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Intravascular ultrasound appearance of normal and mildly diseased coronary arteries: Correlation with histologic specimens

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Intravascular ultrasound imaging was performed *in vitro* on six histologically normal and 104 minimally diseased arteries in patients aged 13 to 83 years. This study tested the hypothesis that normal coronary arteries produce a three-layer image that corresponds to the histologic layers of intima, media, and adventitia. The results showed a very good correlation between area of the echolucent ultrasound layer with the media and the inner echogenic layer with intimal area. In addition, a three-layer appearance was consistently seen when the internal elastic membrane was present with or without intimal hyperplasia. If the internal elastic membrane was absent, a three-layer appearance was still seen if the collagen content of the media was low. However, a two-layer appearance was observed when there was absence of the internal elastic membrane as well as a high collagen content of the media. (*AM HEART J* 1995;130:976-86.)

Studies with intravascular ultrasound (IVUS) in peripheral and coronary vessels have demonstrated a characteristic three-layer appearance in normal and atherosclerotic muscular arteries.¹⁻⁸ The inner echodense layer, the middle echolucent zone, and the outer echodense layer are generally interpreted as the intima, media, and the adventitia, respectively. However, there are conflicting reports on the usual configuration of IVUS images—whether a three-layer appearance is seen in normal arteries—and diverse interpretations of what the echogenic and echolucent structures represent compared with histologic evidence.^{9, 10} This study was conducted to in-

vestigate the histologic tissue characteristics that determine the ultrasound representation of coronary artery microanatomy.

METHODS

Human coronary arteries. Coronary artery segments that appeared grossly normal by palpation were obtained at autopsy from 110 people over a wide age range. Because the arteries were obtained from the coroner's office, little other clinical information was available. At the time of ultrasound imaging, the arteries were fresh and unfixated. An 8F guiding catheter was inserted into the proximal end of the vessel, and normal saline was perfused at 100 mm Hg at room temperature in a saline bath.

Intravascular ultrasound system. A 3.9F, 25 MHz intravascular ultrasound catheter (Intertherapy by CVIS, Sunnyvale, Calif.) was used. The catheter was adapted to an 1800 rpm motor drive that provided real-time cross-sectional images at 30 frames per second. The echo signal was sampled by an 8-bit analog-to-digital converter and converted from radial data format into a rectangular format. The images were viewed on a 640 × 480 pixel grayscale video display, stored on half-inch super-VHS videotape, and accessed later for analysis. A surgical needle was placed in the adventitia as an acoustic reference, and cross-sectional images were obtained.

The ultrasound images were reviewed from the videotape, and the presence and extent of a three-layer appearance around the circumference of the image was identified by two independent observers. A representative cross-sectional image was digitized by a video frame-grabbing board (RasterOps 24 STV, Mediagrabber software, Santa Clara, Calif.) into a Macintosh IICI computer for quantitative analysis and subsequent comparison to the histologic images. From the digitized images, the lumen perimeter, the outer perimeter of the inner echogenic layer, and the outer perimeter of the middle echolucent layer were traced by two independent observers (Fig. 1) using a public domain image processing and analysis program (NIH image). The areas of the inner echogenic layer and the echolucent layer were calculated by the computer program using the magnification scale on the ultrasound images. The extent of the visible three-layer appearance was quantified by measur-

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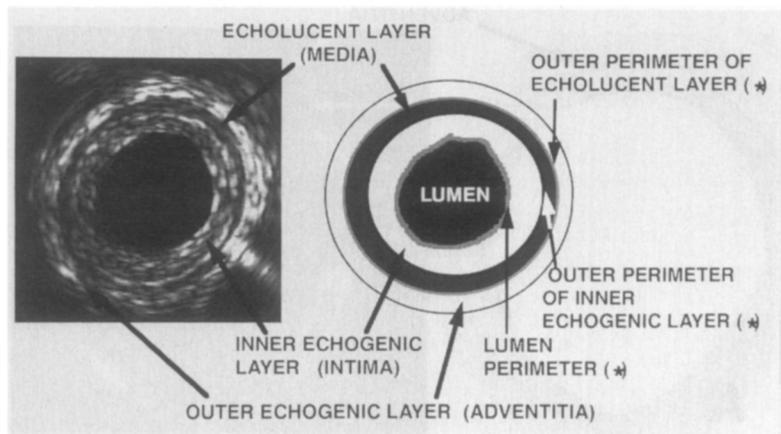


Fig. 1. Orientation of IVUS measurements. The ultrasound image shows a three-layer appearance with the inner echogenic structure 2 to 3 times the width of the middle echolucent band. The schematic drawing shows the perimeters (*) where the measurements were made.

ing the angle subtended by the three layers around the circumference of the ultrasound image. Angles were measured on the digitized image from the center of the lumen. The center was defined mathematically by the computer after the operator outlined the lumen.

Histologic examination. After image acquisition, the saline perfusion was changed to 10% formalin. A constant pressure of 100 mm Hg was maintained for 12 hours. The adventitial needle acoustic reference sites were marked with a suture or India ink. The artery segments were then prepared for histology. Transverse arterial sections were made at the level of the suture or India ink. Vessel cross sections were stained with hematoxylin and eosin, Masson's trichrome stain for collagen, and van Gieson's stain for elastin.

The specimens were examined microscopically (at $\times 100$ magnification) by two investigators who assessed the presence and extent of the internal elastic membrane (IEM) around the circumference of the vessel on the van Gieson stain. The investigators were not blind to the ultrasound images; however, to diminish any reading bias, the histology measurements were performed several weeks later without referring to the ultrasound images. The extent of IEM was scored by identifying the amount of circumference occupied by IEM: 0% to 25%, 1+; 26% to 50%, 2+; 51% to 75%, 3+; 76% to 100%, 4+. The two investigators scored the collagen content of the media on the trichrome stain slides from 0 to +3: 0, absent collagen; +1, less blue collagen than red smooth-muscle cell area; +2, equal collagen and smooth-muscle content; +3, more collagen than smooth-muscle area. The two scores were averaged for each artery.

A low-power microscope (Olympus stereo zoom, model SZHILLD, Lake Success) connected to a video camera (Sony CCD, model DXC 101, Tokyo, Japan), was used to photograph the histologic sections. The histologic images were digitized and stored in the computer. Measurements were made on the digitized images using the same image processing and analysis program used to analyze the

ultrasound images. The perimeters of the lumen, intima-media interface, and media-adventitia interface were traced, and the lumen area and the areas occupied by intima and media were computed. The ultrasound and histologic images of each artery were transferred to a computerized image processing application and placed next to each other at the same magnification and orientation, using the acoustic needle echo and the India ink stain as references. The ultrasound appearance of two or three layers and the thickness of those layers were compared with the corresponding segments around the circumference of the histologic sections. The tissue composition (extent of IEM and collagen content of media) assessed from the higher magnification analysis was also compared with the ultrasound appearance.

Statistics. Data are expressed as mean \pm SD. Correlations between groups were made by linear regression analysis. Analysis of variance with the Bonferroni correction was used to compare mean differences between groups.

RESULTS

Histologic observations. The 110 vessel segments were obtained from people with a mean age of 44 ± 17 years. There were 98 segments from males and 12 from females. Although no artery had palpable coronary atherosclerosis, there was a wide range of intimal hyperplasia. Six arteries were normal as defined histologically by an intimal layer only 1 cell thick (Fig. 2). Intimal hyperplasia was observed in all the men (youngest was age 13). There was a direct correlation between artery age and mean intimal thickness ($r = 0.62$) (Fig. 3).

The IEM was present for a mean of $42\% \pm 31\%$ around the arterial circumference. There was good agreement between the two observers on the percentage of IEM that was present on the histologic sections ($r = 0.98$). The extent of IEM decreased with

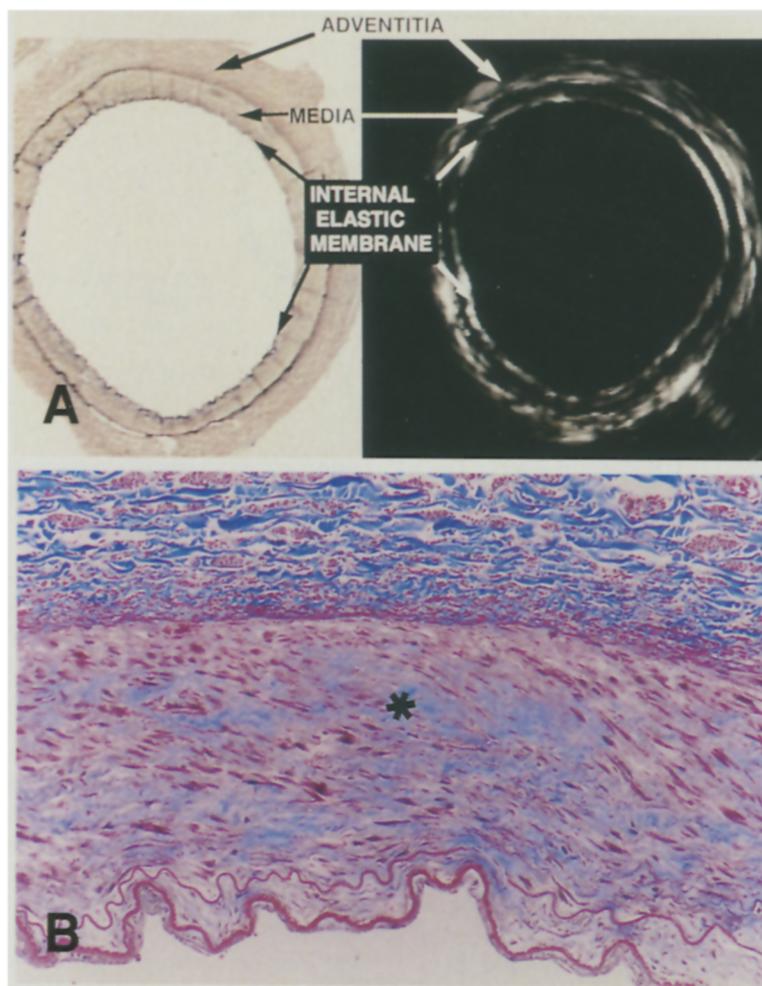


Fig. 2. A, Histologic cross-section of a normal coronary artery from a 16-year-old girl (*left*, elastin van Gieson's stain) compared with the corresponding ultrasound image (*right*). A three-layered appearance is present even when there is no intimal hyperplasia. Inner echogenic structure corresponds to the internal elastic membrane. **B,** Magnified view ($\times 100$) shows high (3+) amount of collagen (blue) within media (trichrome stain). Two wavy bands of elastin comprise internal elastic membrane.

age ($r = -0.46$) (Fig. 4) and with increasing intimal hyperplasia ($r = -0.41$). The mean score for collagen content in the media was 2.0 ± 0.9 . There was a close correlation between the two observers in their assessment of the extent of medial collagen ($r = 0.89$). The amount of medial collagen increased with the age of the artery ($r = 0.42$).

Ultrasound observations. Good quality ultrasound images were obtained in 96.5% of the arteries. Of the 110 cross-sections studied, 59 (54%) demonstrated three layers in more than 75% of the vessel circumference, whereas 94 (86%) of the arteries had a three-layer appearance for more than 50% of the vessel circumference. Of 20 arteries from individuals younger than age 25, 20 (100%) showed a three-layer appearance in more than 75% of the vessel circum-

ference. The intraobserver and interobserver variability was close for all area and perimeter measurements from the ultrasound images (Table I).

Comparison of histologic and ultrasound images. The mean values of all the arteries for the histologic and ultrasound measurements of the lumen, intima, and media areas are presented in Table II. The comparison of the ultrasound and histologic images juxtaposed at the same magnification revealed that the inner echogenic layer in the ultrasound image corresponded to the intima, and the echolucent zone corresponded to the media. The correlation for total (intima plus media) area between ultrasound and histology was very close ($r = 0.96$); however, the correlations for media area and thickness were weak ($r = 0.25$ and $r = 0.15$, respectively). This was be-

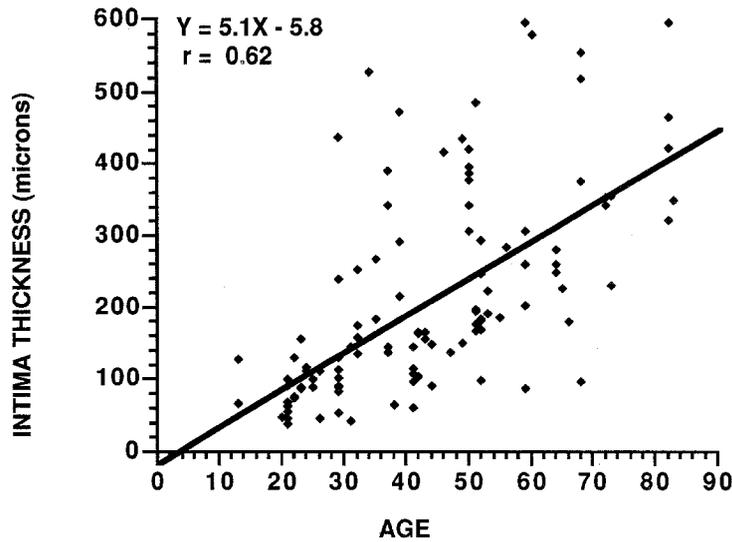


Fig. 3. Correlation of age and intima thickness.

cause of the inability to visualize the media in 31 ultrasound images where only two echogenic layers were visible. When a two-layer ultrasound appearance was seen, the media thickness for that portion of the circumference was measured as zero.

It was noticed that the loss of the IEM and the extent of medial collagen affected the presentation of the ultrasound morphologic features. To clarify this issue, the data were subdivided into four groups according to the extent of IEM (\geq or $<$ 50% circumference) and the amount of medial collagen (0 to 1+, or 2 to 3+) visible on the histologic sections (Table III). Group 1 consisted of 18 arteries with IEM present in the majority ($77.4\% \pm 13.2\%$) of the circumference and a low collagen content in the media. Group 2 consisted of 28 arteries with an intact IEM ($74.8\% \pm 13.5\%$) of the circumference but with an increase in the collagen content of the media (2 or 3+). Group 3 had 29 arteries in which the IEM was fragmented and present for $<$ 50% of the circumference (mean of $24.8\% \pm 12.1\%$). In each of these groups of arteries, the ultrasound image demonstrated a three-layer appearance for $>$ 50% of the circumference. In contrast, the arteries of group 4 had a decreased IEM ($12.7\% \pm 10.3\%$) and an increase in media collagen content. In this group, 31 (89%) arteries had a predominant two-layer ultrasound appearance.

A three-layer ultrasound appearance was produced when either of two conditions were present (Fig. 5). If the IEM was present, it produced an intense echoreflexion that contrasted strongly with

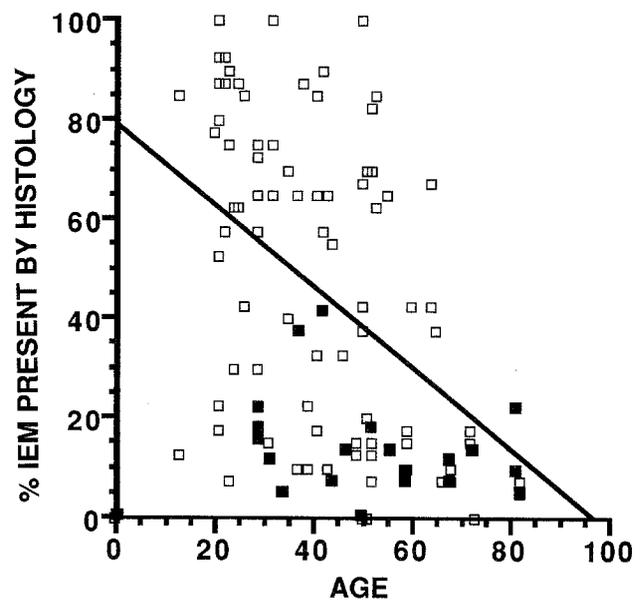


Fig. 4. Correlation of age vs percentage of intact internal elastic membrane. *White squares*, Three layers by ultrasound; *black squares*, two layers by ultrasound. $Y = -0.83X + 79$; $r = -0.46$.

the inner echogenic intima and the generally less echogenic media (Fig. 5, section A). If the IEM was absent around a portion of the circumference, there could still be a three-layer appearance if the media had a low collagen content. In this case, the media was less echoreflexive, and the three-layer appearance was produced by an echogenic intima and adventitia separated by an echolucent middle band

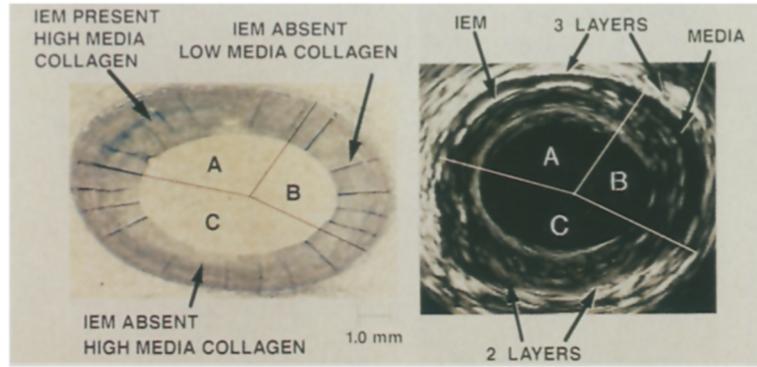


Fig. 5. Mixed pattern 2- and 3-layer IVUS appearance. *Left*, histologic section (trichrome stain) of a 42-year-old man demonstrates intimal hyperplasia. Under higher magnification, the three zones (A, B, and C) had distinct differences in the presence of IEM on van Gieson's stain and the amount of collagen deposited in the media. In section A, the IEM is present with a high (3+) degree of media collagen. The corresponding ultrasound image (*right*, section A) shows a three-layer appearance distinguished by an echogenic inner layer (intima) with an intense, thin echogenic line at the base of the intima that corresponded to the IEM. The media, which has similar echorefectivity as the intima, is separated by the bright band of IEM. In section B, the IEM was absent on van Gieson's stain, and the collagen content on the trichrome stain was low (+1). This produces a three-layer ultrasound image in which the inner echogenic structure corresponds to the intima and the echolucent band corresponds to the media. There is no intense echoreflecting line at the base of the plaque as compared with section A. In section C, the IEM was also absent, and the media collagen content was high. This produces a two-layer appearance on the ultrasound image with no distinction between the echogenicity of the intima or the media. However, there is distinction between the brighter reflections of the adventitia and the combined intima-media. In sections A and B, the measurement of intima and media thickness corresponded closely with the measurements from histology, whereas in section C the total intima plus media thickness correlated to the total wall thickness on histologic examination.

Table I. Ultrasound reproducibility studies

	<i>Intraobserver variability (r)</i>	<i>Interobserver variability (r)</i>
Lumen area	0.99	0.99
Inner echogenic layer area	0.90	0.96
Echolucent layer area	0.92	0.95
Lumen perimeter	0.97	0.97
Echolucent layer inner perimeter	0.99	0.99
Echolucent layer outer perimeter	0.99	0.99

p < 0.001 for all correlations.

of media (Fig. 5, section B). However, if the IEM were absent and the collagen content of the media were high, then the echogenicity of the media was similar to the intima; these structures were indistinguishable (Fig. 5, section C), and a two-layer ultrasound pattern was observed. These histologic and ultrasound patterns could exist contiguously at the same cross-section. These observations were independent of the extent of intimal hyperplasia.

The measurements of intima, media, and total intima plus media areas and the corresponding ultra-

Table II. Quantitative analysis: All arteries (*n* = 110)

	<i>Histologic specimens</i>	<i>Ultrasound</i>	<i>r value</i>
Lumen area (mm ²)	4.6 ± 2.4	4.7 ± 2.3	0.97*
Intima area (mm ²)	2.2 ± 1.5	3.0 ± 2.8	0.77*
Intima thickness (µm)	217 ± 143	293 ± 223	0.79*
Media area (mm ²)	2.2 ± 1.3	1.5 ± 1.0	0.25†
Media thickness (µm)	207 ± 100	128 ± 79	0.15‡
Total intima + media area (mm ²)	4.4 ± 2.4	4.5 ± 2.7	0.96*
Total intima + media thickness (µm)	427 ± 190	421 ± 204	0.96*

**p* < 0.0001.

†*p* < 0.001.

‡*p* = 0.7.

Includes all arteries, even if only two layers were observed.

sound areas of inner echogenic zone and middle echolucent zone for each of the four groups are shown in Table IV.

Group 1. In 18 of the 110 arteries, the IEM occupied at least 50% of the circumference, and the collagen content of the media was low (0 or 1+) by histologic analysis. These arteries had a three-layer appearance on IVUS imaging (Fig. 6). The mean area of intimal hyperplasia occupied 1.07 ± 0.50 mm² on histologic examination. This correlated well with the

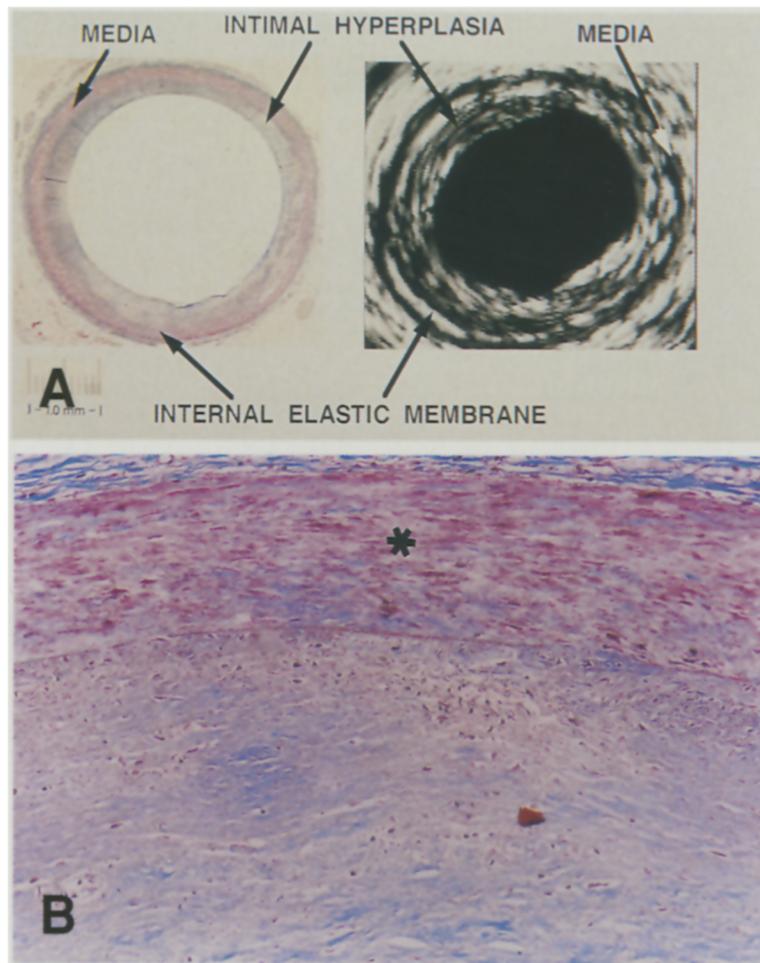


Fig. 6. A, Group 1. *Left*, intimal hyperplasia in a 42-year-old man's coronary artery with 70% of IEM intact and low (1+) medial collagen content. The corresponding ultrasound image (*right*) reveals a three-layered appearance. The echolucent zone underestimates the width of the media, but the total media plus intima thickness corresponds closely. *Scale divisions*, 100 microns. **B**, Magnified image ($\times 100$) of media (*) (middle band) with intact IEM and low collagen (blue) on trichrome stain.

value of the inner echogenic layer on the IVUS images ($r = 0.82, p < 0.0001$). The mean area of the media was slightly underrepresented by the IVUS echolucent zone ($1.72 \pm 0.71 \text{ mm}^2$ vs $1.49 \pm 0.57 \text{ mm}^2$; $r = 0.76, p = 0.0002$).

Group 2. In 28 of the 110 arteries, the IEM was present for $>50\%$ of the circumference on histologic examination, and the media revealed a high (2+ or 3+) degree of collagen. All these arteries also had a three-layer appearance on IVUS (Fig. 7). The correlation was close between the areas of intima and media by histologic examination versus the inner echogenic and echolucent zones on IVUS ($r = 0.92$ and $r = 0.93$, respectively; $p < 0.0001$) (Table IV).

Group 3. In the third group of 29 arteries, the IEM was fragmented and was visible on van Gieson stain for $<50\%$ of the vessel circumference. These arteries

Table III. Influence of IEM and medial collagen as predictors of ultrasound appearance

Group	n	Media collagen content	No. of layers on IVUS	Internal elastic membrane circumference by histologic specimens
1	18	Low	3	$\geq 50\%$
2	28	High	3	$\geq 50\%$
3	29	Low	3	$< 50\%$
4A	4	High	3	$< 50\%$
4B	31	High	2	$< 50\%$

also had a low collagen content in the media by trichrome stain. All these arteries demonstrated a three-layer IVUS image (Fig. 8) with the three layers resulting from an echolucent zone of media contrasted with the echogenic band from IEM seen in

Table IV. Comparison of histologic specimens and IVUS measurements

Area (mm ²)	Histologic specimens	IVUS	r	p Value
<i>Group 1 (n = 18), IEM >50%, low collagen</i>				
Intima	1.07 ± 0.50	1.04 ± 0.42	0.82*	
Media	1.72 ± 0.71	1.49 ± 0.57	0.76†	
TOTAL	2.78 ± 0.83	2.54 ± 0.77	0.86*	
<i>Group 2 (n = 28), IEM >50%, high collagen</i>				
Intima	1.70 ± 1.03	1.87 ± 0.96	0.92*	
Media	2.16 ± 1.28	2.01 ± 1.19	0.93*	
TOTAL	3.87 ± 1.87	3.88 ± 1.89	0.99*	
<i>Group 3 (n = 29), IEM <50%, low collagen</i>				
Intima	2.43 ± 1.62	2.43 ± 1.62	0.99*	
Media	1.72 ± 0.71	1.74 ± 0.71	0.97*	
TOTAL	4.16 ± 2.03	4.17 ± 2.04	0.99*	
<i>Group 4A (n = 31), IEM <50%, high collagen, predominant two-layer on IVUS</i>				
Intima	3.04 ± 1.85	6.31 ± 3.39	0.68†	
Media	3.05 ± 1.65	0	NA	
TOTAL	6.10 ± 2.98	6.31 ± 3.39	0.87*	
<i>Group 4B (n = 4), IEM <50%, high collagen, predominant three-layer on IVUS</i>				
Intima	3.19 ± 1.06	3.02 ± 1.52	0.84	0.16
Media	1.84 ± 1.55	1.42 ± 0.52	0.93	0.07
TOTAL	5.03 ± 2.59	4.44 ± 2.10	0.91	0.09

**p* < 0.0001.†*p* < 0.001.

group 2. There were close correlations between the intimal area on histologic examination and the inner echogenic layer ($r = 0.99$, $p < 0.0001$). These arteries had more intimal hyperplasia compared with the arteries in group 1, with a mean area of 2.43 ± 1.62 mm² ($p < 0.002$). The area occupied by the media was not different than the arteries in groups 1 and 2. The mean echolucent zone area of 1.74 ± 0.71 mm² on the IVUS images correlated closely to the value of the media by histologic examination ($r = 0.97$, $p < 0.0001$).

Group 4. The arteries in group 4 were distinguished by having both destruction of the IEM as well as a high collagen matrix content in the media. These arteries also had more intimal hyperplasia (3.04 ± 1.85 mm²) than the arteries in groups 1, 2, and 3 ($p < 0.002$ by analysis of variance). The media was also larger in this group (3.05 ± 1.65 mm²), which was statistically greater than the media in groups 1 and 3 ($p < 0.005$). On IVUS imaging, the vast majority (89%) of these arteries were represented by only two layers: a brighter echogenic structure corresponding to the adventitia and an inner echogenic layer (Fig. 9). This inner echogenic layer corresponded to the combined width of the intima plus media from histologic examination (6.10 ± 2.98 mm² vs 6.31 ± 3.39 mm²; $r = 0.87$, $p < 0.0001$).

These comparisons between histologic results and

ultrasound were derived from area measurements for each artery. Previous studies have compared measurements of intima and media thickness along a radian in several quadrants.¹¹ Therefore, a second analysis was performed in 32 quadrants from eight randomly selected arteries from groups 1, 2, and 3 to determine the relation of the intima thickness, media thickness, presence of IEM, and media collagen content on histology to the ultrasound image inner echogenic layer width, echolucent layer width, white band of IEM, and appearance of two or three layers along that radian. The histologic sections and ultrasound images were placed in corresponding positions using the India ink mark and the acoustic reference needle reflection. In this analysis by quadrant, there was an excellent correlation between intima thickness and inner echogenic layer width ($r = 0.99$, $p < 0.001$) and media thickness and echolucent layer width ($r = 0.99$, $p < 0.001$). The mean intima thickness was 162 ± 75 μm on histologic examination, and the corresponding mean inner echogenic layer width was 164 ± 73 μm. The corresponding values for media and echolucent zone width were 195 ± 86 μm and 197 ± 85 μm, respectively. The IEM was identified on the ultrasound images as a thin, intense echogenic line at the border between the echogenic layer representing intima and the echolucent layer of media. The percent circumference of the intense

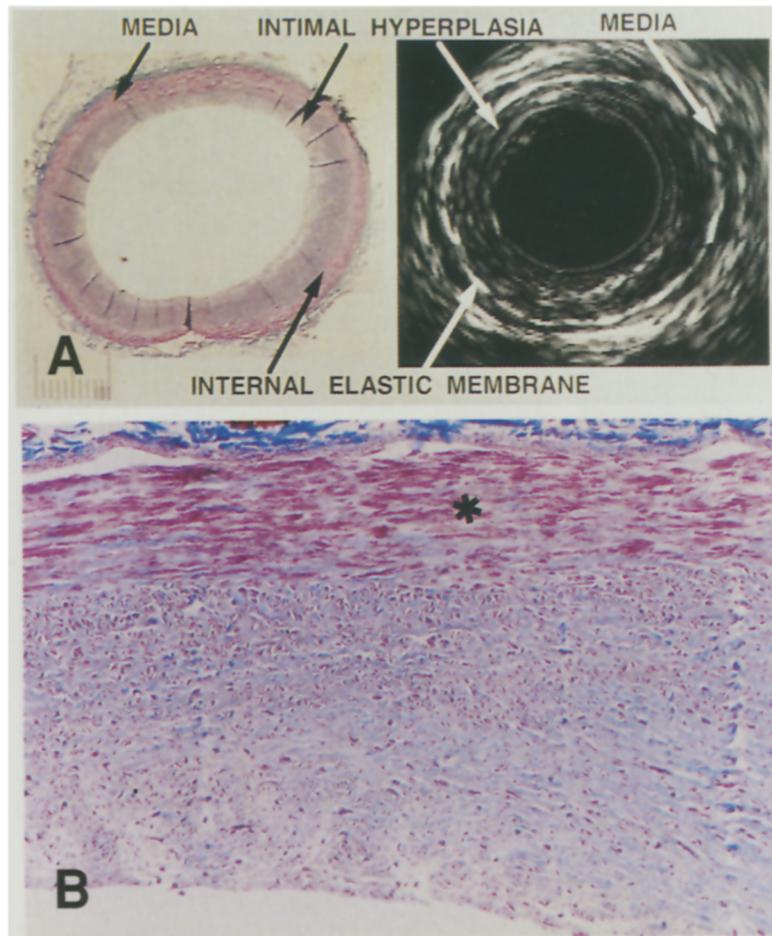


Fig. 7. A, Group 2. *Left*, Intimal hyperplasia in a 31-year-old man's coronary artery with 95% of the IEM intact and moderate (2+) medial collagen content. The corresponding ultrasound image (*right*) demonstrates a three-layered appearance despite the collagen content of the media because the IEM is present and is relatively more echo-intense. **B,** Magnified image ($\times 100$) of media (*).

echogenic thin band on ultrasound correlated closely with the presence of the IEM on histologic examination ($r = 0.92$, $p < 0.001$). As previously described (Fig. 5), the appearance of two or three layers varied among quadrants, but the ultrasound appearance accurately predicted the histologic content of medial collagen and presence or absence of IEM in each of the 32 quadrants.

DISCUSSION

The intravascular ultrasound appearance of a normal human coronary artery has been controversial. Early in vitro studies described normal muscular arteries as having a three-layer appearance with two echogenic zones separated by an echolucent zone.¹⁻⁸ Although there was some variation attributed to "blooming" from intense echogenic signals, the width and position of these zones appeared to correspond to the intima, media, and adventitia of the associated histologic sections.¹²⁻¹⁴ Other investi-

gators, using different ultrasound devices, have questioned these earlier observations.¹⁵⁻¹⁹ St. Goar et al.¹⁷ studied human transplanted hearts in vivo and found that the ultrasound pattern was represented by two layers in younger, presumably more normal arteries, from donors with an average age of 25 years. A three-layer ultrasound appearance was not observed until the intima thickness was greater than 178 μm . They concluded that the three-layer pattern was produced by intimal hyperplasia, and that truly normal arteries are represented by only two layers on ultrasound images.

The results of this in vitro analysis help to explain why there have been different conclusions among IVUS studies. The data indicate that the IVUS representation of an artery varies according to the histologic composition of the artery. The entire cross-section or portion of the circumference may be represented on ultrasound as a two- or three-layer structure for several reasons: (1) Relatively young,

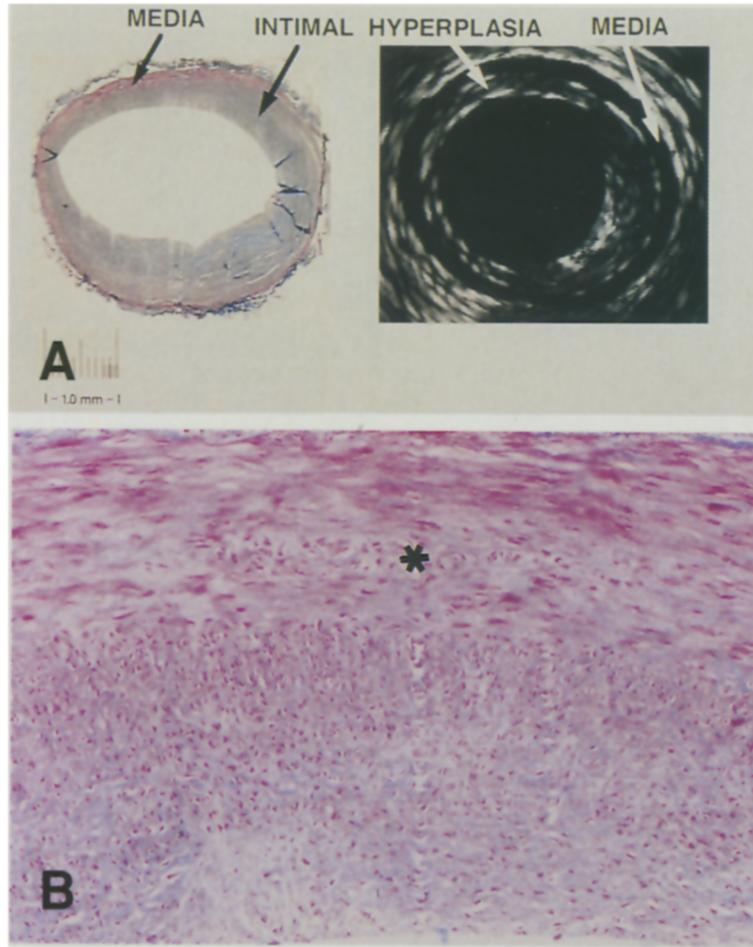


Fig. 8. A, Group 3. Histologic image (*left*) shows intimal hyperplasia in a 29-year-old man's coronary artery. There is absence of the IEM for most of the circumference and a low (0-1+) medial collagen content. The corresponding ultrasound image (*right*) shows a three-layered appearance with intimal thickness and an echolucent zone similar in width to the medial layer thickness. **B,** Magnified image ($\times 100$) of media (*).

grossly normal arteries may differ histologically because of the extent of IEM present, the collagen content of the media, and the extent of intimal hyperplasia. The IEM is a strong echo reflector; if it is present, the muscular media appears relatively echolucent and distinct, whether or not there is intimal hyperplasia present. If the IEM is absent from a portion of the artery, that segment of arc will still appear as three layers if the media is present and does not contain a large amount of collagen. Because collagen is echogenic, as the collagen content of the media increases, the media will reflect more and the image will lose the three-layer appearance. In these cases, the intima and media merge on the ultrasound image, but their combined width corresponds very closely to the intima plus media width on histologic examination.

(2) The three-layer appearance may also be al-

tered in severely diseased vessels by the presence of calcium, which causes acoustic shadowing. In addition, the media is often destroyed at the base of large sclerotic plaques, and therefore the ultrasound image may not show three layers because of the absence of the echolucent media.²⁰

(3) Another explanation for differences in interpretation may be that different ultrasound machines vary in their image resolution or the way in which the gray scale is presented. St. Goar used a 30 MHz transducer and a CVIS ultrasound machine. We used a 25 MHz device developed by Intertherapy. Other investigators using an electronic array catheter have found different correlations between histologic examination and the ultrasound image patterns.²¹

Pathologic studies of age-related changes in coronary arteries demonstrate that the degree of intimal

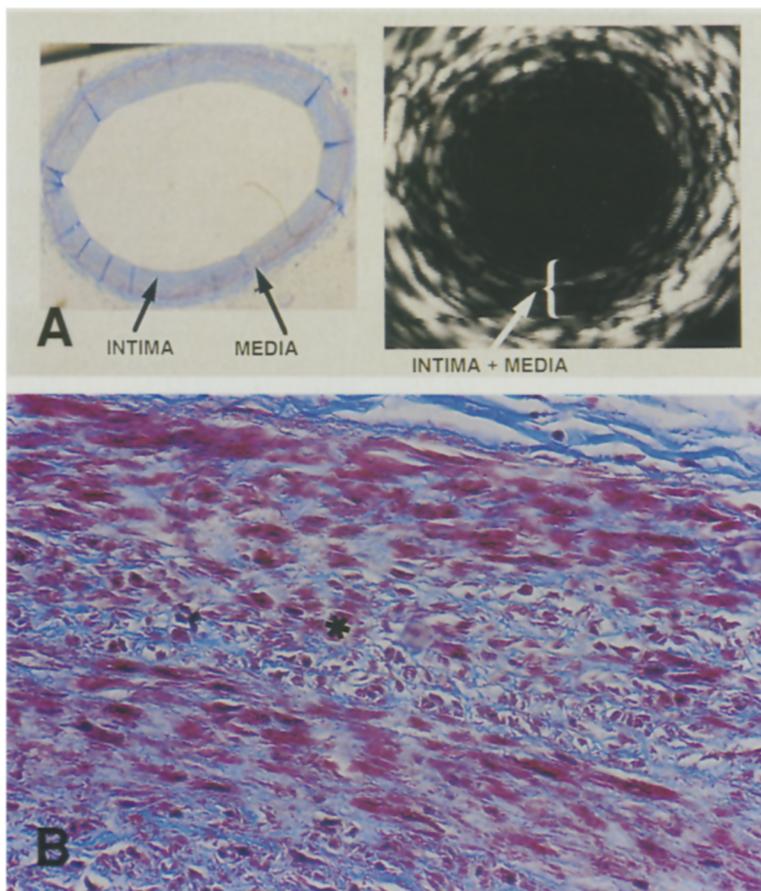


Fig. 9. A, Group 4. *Left*, histologic section from a 66-year-old woman's coronary artery with absence of the IEM for 90% of the circumference. The collagen content is high (3+) through the entire thickness of the media. The corresponding ultrasound image (*right*) shows only a two-layered appearance. The inner echogenic structure corresponds to the thickness of the media and intima combined. **B**, Magnified image ($\times 100$) of media (*).

thickening increases with age,²²⁻²⁴ and that men have thicker intima than women.^{22, 25} The intimal layer thickens through adolescence and exceeds the dimensions of the media near the age of 30.²⁶⁻²⁹ Our observations in 110 normal or minimally diseased human coronary arteries are consistent with these prior histologic studies. Collagen fiber content in the media increases with age.³⁰⁻³³ The increase is initially in the inner layers of the media and then spreads out to the periphery of the media near the external elastic membrane.³⁴⁻³⁶ This observation explains why ultrasound images tend to underestimate the thickness of the media: if the inner portion of the media contains much collagen, only the remaining rim of media will be echolucent.

Porter et al.³⁷ noted a similar increase in the echoreflectivity of the relative echolucent zone when the collagen content of the media was increased. When collagen content was high, there was an overestimation of the intima thickness by ultrasound.

However, the total echogenic layer thickness corresponded closely to the combined intima plus media thickness ($r = 0.89$) in 21 vessels. Our observations are consistent with these results, but they also extend the findings to include the influence of the IEM as well as medial collagen content in determining the appearance of two or three layers on the ultrasound image and their effect on measuring media and intima areas.

These observations should be useful in interpreting IVUS images during clinical studies. Identification of the depth of the intima and the level of the media is crucial, especially when performing atherectomy procedures. The thickness of the inner echogenic layer of human coronary arteries by intravascular ultrasound correlates well with the intima thickness by histologic analysis when the IEM is present. When the IEM is destroyed but the medial collagen density is low, the inner echogenic layer thickness still correlates well with intima thickness.

In vessels in which the IEM is destroyed and the medial collagen density is high, a two-layer appearance is seen with the inner echogenic layer by ultrasound corresponding to the combination of intima plus media. Although there may be differences in observations because of technical disparities among the available ultrasound machines, histologically normal human coronary arteries demonstrated a three-layer appearance on the IVUS imaging system used in this study.

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