fold less than that of intimal fibrous plaques, according to ultrasonic microscopy. Picano et al 14 and Barzilai et al 15 examined human aortas with complex plaques that had a lower frequency and lower resolution ultrasonic systems (10 MHz) and measured IBS within a 2-μs window (≈1.5-mm tissue thickness). They reported that arterial segments with extensive calcification demonstrated greater backscatter than predominantly fibrofatty, fibrous, or normal aortas. Although the sensitivity of the system used in the present study was lower due to lower axial resolution, we could distinguish these 3 types of aortic tissue from each other with acoustic densitometry in conjunction with IMT assessment through conventional B-mode imaging. Acoustic densitometry appeared to provide reliable information about plaque composition in the clinical setting.

Of more interest, the present technique in the clinical setting revealed that risk factors for coronary heart disease were related to the incidence of atheromatous plaque in the carotid arteries. This is quite important because previous reports have shown that weak reflection of ultrasound from a carotid atheromatous plaque is associated with a high lipid content, as well as with an increased risk of cerebral infarction. 19, 20 Several studies have suggested that plaque rupture is related to the lipid content of plaques. Ultrasonic parameters of the carotid artery (IMT, presence of plaque) are considered to be sensitive markers of the earliest stage of systemic atherosclerosis. 1–3, 21–23 In the present study, thick IMT lesions were observed in middle-aged and elderly patients, but a wide distribution of IBS data (especially lower values) was seen only in the high-risk middle-aged group. In addition, the same trend was clearly observed in patients with multivessel disease who had recently had an MI. These findings indicate that it is possible to identify a high-risk group with systemic atherosclerosis, which is a high risk factor for coronary heart attack and stroke, through the use of IBS analysis in conjunction with conventional IMT measurement.

The extrapolation of these findings to in vivo conditions is fraught with several technical problems, including the necessity for perfect alignment of the ultrasound beam, the necessity to account for tissue anisotropy, variable attenuation introduced by anatomic structures between the skin surface (transducer) and the arterial wall, and insufficient dynamic range (signal sensitivity). The present system does not have a sufficient dynamic range to measure the absolute value of IBS (ie, backscatter of plaque/tissue with reference to the backscatter from an adjacent blood pool or vascular cavity). In the present study, we adopted the adventitia of the carotid artery as a reference object to calculate calibrated IBS value. There has not been an established standard reference tissue for quantitative ultrasonic tissue characterization with IBS.
analysis in vivo. Some investigators used the IBS signal from the vessel lumen or left ventricular cavity as a reference object. However, the relative IBS values of the adventitia of pathologically different samples in our ex vivo study were almost same when those values were calibrated with the IBS value of a fixed reference object beside each sample (data not shown). Therefore, adventitia could be one appropriate candidate for a reference object in this study. We could not recognize a difference between IBS of the intima and media due to insufficient axial resolution of the present system. Our data reflect the sum of backscatter signal from the intima-media complex. Improvement in the axial resolution will increase the sensitivity to distinguish atheromatous plaque and fibrous plaque.

At the present time, we can estimate the IBS value of the intima-media complex for a follow-up examination of anti-atherosclerotic therapy, and if the acoustic densitometry analysis package were transferred to an intravascular ultrasound system with >1 order of magnitude improvement in resolution, we could potentially evaluate the stability of the plaque and obtain important information to determine the treatment strategy. This relatively simple, repeatable, and quantitative data analysis will enable us to evaluate the biological and pathophysiological status of an atherosclerotic lesion in the long-term follow-up of vascular structure as a “noninvasive ultrasonic biopsy.”

Acknowledgments

We are indebted to Alwyn D’sa (Hewlett-Packard) and Kenji Kikuda (Kikuda Ltd) for helpful discussion.

References

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