Ultrasonic–Pathological Comparison of the Human Arterial Wall
Verification of Intima–Media Thickness

Maylene Wong, Josef Edelstein, Jerome Wollman, and M. Gene Bond

Recent intravascular ultrasound experience challenges the accuracy of ultrasonic measurement of arterial wall thickness. We reevaluated the correlation between histological and sonographic measurements of intima–media thickness using standard transcutaneous vascular technology. Carotid and femoral arterial segments were imaged before and after fixation using a 7-MHz linear-array vascular transducer. Log compression and beam orientation were varied. Mean intima, media, and adventitia thicknesses were measured and compared with corresponding histological tunica. Tissue processing caused 2.5% shrinkage. Intraobserver reading error was 0.7% for histology and 5.4% for sonography. Ultrasound overestimated the thickness of the intima and adventitia and underestimated the thickness of the media. For combined intima–media thickness, the differences between histology and imaging were insignificant, averaging 4% for the carotid artery and 9% for the femoral artery in the far-wall projection. In the near-wall projection, sonographic intima–media thickness was 20% less than that determined histologically. We conclude that ultrasonography is limited mainly by axial resolution in quantifying the dimensions of individual arterial tunica but is capable of accurately measuring far-wall intima–media thickness. (Arteriosclerosis and Thrombosis 1993;13:482–486)

KEY WORDS • ultrasonography • arterial wall • tunica thickness

The applications of ultrasonography in carotid atherosclerosis range from making clinical diagnoses to determining prevalence in epidemiological studies and monitoring the efficacy of interventions in clinical trials.1,2 In the evolution of these applications, the technology has met rigorous standards of reproducibility for measuring carotid intima–media thickness.3 The validity and accuracy of the measurement are based on pathological and clinical correlations described by Pignoli et al4 in 1986. However, recent data from intravascular ultrasound experience have challenged the accuracy of quantitative transcutaneous vascular sonography.5–9

Our purpose was to reevaluate in vitro measurements of arterial wall segment thicknesses obtained by histology and by current vascular sonography. We found systematic differences that are inherent with ultrasound technology but do not invalidate the quantification of intima–media thickness.

Methods

Segments from common carotid and femoral arteries were taken during the autopsies of 36 male subjects who died at an age of 69±8 (mean±1 SD) years. After removing fat and loose tissue from the adventitia, the vessels were opened longitudinally. They were then clamped to frames for fixation in 10% formalin and for imaging before and after fixation. The specimens were divided longitudinally in the line of the imaging plane. A block of tissue measuring 20×2.5×thickness of the wall (mm3) was taken from the vertical center of either side of the imaging plane, corresponding with the center of the sonogram (Figure 1). The cut edges were photographed on 35-mm slide film to determine the amount of shrinkage incurred during histological processing.

Histology

The sections were marked with india ink for orientation and placed in perforated plastic cassettes for ethanol dehydration, xylene substitute clearing, and paraffin embedding. Four sections (5 μm thick) were sectioned by rotary microtome from the imaging side of the tissue block and were stained with hematoxylin and eosin to assess general histological features, with Masson’s trichrome to identify collagen, with modified Verhoeff–van Gieson’s stain to identify elastic fibers, and with von Kossa’s stain to identify calcium deposition.

The histological slides were reproduced on 35-mm colored photomicrographs and projected on a table top for tracing the boundaries of the intima, media, and adventitia. The total section, intima+media, and media+adventitia were measured by planimetry and...
FIGURE 1. Diagram of in vitro imaging system showing the transducer (B), the frame holding an arterial segment (A), and razor for marking the imaging plane (C). The insert (A) illustrates the tissue blocks taken on either side of the imaging plane. The photo insert (lower left) depicts a scan of an arterial segment and the razor assemblage.

Ultrasonography

The frame suspending the arterial segments was secured 2.5 cm from the imaging port in a Lucite imaging tank filled with a glycerine and water solution. Images of longitudinal views of the arteries were obtained with a 7-MHz linear-array probe (model 128, Acuson, Mountain View, Calif.) and displayed on a system memory of 512×1,500 pixels, eight bits deep with 256 shades of gray. For transmit foci of <35 mm, the pulse length was 0.5 μsec at 6 dB and corresponded with a practical axial resolution of 0.4 mm. The images were recorded on super VHS videocassette tape. Settings for depth-gain-compensation, preprocessing, persistence, and postprocessing were held constant. Images were recorded at two log compressions, 60 and 70 dB. Gain was adjusted to the setting at which the least dense interfaces were just visible. Regional expansion selection was used to assign the pixels to a 20×20-mm² field and to magnify the image ×5.6. Areas of the arteries that were free of gross plaques were imaged as the far wall with the ultrasound beam passing through the intima to the adventitia and as the near wall with the beam traversing from the adventitia to intima by reversing the frame in the tank. To register the imaging plane on the tissue precisely, a razor mounted in the tank on a movable arm fixed in the imaging plane made a shallow longitudinal incision in the adventitia visible to the naked eye (Figure 1). The location of the razor was verified by its focused image in the ultrasound field.

The videotapes were digitized in an image-processing computer (Cine View, Prism Imaging, Inc., Louisville, Colo.) at a final magnification of ×14. The imaged segment was measured by planimetry with an electronic cursor, as described above, to derive mean thicknesses for the intima, media, and adventitia. The boundaries were defined by aligning the cursor's edge on the brim of the leading and trailing edges of the image. The measurements were repeated for three recordings: far-wall projections at log compressions of 70 dB (FW-70) and 60 dB (FW-60) and near-wall projection at a log compression of 70 dB (NW-70). Duplicate measurements were averaged.

Intraobserver errors for measuring histological and ultrasonic wall dimensions were determined by comparing a second reading of the intima–media thickness of 15 arteries with the original reading. Each reading represented an average of two measurements. The mean differences were determined in absolute millimeters, ignoring the sign.

Statistical Analysis

The mean total thicknesses of sonographic images of unfixed and fixed tissue were compared. Histological mean intimal, medial, and adventitial thicknesses were compared with corresponding sonographic measure-
ments recorded at two log compressions and two projections. Paired t tests were used to evaluate the differences.

**Results**

**Unfixed Compared With Fixed Tissue**

Qualitatively, ultrasound images of arterial wall composition in the unfixed and fixed state were similar with few exceptions. Quantitatively, total wall thickness measured sonographically was the same before and after fixation.

**Shrinkage**

The areas of the imaging surfaces of the tissue blocks photographed on 35-mm slide film before processing were measured by planimetry and compared with planimetered areas of the histological sections. The mean shrinkage of 15 comparisons was 2.5%.

**Histology**

Although histological sections were sampled from grossly normal areas of the arteries, they occasionally revealed intimal fat and rarely revealed foam cells. Carotid and femoral intima samples showed intimas of varying thickness, consistent with advancing age. The carotid media samples consisted of elastic laminas, and the femoral media samples consisted of smooth muscle cells.

**Qualitative Characteristics**

The imaged arteries were defined by the presence or absence of an anechoic–hypoechoic media that differentiated the arteries into triple-layered or nonlayered patterns (Figure 2). Sixty-four percent of the carotid and 45% of the femoral arteries were triple layered. The remainder had an echogenic media with loss of the intima–media and media–adventitia interfaces and were not quantified. However, the layered and nonlayered patterns were compared with the corresponding histology, which revealed no differences in morphology or staining characteristics. The angle of interrogation was varied >20° while imaging triple-layered arteries without loss of the laminar pattern.

**Quantitative Sonography**

Figure 2 is a composite of a histological section of a carotid segment and corresponding B-mode scans. Table 1 summarizes the quantitative histological–ultrasonic data. The results show that the echogenic intima and adventitia were thicker than the histological intima and adventitia. The anechoic–hypoechoic media was thinner than the histological media. Table 2 summarizes the comparison between histological and ultrasonic intimal–medial thickness. With far-wall orientation, there was no significant difference between histological and sonographic intima–media thickness. With near-
Intraobserver Error

Sonographic measurements. Values are mean±1 SD.

<table>
<thead>
<tr>
<th>Thickness (mm)</th>
<th>Intima</th>
<th>Media</th>
<th>Adventitia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carotid artery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hist</td>
<td>0.78±0.20</td>
<td>0.70±0.15</td>
<td>0.30±0.12</td>
</tr>
<tr>
<td>FW-70</td>
<td>0.47±0.11†</td>
<td>0.55±0.09*</td>
<td>0.42±0.11†</td>
</tr>
<tr>
<td>Hist</td>
<td>0.16±0.10</td>
<td>0.81±0.03</td>
<td>0.26±0.10</td>
</tr>
<tr>
<td>FW-60</td>
<td>0.33±0.06†</td>
<td>0.48±0.11†</td>
<td>0.55±0.13†</td>
</tr>
<tr>
<td>Hist</td>
<td>0.22±0.12</td>
<td>0.74±0.22</td>
<td>0.42±0.20</td>
</tr>
<tr>
<td>NW-70</td>
<td>0.35±0.13‡</td>
<td>0.45±0.11†</td>
<td>0.57±0.14‡</td>
</tr>
<tr>
<td>Femoral artery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hist</td>
<td>0.24±0.12</td>
<td>0.71±0.21</td>
<td>0.41±0.21</td>
</tr>
<tr>
<td>FW-70</td>
<td>0.43±0.06†</td>
<td>0.45±0.11†</td>
<td>0.57±0.14†</td>
</tr>
<tr>
<td>Hist</td>
<td>0.25±0.13</td>
<td>0.53±0.20*</td>
<td>0.45±0.13</td>
</tr>
<tr>
<td>FW-60</td>
<td>0.35±0.08*</td>
<td>0.53±0.20*</td>
<td>0.45±0.13</td>
</tr>
<tr>
<td>Hist</td>
<td>0.24±0.13</td>
<td>0.77±0.27</td>
<td>0.39±0.20</td>
</tr>
<tr>
<td>NW-70</td>
<td>0.30±0.05</td>
<td>0.50±0.20‡</td>
<td>0.60±0.16‡</td>
</tr>
</tbody>
</table>

Hist, histological data; FW-70, far-wall data at log compression of 70 dB; FW-60, far-wall data at log compression of 60 dB; NW-70, near-wall data at log compression of 70 dB. Values are mean±1 SD.

All arteries were imaged at FW-70, one half were imaged at FW-60, and the other half were imaged at NW-70. Therefore, the histological references are slightly different for each comparison.

Discussion

Carotid sonography has assumed, in addition to its use in diagnosis, a new role as an investigative tool for surveying risk factors of atherosclerosis in epidemiological studies and for monitoring interventional therapies in clinical trials. In these applications, the measurement of carotid intima-media thickness is a primary end point. Pignoli et al first provided the anatomic basis for the ultrasonic interpretation and validated the accuracy of measuring intima-media dimensions. Compared with gross anatomy, sonograms of aortic intima-media thickness differed <20% in 77% of the measurements. B-mode scans of carotid intima-media thickness in cadavers did not differ significantly from in vivo measurements recorded in age-matched subjects. These seminal studies were carried out with an 8-MHz transducer and axial resolution of 0.4 mm.

Recent experiences with 20–25-MHz intravascular probes and axial resolutions of approximately 0.1 mm have challenged the accuracy of ultrasonic quantification. Mallery et al imaged unprocessed arteries and found that imaged medial thickness was the same as the histological preparation but that imaged intimal and total wall thicknesses were 33% and 41% thicker, respectively. Potkin et al imaged unfixed and fixed coronary arteries and reported that imaged total wall and intima-media dimensions were 19% thicker than the processed tissue. Pandian et al probed fresh arteries and found that sonographic total wall thickness was systematically greater than measurements taken from photomicrographs of unprocessed specimens.

Our present study reexamined the accuracy of a lower-frequency probe with an axial resolution of 0.4 mm and found that ultrasound systematically overestimated the thicknesses of the intima and adventitia and underestimated the thickness of the media. However, the intima-media dimensions, when imaged as the far wall, were not

Wall orientation, ultrasonic intima-media thickness was less than that determined histologically. This difference was due entirely to a narrower intima in the ultrasonic intima-media thickness measurement.

Intraobserver Error

Intrareader error for duplicate measurements of histological intima-media thickness was 0.01±0.01 mm, or 0.6±0.8% of the mean. Intrareader error for duplicate measurements of sonographic intima-media thickness was 0.05±0.04 mm, or 5.4±4.3% of the mean.

Discussion

Carotid sonography has assumed, in addition to its use in diagnosis, a new role as an investigative tool for

Table 2. Intima-Media Thickness: Histological Minus Sonographic Measurements

<table>
<thead>
<tr>
<th>Intima-media thickness</th>
<th>FW-70</th>
<th>FW-60</th>
<th>NW-70</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carotid artery (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hist</td>
<td>0.98±0.28</td>
<td>0.86±0.20</td>
<td>1.04±0.31</td>
</tr>
<tr>
<td>Sonographic</td>
<td>0.92±0.15</td>
<td>0.88±0.11</td>
<td>0.83±0.12</td>
</tr>
<tr>
<td>ΔThickness</td>
<td>+0.06±0.29</td>
<td>-0.02±0.19</td>
<td>+0.21±0.35</td>
</tr>
<tr>
<td>p</td>
<td>0.34</td>
<td>0.69</td>
<td>0.05</td>
</tr>
<tr>
<td>Femoral artery (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hist</td>
<td>0.98±0.28</td>
<td>0.96±0.28</td>
<td>1.00±0.32</td>
</tr>
<tr>
<td>Sonographic</td>
<td>0.88±0.11</td>
<td>0.88±0.19</td>
<td>0.80±0.19</td>
</tr>
<tr>
<td>ΔThickness</td>
<td>+0.10±0.24</td>
<td>+0.08±0.24</td>
<td>+0.21±0.28</td>
</tr>
<tr>
<td>p</td>
<td>0.14</td>
<td>0.25</td>
<td>0.06</td>
</tr>
</tbody>
</table>

FW-70, far-wall data at log compression of 70 dB; FW-60, far-wall data at log compression of 60 dB; NW-70, near-wall data at log compression of 70 dB; ΔThickness, histological minus sonographic measurements. Values are mean±1 SD.
statistically different from the histological references, varying by only 2.3–6.0% for the carotid arteries and 8.3–10.2% for the femoral arteries. When imaged as the near wall, the sonographic intima–media measurement was 20% less than the reference measurement.

In summary, ultrasonography overestimates the intimal and adventitial layers of arteries. Tissue shrinkage might account for part of the difference, but documentation of shrinkage has been infrequent. Our 2.5% shrinkage is several fold less than that previously reported. However, our methods differed in that the tissue was stretched during fixation and wedged in rigid casettes during dehydration. In addition, wrinkles and folds in individual sections were allowed to straighten in a water bath before mounting.

Shrinkage notwithstanding, studies have consistently demonstrated that sonographic images of intima are thicker than histological intima. Measurement error is related to the axial resolution of the imaging device, which is determined primarily by the transducer frequency, transmitted pulse duration, and system bandwidth. Higher-frequency probes with shorter pulse length and wider bandwidth obtain a finer resolution. To resolve an intimal thickness of 0.2 mm requires an axial resolution of <0.2 mm to separate the reflecting interfaces (lumen–intima and intima–media). Otherwise, they will appear to merge. Our transducer with an axial resolution of 0.4 mm was unable to resolve the intima but was able to accurately measure the 1.0-mm distance between the leading edge of the intima and leading edge of the adventitia in the far-wall orientation.

Axial resolution is also influenced by display settings such as log compression and image gain. Increasing log compression resulted in the display of lower-amplitude echoes in the gray scale image, especially posterior to a reflecting interface, which served to broaden the intima. Variations in gain also affect the perceived thickness of individual interfaces, and in this study gain was held constant.

With near-wall orientation, intima–media thickness was underestimated because of a change in the reflectivity of the reversed interfaces. The trailing edge of the adventitia extended into the media, reducing its mean thickness in a manner similar to the encroachment by the intimal trailing edge with far-wall orientation. However, the media–intima interface was less reflective than the far-wall lumen–intima interface, resulting in less reverberation and an apparently thinner intima. Wendelhag et al., using the same phased-array equipment, concluded that the trailing edges of the near-wall adventitia–media and intima–lumen interfaces did not correlate with anatomy and could not be used to measure intima–media thickness. Using a mechanical 8-MHz probe and a different analysis, measured near-wall intima–media thickness in an in vitro preparation and found excellent correlation and insignificant differences with histological references. These divergent results emphasize the importance of varying instrumentation and methods and the need for individual laboratories to conduct their own valid experiments.

The finding that both elastic and muscular media are frequently echogenic, resulting in a nonlayered pattern, has been recorded with low- and high-frequency transducers. The reflectivity of each interface depends on differences in acoustic impedance, which in turn depends on differences in tissue composition. Nishimura et al. found that nonlayered muscular arteries imaged with intravascular probes correlated with media containing more collagen–elastin and less muscle and with adventitia containing more muscle and less collagen–elastin. Our work and that of Pignoli et al., performed with 8-MHz transducers, showed that the media of elastic arteries was more often echoruent and, in our hands, that the media of muscular arteries was either echoruent or echogenic. Histology revealed no differences to account for the layered and nonlayered patterns. Possible explanations are that single 5-μm sections are not always representative of corresponding thicker ultrasound scans, even in grossly normal arteries, or that qualitative changes in the collagen matrix are not always detectable under standard light microscopy.

We conclude that quantifying the individual arterial tunica is beyond the axial resolution of low-frequency transducers. However, the intima–media thickness of the carotid artery can be accurately measured with a 7-MHz vascular transducer when the vessel is imaged in the far-wall projection.

Acknowledgments

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References

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