Coronary Artery Imaging With Intravascular High-Frequency Ultrasound

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Safe and effective clinical application of new interventional therapies may require more precise imaging of atherosclerotic coronary arteries. To determine the reliability of catheter-based intravascular ultrasound as an imaging modality, a miniaturized prototype ultrasound system (1-mm transducer; center frequency, 25 MHz) was used to acquire two-dimensional, cross-sectional images in 21 human coronary arteries from 13 patients studied at necropsy who had moderate-to-severe atherosclerosis. Fifty-four atherosclerotic sites imaged by ultrasound were compared with formalin-fixed and fresh histological sections of the coronary arteries with a digital video planimetry system. Ultrasound and histological measurements correlated significantly (all \(p<0.0001\)) for coronary artery cross-sectional area \((r=0.94)\), residual lumen cross-sectional area \((r=0.85)\), percent cross-sectional area narrowing \((r=0.84)\), and linear wall thickness (plaque and media) measured at 0°, 90°, 180°, and 270° \((r=0.92)\). Moreover, ultrasound accurately predicted histological plaque composition in 96% of cases. Anatomic features of the coronary arteries that were easily discernible were the lumen-plaque and media-adventitia interfaces, very bright echoes casting acoustic shadows in calcified plaques, bright and homogeneous echoes in fibrous plaques, and relatively echo-lucent images in lipid-filled lesions. These data indicate that intravascular ultrasound provides accurate image characterization of the artery lumen and wall geometry as well as the presence, distribution, and histological type of atherosclerotic plaque. Thus, ultrasound imaging appears to have great potential application for enhanced diagnosis of coronary atherosclerosis and may serve to guide new catheter-based techniques in the treatment of coronary artery disease. (Circulation 1990;81:1575–1585)

Although coronary arteriography provides adequate imaging for routine diagnostic studies, more precise definition of lumen surface characteristics and identification of transmural components of atherosclerotic coronary arteries may be an important adjunct to standard angiography. Alternative imaging modalities may be especially useful for guidance during new experimental angioplasty procedures such as mechanical atherectomy, placement of expandable permanent stents, and laser angioplasty. The potential value of ultrasound to characterize the morphological features of cardiovascular tissue has been extensively studied in recent years.1–3 Ultrasound tissue characterization can detect the presence and severity of atherosclerotic lesions as well as differentiate normal from fatty, fibrous, and calcified regions within the vessel wall.4–6 Transepicardial high-frequency ultrasound has been used intraoperatorively to image coronary artery anatomic features.7–9 In addition, recent studies suggest that catheter-based intraluminal ultrasound imaging provides similar high-resolution images of blood vessel wall architecture.10–15 The present report is a feasibility and validation study comparing the capability of quantitative histological findings with miniature intravascular high-frequency ultrasound to discern vessel wall geometry and structural composition of diseased necropsy coronary arteries.

Methods

Ultrasound Probe

Ultrasound images were obtained with a prototype intravascular imaging system (InterTherapy, Costa
Mesa, California). A 1-mm diameter transducer ele-
ment (center frequency, 25 MHz), acting as transmitter and receiver, was mounted in a rigid probe (outer diameter, 1.5 mm) designed for in vitro imaging (Figure 1). With pulse-echo techniques, the ultrasound beam was reflected perpendicular to the long axis of the probe by a mirror positioned at 45°. Two-dimensional, cross-sectional images of the coronary artery segments were created by manual rotation of the ultrasound probe inside the vessel lumen.

Ultrasound and Histological Examination

A total of 32 coronary artery 1.5-cm segments were excised from 21 epicardial coronary arteries (three left main, eight left anterior descending, one left circumflex, seven right, one ramus intermedium, and one first diagonal) from 13 patients studied at necropsy (seven men and six women). The mean age at death was 64±17 years (range, 30–83 years). Five patients died from acute myocardial infarction, two from cardiogenic shock after coronary bypass graft surgery, and six from noncardiac causes (three sepsis, two neoplasm, and one stroke). Of the 32 coronary segments, 22 had been excised from heart specimens that were preserved in 10% formaldehyde, and the remaining 10 artery segments were excised from a fresh unfixed heart and later preserved with formaldehde after the ultrasound scanning was performed.

Each of the 32 coronary artery segments was mounted vertically on a stage with cyanoacrylate ester glue applied to the distal end of the excised vessel. A 27-gauge stainless-steel needle was inserted transversely into the adventitia in 58 sites of the 32 mounted artery segments (Figure 2, bottom). The transverse needle served as a spatial marker to precisely identify the ultrasound imaging site that would later be examined by histological study. Each of the 32 mounted coronary segments was placed in a beaker of water (or 0.9% saline for the fresh unfixed specimens), and the ultrasound probe was advanced into the residual lumen with an X-Y-Z micropositioner (1.3 cm travel, 0.025 mm accuracy) until the needle marker was clearly imaged (Figure 2, top). Two potentiometers, placed around the probe, were used to define the position of the ultrasound trans-
ducer in relation to the artery segment. Manual rotation of the probe inside the artery resulted in a cross-sectional image of the artery wall at the needle marker site. The hard copy images were stored on a computer file for digital processing and subsequent analysis.

Of the 58 imaged sites, 14 (24%) were imaged before (fresh) and 5 days after fixation in 10% formaldehyde; the other 44 sites were imaged only in the formaldehyde-preserved state. After all the ultrasound images were obtained, each vessel segment with two needle markers (n=26) was cut transversely in two portions, 2–5 mm above and below the imaged site marked with the needle. Therefore, a total number of 58 coronary artery segments was obtained for histological processing. A 1.5-mm diameter stain-
less steel probe (the same size as the ultrasound probe and housing) was inserted into the residual lumen of any collapsed arteries to help maintain the geometric dimensions present when the vessel was imaged by the ultrasound probe. All 58 artery seg-
ments were then processed in alcohols and xylene; 24 segments had also required partial decalcification for 24 hours in a formic acid and sodium citrate solution to avoid crush artifacts during microtome sectioning. Care was used in preserving the spatial orientation of the vessel specimens when, before paraffin embed-
ning, the needle marker was removed. Of the 58 artery segments, two had lost position markers in the tissue processing, and two were destroyed during the embedding process leaving 54 coronary segments for histological analysis. Each of the remaining 54 seg-
ments was serially sectioned (5 μm thick), and every 50th section was placed on a glass slide and stained with a Movat pentachrome stain to identify anatomic features of the intima, media, and adventitia.16

Quantitative Ultrasound and Histological Measurements

Ultrasound images and the corresponding histo-
logical sections were analyzed with a digital video analyzer (Magiscan, Nikon, Instrument Division,
FIGURE 2. Photograph of the apparatus for in vitro ultrasound imaging of the coronary arteries (top panel). The stage for mounting coronary segments (A), the ultrasound probe (B), and the positioning potentiometers (C) are shown. A proximal right coronary artery segment glued on the stage is shown in long axis (left) and short axis (right) view (bottom panel). Two 27-gauge needles were inserted as spatial markers, 0.75 cm apart from one another, within the adventitia.

Garden City, New York). Each image was acquired by a television camera, linked to a light microscope (×10) for the histological specimen evaluation, and reproduced on the analyzer video screen where perimeters and linear dimensions were traced with a light pen. Areas (mm²) and linear dimensions (mm)
were then calculated by the computer from the tracings. Ultrasound cross-sectional area, which was representative of the coronary artery cross-sectional area (area confined within the external elastic membrane), and the residual lumen cross-sectional area were measured from the ultrasound images and compared with the corresponding histological areas (Figure 3). Linear dimensions of the wall (plaque and media) thickness at 0°, 90°, 180°, and 270° were also measured from the ultrasound images and histological sections (Figure 3). Percent cross-sectional area narrowing caused by atherosclerotic plaque was calculated from the equation: [(coronary artery CSA – residual lumen CSA)/coronary artery CSA]×100.

**Tissue Characterization**

Skills in interpretation of arterial wall structure and plaque composition were learned after repetitive comparisons between the first 54 ultrasound images and the corresponding histological specimens. Histological analysis of specimens indicated that all plaques contained variable amounts of fibrous tissue. Plaques with only fibrous constituents were classified as fibrous. Plaques with fibrous components and discrete areas of extracellular lipid material or calcific deposits were classified as lipid filled or calcified, respectively.

To test the predictive accuracy of ultrasound imaging for tissue characterization, 28 arterial segments excised from 12 additional coronary arteries (seven right coronary, two left anterior descending, one left circumflex, one patent ductus, and one left main) from four patients (two men and two women; mean age at death, 63 ± 18 years) were studied. A total of 28 fresh coronary segments were imaged by ultrasound and then processed with the technique previously described. Plaque composition by ultrasound was determined by an investigator unaware of the histological classification in 112 quadrants obtained by dividing each of the 28 ultrasound images into four equal quadrants. Ultrasound prediction was then compared with the histological analysis previously performed by a second investigator on the corresponding 112 histological quadrants.

**Reproducibility**

Intraobserver and interobserver variability of ultrasound and histological measurements was determined by remeasuring 26 of 54 coronary artery cross-sectional areas, lumen cross-sectional areas, and linear dimensions without knowledge of the original values. The second measurements were performed after 1 month by the original examiner and by a different observer.

**Statistical Analysis**

Correlations were determined by a linear regression analysis for two variables. The regression lines were compared with the line of identity (slope = 1, y intercept = 0) for each correlation to test the level of significance. Analysis of variance was used to test for significant differences among sample means and variances. A probability value less than 0.05 was considered significant.

**Results**

**Arterial Wall Structure and Plaque Characterization**

Ultrasound images of the coronary arteries were presented as a two-dimensional, 360° display of vessel cross-section perpendicular to the long axis of the probe. The typical image pattern consisted of three concentric layers around an echo-free lumen that could be clearly distinguished by sharp changes in ultrasound reflecting properties (Figures 4, 6, and 7). The ultrasound scanning provided an accurate description with high resolution of lumen structure and lumen-intima interface in all vessel specimens.
FIGURE 4. Photomicrograph (×27) of a histological section from the right coronary artery (left panel) and the corresponding ultrasound images before (center panel) and 5 days after fixation with 10% formaldehyde (right panel). The ultrasound image shows a typical concentric three layer pattern consisting of plaque-intima, media, and adventitia. No significant qualitative or quantitative differences are discerned after tissue fixation.

FIGURE 5. Photomicrograph (×24) of a histological section from the left anterior descending coronary artery showing a calcified plaque (black arrow) (left panel). In the corresponding ultrasound image (right panel), the very bright echoes due to the calcium deposits (white arrow) and the acoustic shadow behind the calcium (bracket) are visible. Also shown in the ultrasound image are the echo reverberations from the needle marker inserted in the adventitia (at the seven o'clock position).
FIGURE 6. Photomicrograph (×26) of a histological section from the left anterior descending coronary artery showing concentric mural thickening by a thin fibrous plaque (left panel). The bright and homogeneous echoes, characteristic of fibrous tissue, are shown in the corresponding ultrasound image (right panel). Also, the thin internal elastic membrane (IEM) is represented by a thicker echo-dense zone on the ultrasound image.

(FIGURES 3–8). The tunica media was seen as an echo-lucent zone between the more intense echoes of the intima and adventitia laminae (Figures 4, 6, and 7). The junction between media and adventitia (external elastic membrane) could be identified in all 54 segments (Figures 3–8). However, the junction between intima and media (internal elastic membrane) could be clearly seen only in less-diseased coronary arteries that had minimal or moderate fibrous intimal thickening. In these arteries, the intima-media interface, corresponding histologically to the internal elastic membrane, appeared as a thin echo-dense layer (Figures 4, 6, and 7).

From comparative analyses between the first 54 ultrasound images and corresponding histological sections, plaque morphological subtypes were characterized. Plaques with calcific deposits were clearly identified by the presence of bright echoes casting echo-free shadows onto deeper tissue zones (Figure 5). Fibrous lesions yielded dense, homogeneous echo reflections without echo-free shadowing (Figures 4, 6, and 7), whereas extracellular lipid components were much less echogenic (Figure 8).

The histological analysis of the 112 quadrants obtained from the 28 additional arterial sections showed that 84 (75%) were composed of fibrous tissue, 19 (17%) were composed of calcific deposits, and nine (8%) were composed of lipid. Of the 84 fibrous plaque quadrants areas, 81 (96%) were correctly identified by ultrasound. Of the three areas incorrectly diagnosed, the low echo density of the images, probably due to technical echo dropout, was erroneously interpreted as lipid deposits. Of the 19 calcific plaque quadrants areas, all were correctly identified by ultrasound. Of the nine lipid quadrants areas, seven (78%) were correctly identified by ultra-
sound. The two (22%) areas incorrectly diagnosed were identified as fibrous plaque with echo dropout rather than lipid deposits. Thus, ultrasound accurately predicted histological plaque composition in 96% of quadrants analyzed.

**Coronary Artery Cross-Sectional Area**

The mean coronary artery cross-sectional area of the 54 coronary artery sites was $9.43 \pm 3.70$ mm$^2$ by ultrasound and $8.25 \pm 3.18$ mm$^2$ by histological analysis ($r=0.94$, $p=0.0001$) (Table 1, Figure 9A). The histological area was smaller than the corresponding ultrasound area in 43 of 54 segments (80%), and the average decrease was 10±13%.

**Residual Lumen Cross-Sectional Area**

The mean residual lumen cross-sectional area of the 54 coronary artery sites was $2.65 \pm 1.19$ mm$^2$ by ultrasound and $2.86 \pm 0.90$ mm$^2$ by histological analysis ($r=0.85$, $p=0.0001$) (Table 1, Figure 9B). The histological lumen area was equal to or larger than the corresponding ultrasound lumen area in 39 of 54 sites (72%), and the average increase was 18±36%.

**Percent Narrowing of Cross-Sectional Area**

The mean percent narrowing of cross-sectional area by plaque of the 54 coronary artery sites was 70±10% by ultrasound and 63±11% by histological analysis ($r=0.84$, $p=0.0001$) (Table 1, Figure 9C). The histological percent area narrowing was less than the corresponding ultrasound area narrowing in 48 of 54 sites (89%), and the average decrease was 11±9%.

**Wall Thickness**

The mean wall thickness (plaque and media) of the 54 coronary artery sites (216 measurements obtained at 0°, 90°, 180°, and 270°) was $0.75 \pm 0.38$ mm by ultrasound and $0.61 \pm 0.36$ mm by histological analysis ($r=0.92$, $p=0.0001$) (Table 2, Figure 9D). The histological wall thickness was less than the corresponding ultrasound wall thickness at 184 of 216 sites (85%), and the average decrease was 19±19%. An excellent correlation was also found when ultrasound and histological measurements performed at the four different locations were compared individually ($r=0.85$ at 0°, 0.91 at 90°, 0.94 at 180°, and 0.91 at 270°; $p=0.0001$ for each).

**Intraobserver and Interobserver Variability**

The mean percent difference between the initial and the second measurement by the same examiner for ultrasound images and histological sections, respectively, was $-0.5 \pm 5\%$ and $1 \pm 3\%$ for coronary artery cross-sectional area, $2 \pm 9\%$ and $2 \pm 2\%$ for residual lumen cross-sectional area, and $-3.6 \pm 18\%$ and $0.1 \pm 6\%$ for wall thickness. The mean percent difference between the two different observers for ultrasound images and histological sections, respectively, was $2.8 \pm 7\%$ and $0.2 \pm 1\%$ for coronary artery cross-sectional area, $1.8 \pm 15\%$ and $2 \pm 9\%$ for residual lumen cross-sectional area, and $12 \pm 15\%$ and $0.9 \pm 12\%$ for wall thickness.

**Effect of Formaldehyde Fixation and Tissue Processing**

In 14 arterial sites from 10 coronary segments, no significant qualitative or quantitative differences were noted when imaging of fresh specimens was repeated after 5 days of fixation with 10% formaldehyde (Figure 4). The mean percent difference between the fresh and fixed coronary artery measurements, respectively, was $4 \pm 5\%$ for coronary artery cross-sectional area, $2 \pm 10\%$ for residual lumen cross-sectional area, and $1 \pm 13\%$ for wall thickness.

On the other hand, analysis of the first 54 arterial sites indicated that tissue processing after fixation elicited important systematic changes in vessel wall dimensions. The histological cross-sectional area and

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**Figure 8.** Photomicrograph ($\times 27$) of a histological section from the right coronary artery showing a fatty plaque that is partially dissolved by tissue processing (left panel). The relative echo lucency of the lipid material is discernible in the corresponding ultrasound image (right panel).
wall thickness were reduced after tissue processing an average of 10% \( (p<0.001) \) and 19% \( (p<0.001) \), respectively, compared with the ultrasound images. In contrast, lumen cross-sectional area was 18% larger than the ultrasound areas \( (p<0.05) \).

**Discussion**

Contrast angiography of coronary arteries is currently the standard technique for in vivo quantification of the presence and severity of coronary atherosclerosis. However, several studies have provided anatomic and physiological evidence that angiography consistently underestimates the severity of coronary atherosclerosis.\(^17\)\(^{-19}\) These findings result from the inaccuracy of visual interpretation based on axial contrast angiograms. Angiography estimates coronary diameter narrowing as the difference between markedly narrowed and adjacent less narrowed sites without considering the absolute lumen size or the true cross-sectional area of the vessel. In addition, there is evidence that atherosclerosis is associated with vessel wall dilatation, and therefore, even in diffusely diseased arteries, the residual lumen diameter may appear angiographically normal.\(^20\)\(^,21\) These important limitations suggest that standard angiography does not provide a reliable quantitative assessment of coronary atherosclerosis and is grossly inadequate for evaluating vessel wall structure. Thus, new imaging modalities with the capacity for enhanced analysis of coronary atherosclerosis may provide valuable diagnostic and pathological insights and could be used for rea-
TABLE 1. Coronary Artery Cross-Sectional Area, Residual Lumen Cross-Sectional Area, and Percent Cross-Sectional Area Narrowing by Ultrasound and Histological Analysis in 54 Coronary Artery Sites

<table>
<thead>
<tr>
<th></th>
<th>Cross-sectional area</th>
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<tr>
<td></td>
<td>Coronary</td>
<td>Lumen</td>
<td>% Narrowing</td>
<td></td>
</tr>
<tr>
<td>Ultrasound</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>9.43±3.70</td>
<td>2.65±1.19</td>
<td>70±10</td>
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</tr>
<tr>
<td>Range</td>
<td>3.77–18.71</td>
<td>0.71–7.24</td>
<td>45–91</td>
<td></td>
</tr>
<tr>
<td>Histological</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>8.25±3.18</td>
<td>2.86±0.90</td>
<td>63±11</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>3.20–17.95</td>
<td>1.53–6.17</td>
<td>31–85</td>
<td></td>
</tr>
<tr>
<td>Correlation (ultrasound vs. histological)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$r$</td>
<td>0.94</td>
<td>0.85</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>$p$</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Histological&lt;ultrasound ($n,%$)</td>
<td>43, 80</td>
<td>15, 28</td>
<td>48, 89</td>
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</tr>
<tr>
<td>Histological&gt;ultrasound ($n,%$)</td>
<td>11, 20</td>
<td>39, 72</td>
<td>6, 11</td>
<td></td>
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<tr>
<td>% Difference*</td>
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<tr>
<td>Mean</td>
<td>$-10±13$</td>
<td>18±36</td>
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<td>Range</td>
<td>$-33$ to +26</td>
<td>$-33$ to +161</td>
<td>$-33$ to +6</td>
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</tr>
</tbody>
</table>

All areas are cross-sectional measured in millimeters squared.

*% Difference = [(histological−ultrasound)/ultrasound]×100.

time guidance of new catheter-based therapeutic intracoronary devices.

Ultrasound Plaque Geometrical and Morphological Features

In this in vitro study, a new intravascular ultrasound imaging system was used to create high-resolution, two-dimensional, cross-sectional images of human atherosclerotic coronary arteries that were quantitatively and qualitatively compared with corresponding histological specimens. Significant correlations were found between the ultrasound and histological paired measurements of coronary artery cross-sectional area, residual lumen cross-sectional area, percent cross-sectional area narrowing by atherosclerotic plaque and linear wall thickness dimensions (Figure 9). In addition to these significant morphometric correlations, ultrasound images accurately predicted the plaque distribution and its histological composition in most of the examined sites.

Heavily calcified lesions were characterized by bright echoes and a significant acoustic "shadow. First, Calcium salts, having the highest acoustic impedance among biological materials,22,23 behave as total reflectors, and preclude ultrasound interrogation of deeper structures. This results in an echo-free space behind the calcified lesion. Thus, calcified coronary

TABLE 2. Wall Thickness (Intima and Media) at 0°, 90°, 180°, and 270° by Ultrasound and Histological Analysis in 54 Coronary Artery Sites

<table>
<thead>
<tr>
<th></th>
<th>0°</th>
<th>90°</th>
<th>180°</th>
<th>270°</th>
<th>Sum of all sites</th>
</tr>
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<tr>
<td>Sites ($n$)</td>
<td>54</td>
<td>54</td>
<td>54</td>
<td>54</td>
<td>216</td>
</tr>
<tr>
<td>Ultrasound</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.64±0.24</td>
<td>0.78±0.38</td>
<td>0.87±0.49</td>
<td>0.72±0.36</td>
<td>0.75±0.38</td>
</tr>
<tr>
<td>Range</td>
<td>0.24–1.33</td>
<td>0.29–1.71</td>
<td>0.26–2.76</td>
<td>0.21–1.60</td>
<td>0.21–2.76</td>
</tr>
<tr>
<td>Histological analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.49±0.21</td>
<td>0.68±0.04</td>
<td>0.71±0.45</td>
<td>0.57±0.31</td>
<td>0.61±0.36</td>
</tr>
<tr>
<td>Range</td>
<td>0.19–1.27</td>
<td>0.16–1.95</td>
<td>0.15–2.35</td>
<td>0.19–1.50</td>
<td>0.15±2.35</td>
</tr>
<tr>
<td>Correlation (ultrasound vs. histological)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$r$</td>
<td>0.85</td>
<td>0.91</td>
<td>0.94</td>
<td>0.91</td>
<td>0.92</td>
</tr>
<tr>
<td>$p$</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
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</tr>
<tr>
<td>Histological&lt;ultrasound ($n,%$)</td>
<td>49, 91</td>
<td>49, 76</td>
<td>47, 87</td>
<td>47, 87</td>
<td>184, 85</td>
</tr>
<tr>
<td>Histological&gt;ultrasound ($n,%$)</td>
<td>5, 9</td>
<td>13, 24</td>
<td>7, 13</td>
<td>7, 13</td>
<td>32, 15</td>
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<tr>
<td>% Difference*</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Mean</td>
<td>$-23±17$</td>
<td>$-15±21$</td>
<td>$-18±20$</td>
<td>$-20±18$</td>
<td>$-19±19$</td>
</tr>
<tr>
<td>Range</td>
<td>$-58$ to +25</td>
<td>$-51$ to +40</td>
<td>$-54$ to +63</td>
<td>$-53$ to +33</td>
<td>$-58$ to +63</td>
</tr>
</tbody>
</table>

Plaque thickness measured in millimeters.

*% Difference = [(histological−ultrasound)/ultrasound]×100.
plagues, though easily identified by ultrasound, impair quantitative analysis of circumferential and linear dimensions of the vessel wall.

Bright and homogeneous ultrasound reflections were also seen in lesions characterized histologically by fibrous tissue accumulation. Experimental and clinical studies have demonstrated that ultrasound properties of fibrous tissue are related to a high concentration of collagen, which has been shown to possess high acoustic reflectivity. However, because fibrous tissue has lower density than calcium deposits, and therefore less acoustic impedance, fibrous plaques do not manifest shadow artifacts and are less echo dense. Unlike calcified and fibrotic lesions, lipid deposition within the plaque was more difficult to identify by ultrasound techniques. Although lipid-filled areas have relatively less echo density than other plaque components, the ultrasound appearance of lipid lesions was variable depending largely on the presence and magnitude of surrounding fibrous tissue.

These findings are consistent with previous in vitro studies identifying the predominant constituents of atherosclerotic plaque, with quantitative ultrasound indexes based on attenuation or backscatter. These studies also demonstrated a significant increase in integrated backscatter and attenuation in calcified and fibrotic regions of human aorta; in contrast, ultrasound interrogation of lipid deposits demonstrated a reduction in both these indexes.

A constant ultrasound feature in our images was the presence of prominent lumen-intima and media-adventitia interfaces that were separated by reduced echo signals from the media. These observations have been previously reported in vitro and in vivo in normal and diseased human arteries, and this distinctive pattern appears to be caused by relatively sharp changes in acoustic impedance at these interfaces. The increased echo density of adventitia, compared with the relatively silent acoustic behavior of media, may be related either to multiple impedance mismatches from inhomogeneous loose connective tissue or to the scattering effect of collagen whose content is increased in the adventitia. In vessels with mild-to-moderate atherosclerotic disease, there was also a thin echo-dense layer at the intima-media interface corresponding histologically to the internal elastic membrane. Previous reports, based on ultrasound imaging and backscatter, have suggested that the thin elastic membranes, present within the media and oriented perpendicular to the ultrasound beam axis, result in multiple specular scattering and therefore, despite their thinness, are seen as bright reflecting structures. The external elastic membrane is less well seen as a discrete layer because of its proximity to more echo-dense adventitial structures.

**Effect of Tissue Fixation and Processing**

The influences of formaldehyde fixation on the ultrasound properties of coronary arteries were qualitatively and quantitatively minor, which is in agreement with the findings of other investigators examining fixation effects in vascular and nonvascular tissues. Of importance, significant changes of vessel geometric features were seen after tissue processing. Tissue processing (dehydration with alcohols, cleaning with xylene, and embedding in paraffin) is known to cause significant shrinkage artifacts. Nevertheless, Siegel and colleagues compared measurements in 61 human coronary artery segments before and after fixation and tissue processing. Although no significant changes were observed after fixation, tissue processing resulted in a 19% decrease in coronary artery cross-sectional area in 29 sites with less than 50% cross-sectional area narrowing and a 31% decrease in 32 sites with more than 50% cross-sectional area narrowing. Those findings are in agreement with the present study, in which histological cross-sectional area and wall thickness measurements of necropsy coronary arteries after tissue processing were reduced an average of 10% and 19%, respectively, compared with the ultrasound images. Plaque composition probably contributes greatly to the degree of shrinkage with tissue processing. In this study, although not statistically significant, the linear dimensions and vessel cross-sectional area of fibrous and fatty plaques manifested increased shrinkage after tissue processing compared with plaques containing large calcific deposits. These differences are probably due to variations in plaque water content that are directly proportional to the degree of tissue shrinkage during processing.

Unlike the coronary artery cross-sectional area and the wall thickness, the histological residual lumen cross-sectional areas of the 54 coronary sites were 18% larger than the corresponding ultrasound values. This may be explained by the much more pronounced shrinkage of the vascular tissue in the radial direction (18.7%) compared with that in the circumferential direction (0.9%), after fixation and processing, resulting in a larger residual lumen cross-sectional area of the histological sections. These anisotropic shrinkage changes had important artifactual effects on the calculated histological percent narrowing of cross-sectional area, which was 11% less than that determined from the corresponding ultrasound images. Thus, inhomogeneous tissue shrinkage during processing of diseased coronary arteries causes an unpredictable underestimation of percent narrowing of cross-sectional area measured from histological sections. Although additional corroborative studies are needed, our data suggest that quantitative ultrasound imaging of atherosclerotic coronary arteries may more accurately represent true vessel wall geometric features and disease severity than previous histological techniques.

**Conclusion**

This study demonstrates that intracoronary ultrasound imaging with a 25-MHz transducer of necropsy human coronary arteries can precisely determine coronary artery cross-sectional area, percent cross-sectional area narrowing by atherosclerotic plaque, and wall thickness. Ultrasound characterization of plaque composition is feasible and may provide important new perspectives on anatomic features of coronary artery disease.
Future studies are necessary to determine the feasibility and the clinical application of catheter-based intra-arterial ultrasound. Compared with an in vitro setting, where optimal coaxial position can be achieved by accurate probe manipulation, in vivo imaging inside a pulsatile artery presents new challenges. Technical improvements will be required to develop flexible and steerable miniature ultrasound probes capable of precise imaging within pulsatile tortuous coronary vessels overlaying a beating heart.

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