Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging

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ABSTRACT A study in vitro of specimens of human aortic and common carotid arteries was carried out to determine the feasibility of direct measurement (i.e., not from residual lumen) of arterial wall thickness with B mode real-time imaging. Measurements in vivo by the same technique were also obtained from common carotid arteries of 10 young normal male subjects. Aortic samples were classified as class A (relatively normal) or class B (with one or more atherosclerotic plaques). In all class A and 85% of class B arterial samples a characteristic B mode image composed of two parallel echogenic lines separated by a hypoechoic space was found. The distance between the two lines (B mode image of intimal + medial thickness) was measured and correlated with the thickness of different combinations of tunicae evaluated by gross and microscopic examination. On the basis of these findings and the results of dissection experiments on the intima and adventitia we concluded that results of B mode imaging of intimal + medial thickness did not differ significantly from the intimal + medial thickness measured on pathologic examination. With respect to the accuracy of measurements obtained by B mode imaging as compared with pathologic findings, we found an error of less than 20% for measurements in 77% of normal and pathologic aortic walls. In addition, no significant difference was found between B mode–determined intimal + medial thickness in the common carotid arteries evaluated in vitro and that determined by this method in vivo in young subjects, indicating that B mode imaging represents a useful approach for the measurement of intimal + medial thickness of human arteries in vivo.

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EXPERIMENTAL STUDIES on nonhuman primates and on human subjects indicate that atherosclerotic lesions may progress without a reduction in luminal size because of dilatation of the arterial wall. The correct estimation of the size of atherosclerotic lesions therefore requires the simultaneous measurement of arterial wall thickness and residual luminal size. Atherosclerotic lesions in peripheral and carotid arteries are generally evaluated by the measurement of luminal size by invasive methods such as contrast angiography or by noninvasive ultrasound techniques. Gross and microscopic pathologic examination allows measurement of arterial wall thickness in tissue specimens only.

In our laboratory the measurement of the intimal + medial thickness has been attempted by the use of the noninvasive technique of B mode real-time imaging. This approach is currently used to measure organ dimensions as well as atherosclerotic lesions. A multicenter validation trial is now being conducted to determine the accuracy of B mode imaging vs that of angiography and histology. However, the potential of B mode imaging for direct measurement (i.e., not that from residual luminal size) of arterial wall thickness has not been assessed as yet.

In a previous study we found a significant correlation between results of gross pathologic evaluations and measurements by B mode imaging of arterial wall thickness. This study, however, was conducted on a limited number of normal or moderately diseased arterial segments.

The objectives of this study were (1) to determine the anatomic structures involved in ultrasound energy reflection in the arterial wall, (2) to determine the accuracy of intimal + medial thickness measurements by B mode imaging by comparison with findings of gross and microscopic pathologic examinations in nor-

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nal and pathologic arterial segments, and (3) to assess the feasibility of the measurement of intimal + medial thickness of arterial walls not only in vitro but also in vivo in human subjects.

The investigations were carried out in vitro with specimens of human aortas and common carotid arteries and in vivo in common carotid arteries of normal human subjects.

Materials and methods

Selection and procession of autopsy material. Abdominal aortas and common carotid arteries were removed at autopsy from 18 male subjects (age range 20 to 74 years), 12 to 20 hr after death.

Processing of aortic tissue. Aortic tissue was processed as previously described. Briefly, after longitudinal opening, rectangular strips (2 × 4 cm) of washed aortic tissue were longitudinally distended with a Plexiglas holder and fixed with formalin (10% for 10 hr). The segments to be studied were held in place with two metallic pins.

Processing of the common carotid artery. Common carotid arteries from young subjects (age range 20 to 25 years) were cannulated and perfused under pressure (90 mm Hg) with 10% buffered formalin for 10 hr. After ultrasound interrogation, arteries were longitudinally opened and processed for gross and microscopic examination.

For experiments in situ common carotid arteries were cannulated during autopsy (24 to 30 hr after death) with a Foley catheter (Rusch-Gold Balloon catheter, SILKOLATEX, West Germany) and flushed under pressure (∼120 mm Hg) with saline. The arteries were processed as described for aortic tissue.

Gross and microscopic pathologic evaluation and classification. The specimens were cut longitudinally between two metallic pins previously fixed 10 mm apart to define the segment of interest. After ultrasound interrogation the specimens were stained for 12 hr in a supersaturated Sudan IV 38% isopropanol alcohol solution and then washed for 1 hr. After lipid staining, gross pathology of the artery was evaluated by a Zeiss operative microscope at 10× magnification. The media was differentiated from the adventitia on the basis of the color and consistency. The calibration of the ocular graduate scale to be used for thickness measurements was performed with a Zeiss test microobject. The resolution of the microscope was 20 paired lines per millimeter. After gross pathologic evaluation the specimens were cut, decalcified with a Standard Cal Ex Solution (Fisher Inc.) for 4 hr, and embedded in paraffin after dehydration. The histologic slices were stained with Verhoeff–Van Gieson and with hematoxylin-eosin stains and evaluated by optical microscopy.

Each sample was classified into one of two arbitrarily defined categories (A or B) on the basis of gross and microscopic characteristics. Class A included aortic segments that were macroscopically normal or had fatty streaks. Microscopically these samples showed homogenous intima with varying amounts of intimal thickening, intimal fibrosis, internal elastic lamina fragmentation, and duplication. The arterial segments included in class B showed a fibrous/muscular cap with a lipid and/or necrotic core. Microscopically, the intima showed focal areas of fibrotic smooth muscle cell proliferation, microcalcification, and necrosis.

The intimal + medial thickness of the arterial segments was determined by gross pathologic and histologic examination. The thickness of adventitia and of the overall complex intima + media + adventitia was measured by gross pathologic evaluation, whereas the intimal and the medial thicknesses were determined by histologic examination only.

Ultrasound instrumentation. A high-resolution small-part real-time scanner (Biosound, Biodynamics Inc, Indianapolis) was used. This instrument generates a wide-band ultrasonic pulse with a midfrequency of 8 MHz. The measured pulse length at 6 dB is 0.5 μsec, corresponding to an axial resolution of approximately 385 μm for an ultrasonic speed of 1540 m/sec. The reported dynamic range is at least 70 dB. The lateral resolution is 0.5 mm. A standard 15 inch television monitor allowed magnification of the objects by 10×. Marker lines were present on the display for depth measurements, which were performed with a mechanical caliber.

Experiments in vitro were carried out by placing the aortic tissue specimens in a Plexiglas tank filled with water at room temperature (22° to 25°C). A mechanical system allowed optimal positioning of the ultrasound incident beam with respect to the area under evaluation. The intimal surface was exposed to the incident incoming pulse. Common carotid arteries were perfused with saline under pressure and ultrasound interrogation was carried out along their longitudinal axes. For each carotid artery four longitudinal measurements were obtained by rotating (90 degree increment) the vessels along the axis.

Experiments in situ were performed on common carotid arteries during autopsy (n = 3). B mode evaluations were carried out in situ on the same carotid arteries with and without the tissues located between the outer side of the vessels and the ultrasound probe (skin, subcutaneous tissue, and muscles). Scans were carried out in the anteroposterior and laterolateral planes of the lower and higher third of the common carotid arteries. After ultrasound interrogations the carotid arteries were removed and processed.

Experiments in vivo were carried out in 10 young subjects (age range 20 to 29 years) with no clinical sign of atherosclerotic disease.

The subjects were kept supine with the head slightly extended. Two longitudinal scans were performed in the anteroposterior and coronal planes of the lower and higher third of the right and left common carotid arteries. In both studies in vitro and in vivo only the far (deeper) wall image was evaluated.

Identification of the anatomic structures generating the B scan pattern. To define the anatomic structures generating the B scan pattern 10 of the 116 aortic specimens (five from class A and five from class B) were examined in dissection experiments. A 1.5 to 2.0 mm deep excision of the intima was performed in five class B arteries. These specimens were then interrogated by ultrasound and evaluated by microscopy. The findings on the B scan images obtained in the region of the intimal duplication were compared with the structural changes induced by dissection in the arterial wall. To define the anatomic structures generating the outer line, arterial segments with and without the adventitia and the periarterial tissues were interrogated by ultrasound and evaluated by light microscopy.

Quantitative ultrasound and pathologic measurements were also made to determine the correlation between the B mode image and the anatomic structures. The distance separating the inner and outer lines shown in figure 1 was measured in 45 class A aortic specimens and defined as the B mode image of intimal + medial thickness. Without prior knowledge of ultrasound data, gross pathologic and histologic evaluations of the different tunicae were performed in all the aortic specimens (45 class A and 49 class B), and the results were correlated with those obtained by B mode imaging of the intimal + medial thickness.

To ensure that B mode, gross pathologic, and histologic evaluations were performed in the same location, the following criteria were used. (1) From each arterial specimen a segment
showing homogenous thickness was selected, and a distance of 10 mm was delimited with two metallic pins. This procedure was undertaken to avoid the error that might result from making measurements with the three methods at slightly different points. (2) B mode and gross pathologic evaluations were carried out in the middle of the segment under study, which was identified by the metallic pins. (3) Histologic measurements were obtained in the middle of longitudinal sections identified by the holes left by the pins.

The overall error in the identification of the site at which the different measurements (B mode, histologic, and gross pathologic) were made was estimated to be ±0.5 mm. For all the aortic specimens showing a characteristic B scan pattern (45 class A and 49 class B), the absolute and percent differences between results of B mode and gross pathologic evaluations of intimal + medial thickness were determined.

Intimal + medial thickness values obtained in vitro in common carotid arteries with B mode imaging were compared with those determined in vivo in common carotid arteries of young living subjects.

Statistical analysis of the experimental data. The data are expressed as the mean ± SD. Statistical analysis was carried out by paired two-tailed t test. Correlation coefficients were calculated by plotting the values obtained with B mode imaging against those obtained by pathologic techniques.

Results

Gross pathologic and histologic evaluations of aortic specimens and common carotid arteries. One hundred sixteen aortic segments were studied. Fifty of the 116 segments (43%) were grouped in class A (mascroscopically normal or with fatty streaks), whereas 66 (57%) were grouped in class B (aortic segments with atherosclerotic lesions). Histologic evaluations of these segments showed that intimal thickness of class A and class B arteries was 0.25 ± 0.12 and 1.11 ± 0.27 mm, respectively, whereas intimal + medial thickness was 1.03 ± 0.27 and 1.72 ± 0.71 mm, respectively. Results of gross pathologic evaluations were comparable (class A intimal + medial thickness 1.13 ± 0.26 mm and class B 1.93 ± 0.84 mm). The mean intimal + medial thickness measured by histologic examination of common carotid arteries in vitro (n = 44) was 0.48 ± 0.06 mm. The gross pathologic evaluation resulted in comparable data (0.50 ± 0.06 mm).

The typical B mode image of the arterial wall and its prevalence in class A and class B aortic specimens. In all the class A and in 85% of class B aortic specimens a similar B scan pattern was found. This pattern was characterized by two parallel echogenic lines separated by an hypoechoic or anechoic space (figure 1). This B scan pattern was defined as the “double line pattern.” The inner (luminal) line was generally more regular, smooth, and thin than the outer one. In 20% class B
aortic segments (12 of 61) the double line pattern was absent, whereas it was present but more complex in 9% and in 31% of class A and B aortic segments, respectively. The characteristic double line pattern was found in the far wall of all the common carotid arteries evaluated in vitro (figure 2).

**Identification of the structures generating the double line pattern.** Five class B aortic specimens were prepared to identify the structures generating the inner echogenic line of the double line pattern. A small portion of the arterial wall was excised from the luminal side. The excision was found, by light microscopy, to be confined within this tunica. In these specimens the intimal/medial transition and the more peripheral layers were unchanged (figure 3). The B scan image generated by the five dissected arteries showed the disappearance of the inner line at the level of the inlay. This finding demonstrates that the inner line is generated by the intimal surface. In all of the five class A arteries the adventitia was completely removed from the media as confirmed by histologic examination (figure 4). The B scan image of each of these specimens showed the disappearance of the outer line in the region without adventitia, indicating that this line was generated by the adventitia.

To determine the anatomic structures delimited by the inner and the outer lines of the double line pattern, the distance between these two lines was measured with the use of B mode imaging. Values for intimal + medial thickness determined by B mode imaging were correlated with histologic and gross pathologic thickness values for the different tunicae. The B mode measurements of intimal + medial thickness showed a significant correlation with values obtained by gross pathology and by histology in both class A and class B aortic samples (figure 5). In contrast, B mode intimal + medial thickness values appeared to correlate less well with the values for other components of the vessel wall, alone or in combination (intima + media + adventitia and adventitia alone as measured by gross pathology and intima and media measured by histology; figure 6).

It should also be noted that evaluations of intimal + medial thickness by B mode imaging and gross pathologic examination did not significantly differ in either class A or class B aortic samples (class A: B mode intimal + medial thickness, 1.22 ± 0.37 vs 1.13 ± 0.26 by gross pathology, NS; class B: B mode, 2.06 ± 1.02 vs 1.93 ± 0.84 by gross pathology, NS).

**Accuracy of B mode measurements.** To determine the
To assess whether intimal + medial thickness measured in vitro in arterial specimens was similar to that in situ, experiments were carried out during autopsy on carotid arteries that had been cannulated and flushed with saline. For this purpose common carotid arteries were cannulated with an inflatable balloon catheter, and the position of the balloon was verified on the monitor of the instrument. The catheter was kept in the same position during all the experiments, working as a position marker. A clip was placed on the anterior wall of the artery, just over the balloon, to allow the correct identification of the scanning plane after the excision of the artery.

Measurements of intima + media in situ obtained with intact superficial tissues yielded values not significantly different from those obtained in exposed carotid arteries (1.11 ± 0.14 and 1.08 ± 0.19 mm for in situ and exposed arteries, respectively, n = 3; absolute error 5.25 ± 1.65%). Thickness data obtained in vitro in pressure-fixed vessels on the arterial segment identified by the balloon and the clip were 10% lower than those obtained in situ or in the exposed arteries (0.99 ± 0.29 mm, n = 3). This difference could be attributable to an effect of fixation.

B mode experiments to evaluate intimal + medial thickness of common carotid arteries in 10 living subjects showed that the double line pattern was present at the far wall of all the common carotid arteries considered (n = 20). B mode intimal + medial thickness measured in vivo was 0.53 ± 0.05 mm (n = 20). No significant difference was found between the mean values in common carotid arteries evaluated in vitro and in vivo by B mode imaging.

**Discussion**

Our investigations show that a characteristic B mode image is consistently generated in vitro at the far wall level by most arterial walls (100% of class A and 85% of class B arteries).

It is relevant to note that, most likely due to the different order in which the interfaces of the intima/lumen and media/adventitia are exposed to the incoming ultrasound beam, the B mode images of the near (more superficial) and far (deeper) walls are different. Our experiments were performed only on far walls because they could be more constantly and repeatably visualized, and therefore our conclusions can be applied only to far walls.

The characteristic B scan pattern of class A arterial walls shows two parallel echogenic lines separated by a relatively hypoechoic space (the double line pattern) (figure 1). A similar acoustic behavior of the arterial

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**FIGURE 4.** B mode image (top) and histologic section (bottom) of a class A arterial wall with a region without adventitia (wa). Magnification in both images (×10). P = pin; H = hole.
PIGNOLI et al.

Wall has been previously reported in vitro\(^9, 12-20\) and in vivo\(^21, 22\) in normal and diseased human carotid arteries. In experiments in which a dissection confined to the intima was produced in class B aortic specimens the luminal intimal transition was profoundly modified, but the intimal medial transition remained unmodified (figure 2). The inner line change found on the B mode image must be caused by an alteration in the generating structure: this line can thus be identified as the luminal/intimal transition. These experiments were performed only in class B arteries, since the intimal thickness of class A specimens did not allow a careful dissection. On the other hand, the removal of the adventitia from the media resulted in the disappearance of the outer line, indicating that the adventitia was responsible for the echoes representing this line.

Since the time-gain compensation setting of the instrument may influence the appearance of the hypoechoic space between the two lines, the double line pattern of each arterial specimen was evaluated over a large range of time-gain compensation settings. In our experience, however, the typical double line pattern was easily recognizable in the same class A arterial specimens over a large range of time-gain settings. Thus, a time-gain compensation in the usual range does not appreciably influence the distance between the two echogenic lines.

In class B arteries, however, even with optimal adjustment of the time-gain compensation setting, high-amplitude echoes were frequently present between the inner and outer lines. In 12 of 61 arterial specimens (19.7%) these echoes, sometimes followed by acoustic shadows, did not allow the identification of the typical double line pattern.

**FIGURE 5.** Correlation of intimal + medial thickness measured with B mode imaging (BMIMT) and that evaluated by histology (HIMT) in class A and B arterial specimens. The correlation coefficients and the regression lines are shown. GPIMT = intimal + medial thickness measured by gross pathology.
In class A and B aortic specimens a good correlation between gross pathologic and histologic intimal + medial thickness \((r = .77)\) was found. In addition, these data were very similar to those obtained by B mode imaging. The difference between intimal + medial thickness measured by B mode imaging and gross pathology was not statistically significant, indicating that B mode measurements represent the distance between the luminal surface and the inner part of the adventitia. This distance represents the intimal + medial thickness (figure 1). In contrast, the difference between B mode intimal + medial thickness and that measured by histology in class A and B specimens was highly significant \((p < .001)\). This discrepancy was due to artifacts introduced during procession of tissue for histologic evaluation.2,23

The B mode image pattern may be complicated by the presence of additional echoes between the two echogenic lines and/or by a fuzzy appearance or discontinuities in the outer line. The B mode image was complex in 15 of 61 class B (25%) and in four of 45 class A (9%) specimens and these arterial wall measurements were less accurate. The absolute and percent errors in B mode measurements, as compared with gross pathologic findings, in class A and B arteries with a complex ultrasound image were significantly different from those for arterial specimens with a typical ultrasound picture.

Therefore, evaluation of intimal + medial thickness can be carried out more precisely in the early stages of atherosclerotic disease, when other available methods (Doppler ultrasound and contrast angiography) cannot be used. All the normal young subjects studied in vivo and most of the patients with angiographically and surgically proven atherosclerotic lesions had the characteristic double line pattern at the level of the common carotid artery. The results of experiments in situ indicate that the presence of superficial tissues does not influence the B mode image of the arterial wall or the thickness of the intima + media. In addition, B mode measurements of intimal + medial thickness of common carotid arteries obtained in vivo were almost identical to those obtained in vitro with both B mode imaging and histologic techniques. We may therefore
conclude that the results in vitro reported in this study can be extended to conditions in vivo.

The present study demonstrates that: (1) a characteristic B mode image (the double line pattern) is consistently generated in vitro by the far walls of most of the arteries, (2) the distance between the transducer-facing edges of the inner and outer lines of the B mode image correlates with and does not differ significantly from the intimal + medial thickness, (3) regarding the accuracy of B mode imaging, a percent error of less than 20% has been found in 77% of the arterial specimens of both classes (normal and pathologic), and (4) the accuracy of B mode imaging depends on the presence of a typical B scan image. In 85% of class B arterial specimens a characteristic B mode image was found and in 59% of specimens of the same class the image was typical and easy to interpret.

We conclude that B mode imaging represents a useful tool for the detection and monitoring of changes in intimal + medial thickness, allowing the evaluation of changes in the arterial wall in areas without localized plaques. At the present time, it is difficult to determine whether this information will be of clinical relevance. It is possible that early changes in vascular thickness will later result in atheroma; they could on the other hand evolve only in a diffuse intimal thickening and have no hemodynamic relevance. It is also not known whether a relationship exists between the morphologic changes in the vessel wall detected by our method (thickening) and local damage to the endothelial layer, which can induce alterations of potential clinical relevance. The noninvasive nature of this new approach is a recommendation for its use in the preclinical diagnosis and follow-up of patients with atherosclerosis.24, 25

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