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Intravascular echographic assessment of vessel wall characteristics: a correlation with histology

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Summary

In vivo application of intravascular high frequency ultrasonic imaging for peripheral and coronary artery disease is a promising technique for vascular surgeons, radiologists and cardiologists. This report demonstrates in vitro results obtained with a high frequency imaging catheter (40 MHz) in 70 human specimens including arteries with and without atherosclerosis, veins, coronary artery bypass grafts and vascular prosthetic material. Correlation between the ultrasonic images and the histologic characteristics of the corresponding vessel wall tissue and lumen geometry was established. In addition, the effect of intervention techniques i.e. balloon angioplasty, spark erosion and laser were studied with ultrasound and histology. It is anticipated that development of such a catheter imaging technique has potential for diagnostic imaging and for combination with therapeutic systems.

Introduction

The ultimate advantage of intravascular ultrasonic catheters is that its unique position allows the use of high frequency transducers (> 20 MHz) which provide detailed information of both vessel geometry and morphology.

The ability of intravascular ultrasound to assess in vitro the diameter, area and wall thickness of the artery and to distinguish calcified from non-calcified atherosclerotic lesions has been reported [1–5]. Our own experience revealed the possibility to distinguish muscular from elastic arteries as well as to differentiate between the basic components of atherosclerosis [6–7].

This report has two principle aims. First, to acquaint future intravascular ultrasonographers with the **histologic** characteristics of arteries, veins, aorta-coronary artery bypass grafts, vascular prostheses and atherosclerosis. Subsequently, these data are compared with the **ultrasonic** characteristics obtained in a series of in vitro studies using human vascular specimens.

Second, the basic aspects of intervention techniques i.e. balloon dilatation, spark erosion and laser are outlined. Subsequently, the effect of these intervention techniques as visualized by ultrasound using human vascular specimens are evaluated. The results are compared with those from the corresponding histologic sections.

Technique

All studies were performed with a high frequency intravascular device currently under development at our laboratory. A 40 MHz single element transducer mounted on a 8F catheter tip was used. Axial

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resolution of the system was 75 μm. Lateral resolution was better than 200 μm at a depth of 1 mm. Cross-sectional images were obtained by motor-driven catheter tip rotation. Data acquisition time to complete one image was 20 seconds. The resulting images were displayed on a monitor via a video scanned memory.

Human specimens

Specimens were either removed at surgery or at autopsy. The specimens of approximately 10 mm length were isolated from redundant surrounding tissues and were stored at −20° C. For in vitro studies a total of 70 specimens were selected; arteries (54) (common carotid, subclavian, internal thoracic, brachial, coronary, superior mesenteric, splenic, renal, iliac and femoral); veins (4) (splenic, iliac, femoral, and saphenous); aorta-coronary bypass grafts (4) and segments of vascular prosthetic material (Goretex; Stent) (8). Atherosclerotic lesions were present to a variable degree.

For in vitro studies the specimens were thawed and embedded in a 1.2% solution of agar-agar. The specimens were positioned vertically in order to allow adequate access of the ultrasound catheter. The lumen of the specimens was filled with purified water.

In vitro experiments were carried out by placing the catheter in the lumen of the specimen. Subsequently, ultrasonic cross-sections were obtained from proximal to distal with 1 mm interval between each.

Microscopic investigation

The proximal cross-sectional sites of the specimens were correspondingly marked with ink. Subsequently they were fixed in 10% buffered formalin, decalcified and processed for routine paraffin embedding. Transverse sections of 5 μm thick perpendicular to the longitudinal axis of the vessel were cut at 1 mm intervals. The sections, arranged from proximal to distal, were stained with a Verhoeff's elastin van Gieson and the Haematoxylin azophloxine technique [8].

Data analysis

From each of the 70 specimens studied the characteristics, as documented by histology, were compared with the corresponding echo cross-section.

Results

Arteries

Histology
Basically there are 2 types of arteries – elastic and muscular [9]. The 2 types are not sharply divided since elastic arteries gradually merge into muscular arteries.

The aorta, pulmonary trunk and its main branches, and the proximal segments of the brachiocephalic, carotid, subclavian and common iliac arteries belong to the elastic type. All other arteries such as the coronary, renal and femoral, are muscular.

Histologically, the wall of both types of artery is composed of an intima, an internal elastic lamina, a media, an external elastic lamina and an adventitia. The main difference between the 2 types of arteries is found in the composition of the media.

In the elastic artery the media consists of circularly arranged elastin fibers which have smooth muscle cells sandwiched in between (Fig. 1). This makes the media of this type of artery elastin-rich. There may be a varying amount of intercellular connective tissue and mucopolysaccharide-rich ground substance. In the muscular artery the media predominantly consists of circularly arranged smooth muscle cells (Fig. 1). Only a few elastin fibers may be present, particularly within the larger arteries, as well as some intercellular connective tissue.

Echography
Based on the typical histologic differences found in the composition of the media, accurate distinction was possible between the different types of arteries studied. An elastic artery was recognized by a media which was as echogenic as the surrounding intima and adventitia. The presence of circularly

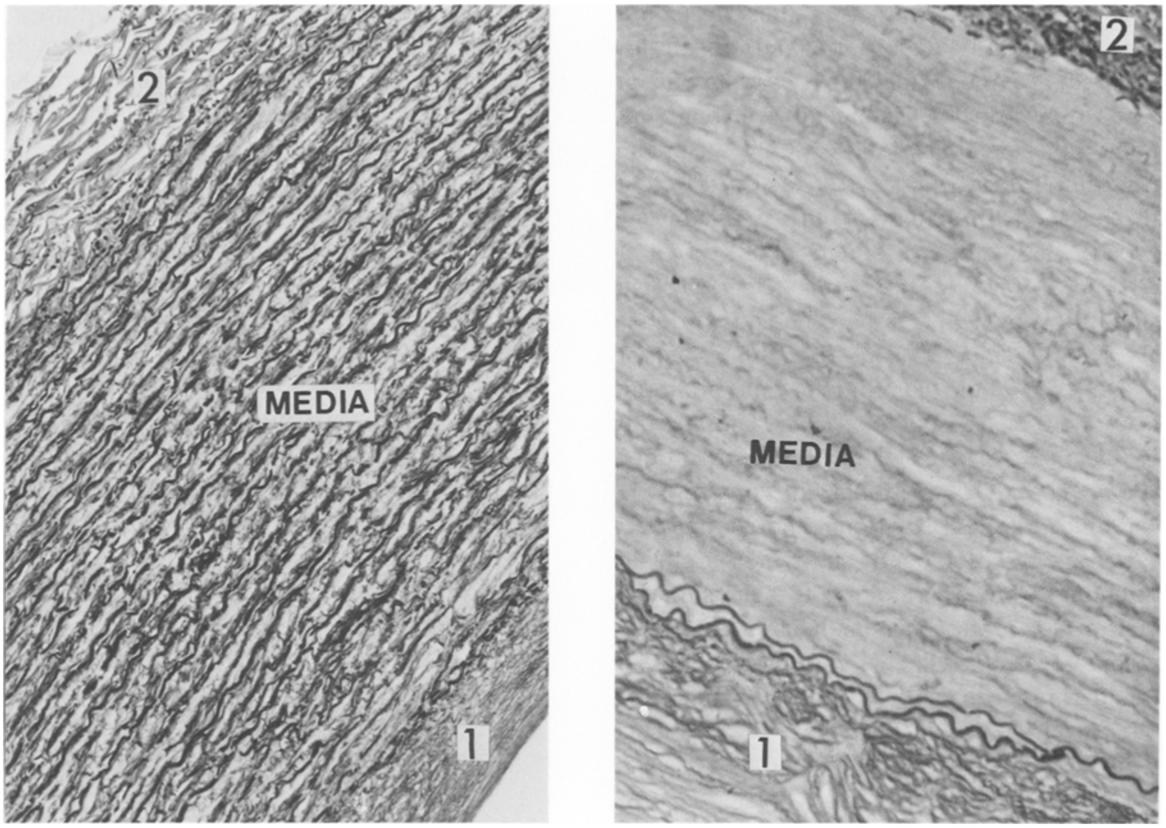


Fig. 1. Photomicrographs showing detailed characteristics of the media of an elastic artery (left panel) and a muscular artery (right panel) interposed between intima (1) and adventitia (2). The media of an elastic artery is densely packed with elastin fibers (black stained) whereas the media of a muscular artery is mainly composed of smooth muscle cells. Verhoeff van Gieson stain. Original magnification 60x.

arranged elastin fibers in the media of an elastic artery resulted in a significant amount of acoustic backscatter with a power level comparable to that from the surrounding tissues (Fig. 2).

Conversely, muscular arteries presented as a typical three-layered wall: an hypoechoic media amidst the intima and adventitia, both showing bright echoes (Figs. 2, 3).

Based on the ultrasonic appearance of the media (bright or hypoechoic) intravascular ultrasound provided a correct distinction between elastic (22) and muscular arteries (32). The aorta, carotid, subclavian and internal thoracic arteries presented as elastic arteries (Fig. 2). The brachial, coronary, mesenteric, splenic, renal, iliac (distal) and femoral arteries were of a muscular nature (Figs. 2, 3).

With ultrasound we were able to observe that an elastic artery gradually merges into a muscular artery based on the echo characteristics of the media. Studies in proximal coronary artery specimens revealed that the media of the artery was of an elastic nature, whereas 2–3 mm distally the media became progressively more muscular (Fig. 2).

In our experience both the intima and internal elastic lamina and the external elastic lamina and adventitia could not be discriminated by ultrasound.

Veins

Histology

Veins have thinner walls than their arterial companion. They contain an abundance of connective

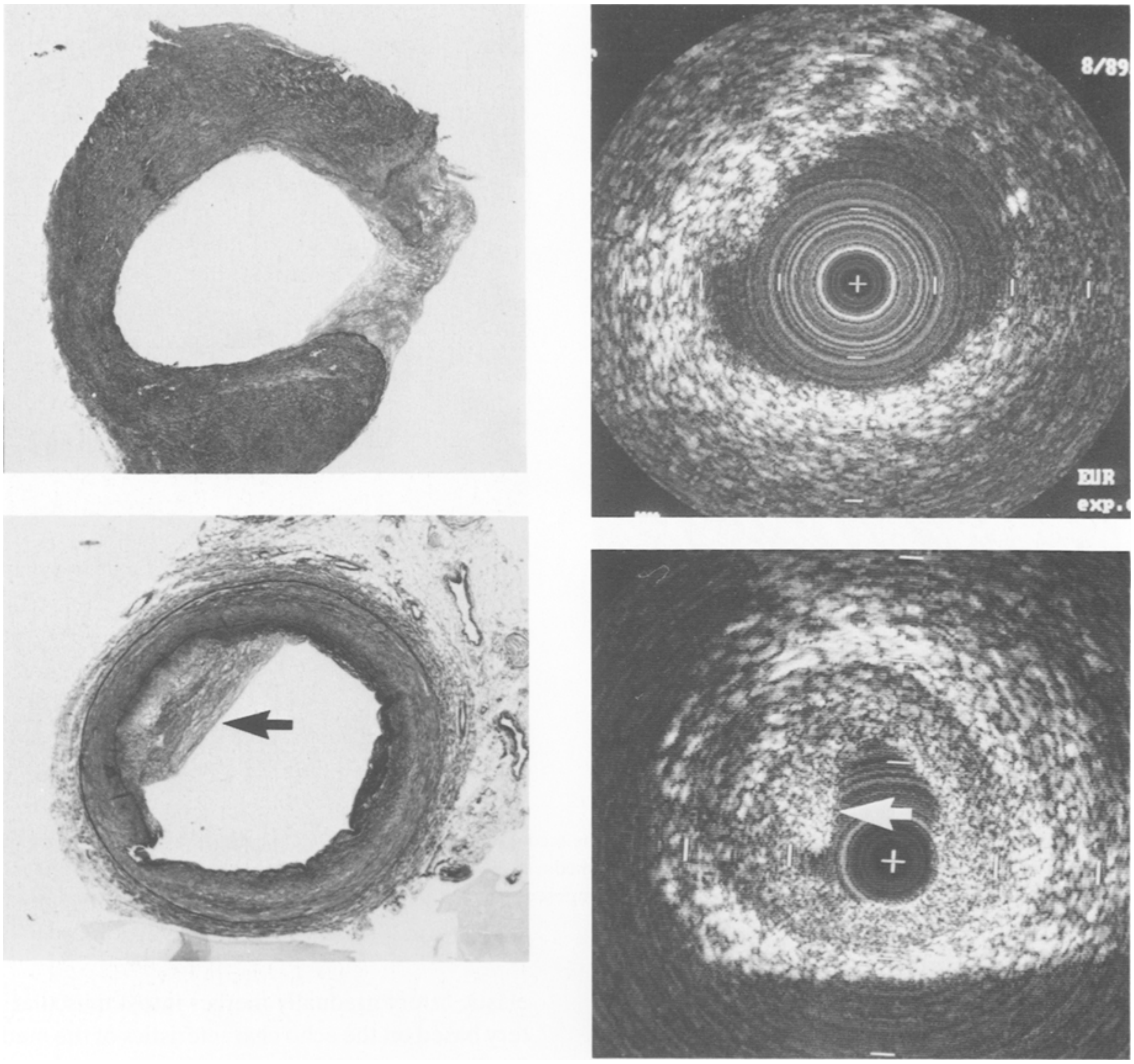


Fig. 2. Photomicrographs showing the difference in architecture of the media in the proximal left main coronary artery. At the junction of the aortic root the media is of elastin nature (upper panel). It consists of densely-packed multi-layered elastin fibers, an observation much facilitated by utilizing a selective elastin staining technique. On the corresponding ultrasonic cross-section the media appears to be as bright as the intima and adventitia.

The section obtained 2 mm distally (lower panel) shows the media to be of muscular nature. It is mainly composed of smooth muscle cells whereas the elastin fibers are more or less disorganized. The media on the corresponding ultrasonic cross-section appears relatively hypoechoic. An eccentric atherosclerotic lesion, best seen between 9 and 12 o'clock in the lower panel, is composed of fibromuscular tissue and lipid (arrow). On ultrasound the lesion (arrow) was recognized by soft echoes with bright particles. Verhoeff van Gieson stain. Magnification 8x.

tissue, whereas elastin fibers and smooth muscle cells occur in smaller numbers. Consequently their lumen is often collapsed during routine preparation for light microscopy.

The structure of small and medium sized veins varies greatly. Histologically, the wall consists of basically 3 unclearly defined layers: intima, media and adventitia. The intima consists of endothelial cells which either rest directly on a poorly defined internal elastic lamina or are separated from it by a

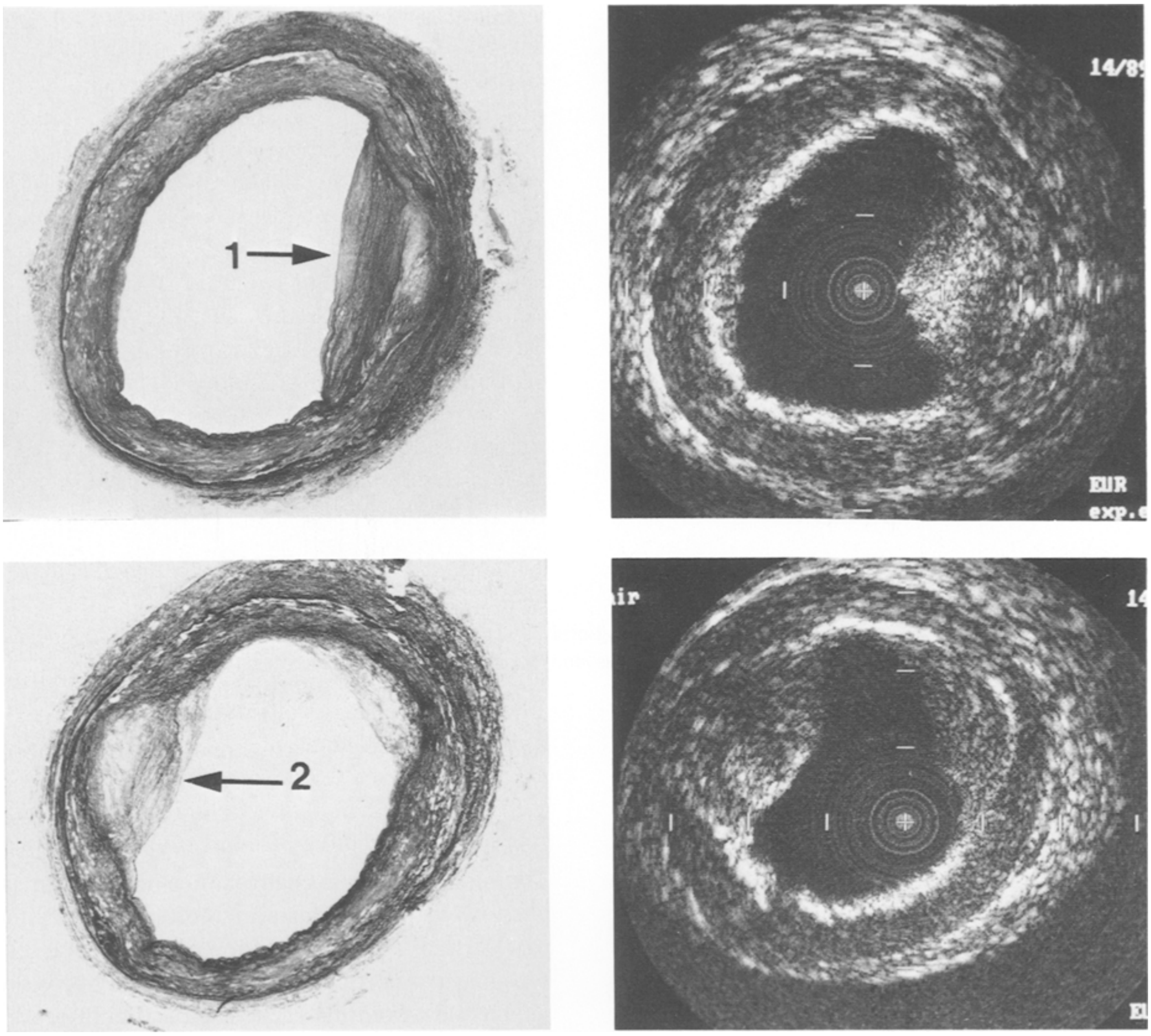
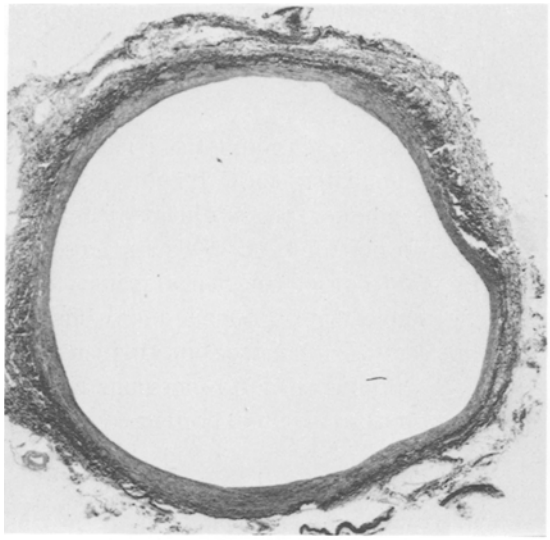


Fig. 3. Photomicrographs of histologic cross-sections obtained from the mesenteric superior artery together with the corresponding ultrasonic cross-sections. The media of this typical muscular artery mainly composed of smooth muscle cells, appears characteristically hypoechoic on ultrasound. Note that the eccentric atherosclerotic lesion barely visible at 9 o'clock (upper panel) is of marked larger size compared to the cross-section obtained 4 mm distally (lower panel). Both lesions (arrows 1 and 2) were of amorphous non-calcific tissue character containing fibromuscular tissue and lipid. Bright echoes of intima and adventitia circumscribing the hypoechoic media. Verhoeff van Gieson stain. Magnification 8x.

slight amount of subendothelial connective tissue. The media consists of circularly, or circularly and longitudinally, disposed smooth muscle cells and collagen, and few elastin fibers. The external elastic lamina is poorly defined. The adventitia consists mainly of thick collagen-rich connective tissue arranged longitudinally. It is the thickest part of the wall blending with the media [9].

Echography

Compared to the ultrasonic gain setting necessary to image arterial wall specimens, a higher gain setting was required to get a proper image of the veins. The walls of the veins studied produced soft echoes which did not allow any ultrasonic distinction between intima, media and adventitia (Fig. 4). This is intimately related to the composition of the



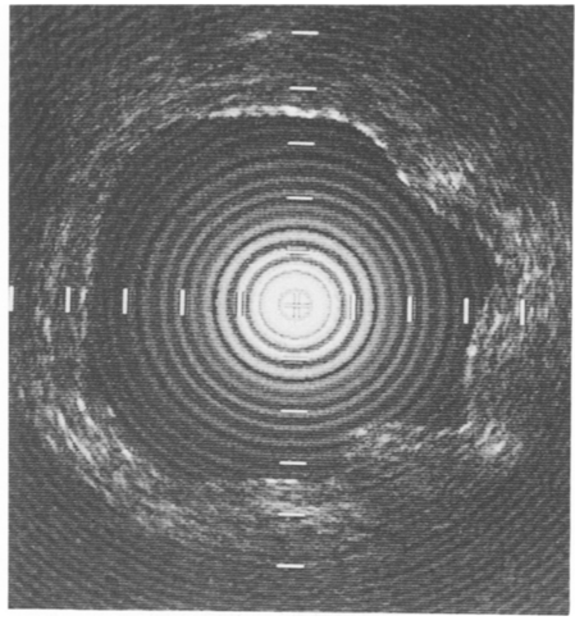


Fig. 4. Microphotograph of a histologic section and corresponding intravascular ultrasonic cross-section obtained from the femoral vein. Intima, media and adventitia cannot be distinguished with ultrasound. Verhoeff van Gieson stain. Magnification 8x.

wall: predominantly composed of fibrous connective tissue and smooth muscle cells. Whereas elastin fibers, commonly present in the internal and external elastic lamina, were practically absent.

Aorta-coronary artery bypass grafts

Histology

Following aorta-coronary bypass grafting the autologous saphenous vein, used in this procedure, may show adaptive and reparative changes [10]. These are superimposed upon any preexisting changes in the vein. Damage to endothelial cells in the early phase after grafting leads to fibrin deposits on the intimal surface and edema of the wall. After 4–6 weeks proliferation of myointimal cells may cause intima thickening. Over years this may progress to classical atherosclerotic lesions [10].

The media is affected by interference with its blood supply caused by transplantation and the muscle cells may die and be replaced by connective or collagen-rich connective tissue (fibrosis). Remaining muscle cells may hypertrophy. The adventitia on the other hand becomes replaced by scarred and collagen-rich connective tissue (fibrosis).

Echography

The main ultrasonic changes that occurred in saphenous veins used for aorta-coronary artery bypass surgery were proliferation of myointimal cells recognized as a zone of soft echoes, and fibrosis of media and adventitia recognized as bright echoes (Fig. 5). Both latter structures could not be differentiated from each other with ultrasound, as was the case with normal veins.

Vascular prostheses

Histology

The synthetic Goretex material appeared on histology as well-arranged, short-fiber elements. For obvious reasons stents are not amenable to be sectioned histologically. In the early postoperative phase the lumen surface of a vascular prosthesis becomes covered by fibrin deposits; later to be replaced by fibromuscular tissue.

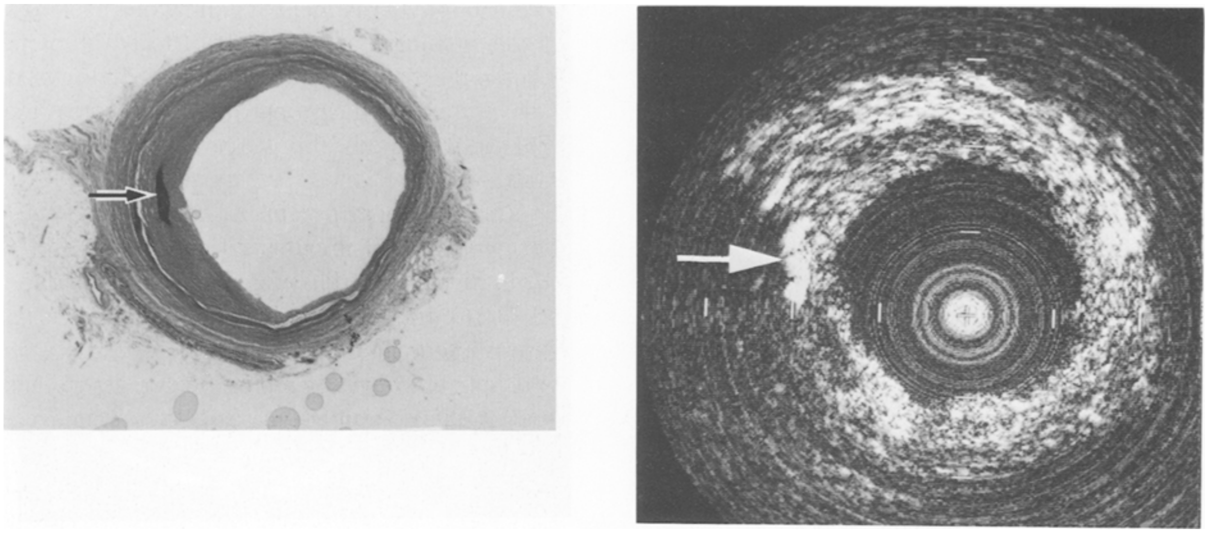


Fig. 5. Photomicrograph showing the architecture of an implanted venous bypass graft and corresponding ultrasonic cross-section. Histologically, the adventitia is composed of collagen-rich connective tissue and the media shows diffuse fibrosis. Between 6 and 12 o'clock a superimposed atherosclerotic lesion is seen which mainly consists of connective tissue with diffuse lipid deposits. On ultrasound a more echoreflective zone is seen between 11 and 3 o'clock reflective of scarred adventitial tissue. The scarred adventitia between 3 and 11 o'clock is not detected with ultrasound presumably due to an inappropriate angle of the ultrasound beam in respect to the adventitia. The atherosclerotic lesion appears with soft echoes. Calcium present at 9 o'clock (arrow) causes ultrasonic shadowing. Haematoxylin azophloxine stain. Magnification 8x.

Echography

On ultrasound the Goretex prosthesis as well as the stent were recognized as a highly reflective, relatively thin structure. Fibrin deposits as well as fibromuscular tissue were recognized as soft echoes (Fig. 6).

Atherosclerotic lesions

Histology

Early atherosclerotic lesions are known as fatty streaks and are composed of agglomerates of foam cells containing lipids. The advanced lesion, the atherosclerotic plaque, shows extensive variability in its composition. At one end of the spectrum the atherosclerotic plaque may be almost entirely composed of fibromuscular tissue i.e. connective tissue, smooth muscle cells and disorganized elastin fibers with an important collagenous component. At the other end, the plaque may consist of a large central atheroma with a scanty fibrous capsule composed of collagen-rich connective tissue.

The classical atherosclerotic plaque is localized in the intima and is composed of a lake of fatty debris with cholesterol crystals, sometimes fibrin and calcium deposits – the atheroma surrounded by macrophages and lymphocytes and a fibromuscular layer. Towards the lumen the lesion is bordered by a fibrous tissue capsule covered by endothelium. Towards the media the border of the plaque may be easily recognizable by a still present internal elastic lamina, but may also be vague due to the presence of a fibromuscular layer. In some instances the internal elastic lamina and the media may even totally disappear and the atherosclerotic lesion can reach as far as the adventitia. Such a lesion will evoke an inflammatory infiltrate in the adventitia [11]. When the fibrous capsule becomes extremely thin it may rupture and allow blood and blood components to enter the plaque leading to plaque haemorrhage.

A complication of a ruptured or cracked plaque is thrombus formation on its surface. The thrombus is composed of a meshwork of strands of fibrin in which red cells, leukocytes and platelets are en-

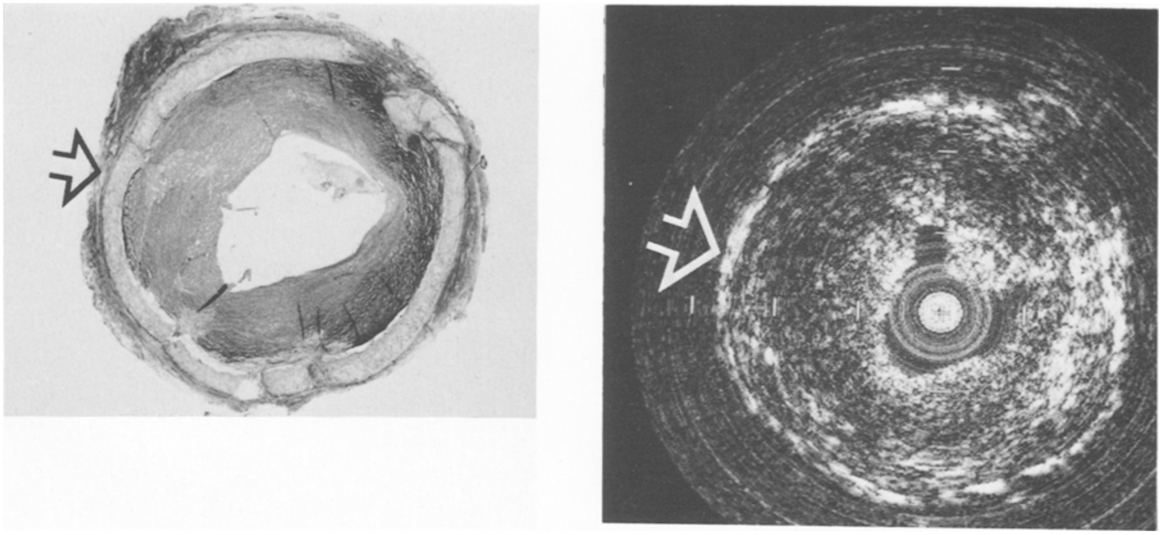


Fig. 6. Microphotograph showing a vascular Goretex prosthesis in cross-section with an obstructive lesion. The lesion consists mainly of loose connective tissue. On the corresponding ultrasound cross-section the Goretex prosthesis is recognized as a reflective structure (open arrow). The lesion itself generates relatively soft echoes. Verhoeff van Gieson stain. Magnification 8x.

trapped. If the thrombus does not dissolve it becomes organized by ingrowth of fibrovascular tissue [12].

Echography

Based on the echogenicity of the atherosclerotic lesion, ultrasound could distinguish 4 basic types of plaque components:

1. hypoechoic – a reflection of a significant deposit of lipid (Fig. 7).
2. soft echoes – reflective of fibromuscular tissue (intimal proliferation as well as lesions that consist of fibromuscular tissue and diffusely dispersed lipid: the atherosclerotic lesion, Figs. 2, 3, 5 and 8).
3. bright echoes – representative for collagen-rich fibrous tissues (Fig. 7).
4. bright echoes with shadowing behind the lesion, representative for calcium (Figs. 5 and 7).

Of the 54 arteries studied there was no histologic evidence of atherosclerosis in 4: this was confirmed with intravascular imaging. In 7 specimens fibromuscular tissue was the only underlying disorder and on ultrasound it was recognized as soft echoes adjacent to the intimal surface. In the remaining 43 arteries histology revealed the presence of an atherosclerotic lesion, either restricted to one particular area (19) or involving the entire arterial vessel circumference (24). Ultrasound examination had correctly assessed the presence and extent of the lesion involved. By systematically scanning the arteries with ultrasound from proximal to distal, at a 1 mm interval, it was noted that the location and composition of atherosclerotic plaques revealed marked variations (Figs. 3 and 7).

Interventional techniques in relation to intravascular echography

In a separate series of 16 human specimens with an obstructive atherosclerotic lesion the effect of balloon dilatation, spark erosion and laser on the tissue interrogated was assessed echographically and compared to histology.

Balloon dilatation

With help of a balloon catheter, obstructive lesions in both femoral and iliac arteries were dilated. For this purpose a Cordis 4F (femoral) and 7F (iliac)

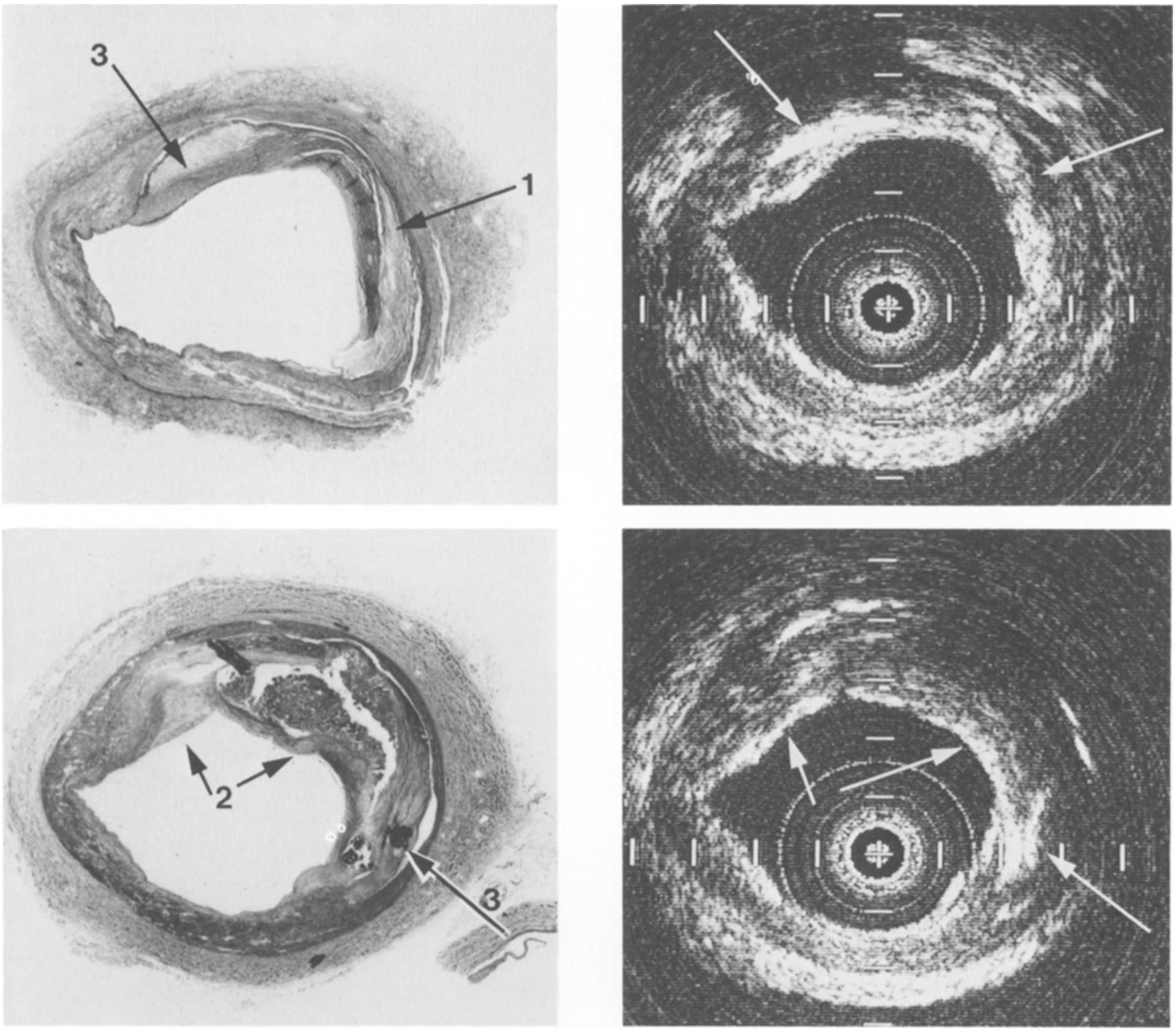


Fig. 7. Histologic cross-sections of a femoral artery showing a classical obstructive atherosclerotic lesion with corresponding ultrasonic cross-sections. Fatty debris appearing as hypoechoic (arrow 1), collagen as bright echoes (arrow 2) and calcium as bright echoes with shadowing (arrow 3), are the major plaque constituents. Distribution of calcium within the lesion varies significantly (11 o'clock in upper panel, 4 o'clock in lower panel). Verhoeff van Gieson stain upper panel. Haematoxylin azophloxin stain lower panel. Magnification 8x.

catheter were used and inflated to 10 Bar for one minute. Echographic investigation was performed before and 2 minutes following intervention. Care was taken to make the cross-sections, before and after the intervention, at comparable levels.

Histologically, a typical laceration of the plaque from the media was observed in all 4 specimens studied (Fig. 8). This observation is highly specific for a successful angioplastic procedure [13]. Ultrasound examination, however, revealed a distinct dissection in one of total 40 cross-sections studied, as an echo-free zone between the lesion and the original wall (Fig. 8). In 2 other cross-sections a dissection was questionable. It should be realized that these investigations were performed under non-physiologic conditions and might not be comparable to the situation in vivo. Therefore, it may be anticipated that after dilatation the dissected lesion remained adherent to the vessel wall. Furthermore, it was noted that the increase in lumen

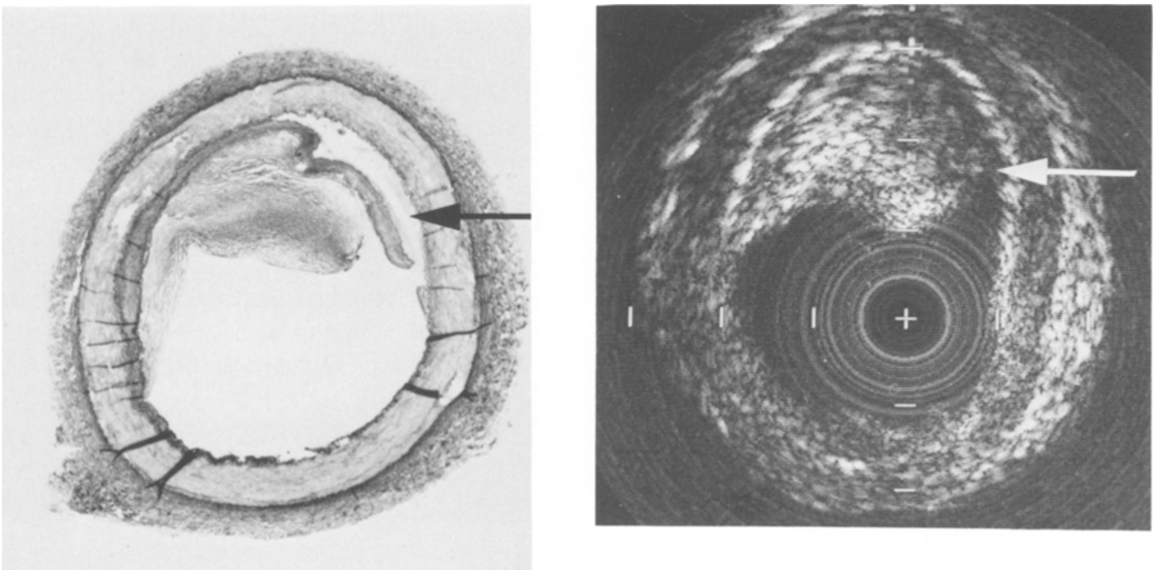


Fig. 8. Histologic and corresponding ultrasonic cross-sections obtained from the femoral artery after angioplasty. The artery presents an eccentric obstructive atherosclerotic lesion recognized as relatively soft echoes. The corresponding histologic cross-section shows that the lesion mainly consists of connective tissue and fatty deposits. The dilatation procedure had resulted in a dissection (arrow). Verhoeff van Gieson stain. Magnification 8x.

diameter after the procedure varied from no measurable increase to a lumen diameter increase twice its original size.

Spark erosion

Spark erosion was accomplished with a 2 mm diameter, parabolically shaped electrode which rotated at 750 rpm. The active electrode area used for spark erosion was 1 square mm. Sparking was applied during 40 ms periods at 1 sec intervals by application of 500 kHz alternating voltage with an effective value of 360 Volt [14].

This procedure was performed in 8 human arteries. In 6 of these arteries the lumen was totally occluded by an atherosclerotic lesion. For this reason spark erosion was performed in order to create an access for the ultrasonic transducer. One hour following the intervention the arteries were systematically studied with ultrasound. The presence of a distinct hole could be precisely identified and its location was subsequently confirmed by histology (Fig. 9). In all instances erosion of the plaque surface was seen. Some of the histologic cross-sections revealed evidence of coagulation. This phenomenon resulted in increased ultrasonic backscatter.

Laser

A 1.8 mm diameter rounded sapphire contact probe mounted on a 0.6 mm core silica fiber was used. The silica fiber was connected to a continuous wave Nd-YAG laser (Medilas-2, MBB). A saline flush (3 ml/min) prevented blood from entering the fiber-sapphire interface and cooled the sapphire crystal and the metal connector [15, 16]. Laser shots of 14 Watt power and exposure time of 20–40 seconds duration were used in 4 arteries, with lumens that were totally occluded by atherosclerotic lesions. This was done in order to create a lumen accessible for the ultrasound probe. Similar to the spark erosion technique, laser caused erosion of the plaque surface as well as some coagulation effect. The amount of coagulation was, in our experience, too minute to be detectable with ultrasound.

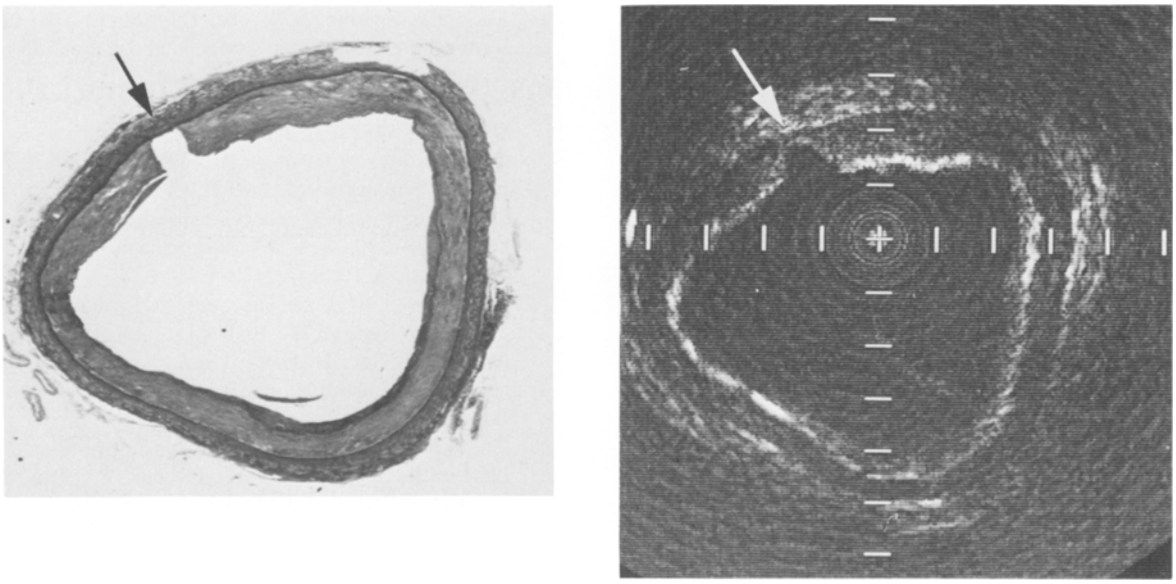


Fig. 9. Histologic and corresponding ultrasonic cross-section obtained from the iliac artery after spark erosion. At 11 o'clock a distinct hole is seen (arrow) created by spark erosion. Verhoeff van Gieson stain. Magnification 8x.

Limitations

The ultimate ultrasonic response is intimately dependent on the angle of insonification. Perpendicular insonification results in an optimal response (see also Figs. 2, 3 and 5). Only under these conditions are we able to make adequate distinction between the size and composition of the atherosclerotic lesion involved. This observation should be taken into account when in vivo application is considered.

Identification of atherosclerotic lesions in a muscular artery in general is facilitated by the presence of a highly reflective intima and a hypoechoic media. Conversely, adequate discrimination of the lesion from the underlying elastic artery may be prevented when the echo characteristics of the lesion are similar to the echo response generated by the intima and media in these elastic arteries (see also Fig. 2).

Conclusions

This report briefly reviews the histologic characteristics of human vascular specimens. The relationship between histology and cross-sectional ultrasonic images obtained with a high frequency transducer was studied in vitro.

A clear difference between elastin and muscular arteries was observed with ultrasound which was caused by the presence or absence of elastin tissue in the media. Moreover, the technique revealed characteristic differences in architecture of normal veins and veins used for aortacoronary bypass grafting. Vascular prostheses have a distinct influence on ultrasound as has its internal lumen surface.

Comparison between ultrasonic images and corresponding histologic cross-sections showed that characteristics of the echo image of an atherosclerotic lesion relate to the histologic composition of the lesion.

The effect of intervention techniques was studied. A typical plaque rupture following balloon dilatation may be observed. Spark erosion as well as laser intervention may produce coagulation which might result in an area of increased echoreflectivity.

This unique high resolution ultrasonic imaging device enables determination of the vessel and plaque morphology as well as assessment of the extent of the disease. Application of such a technique is two-fold. First, for diagnostic imaging, the lumen area as well as characteristics of the type of lesion can be depicted. Knowledge of the plaque type enables prediction of the intervention procedure of choice and potential areas of difficulty. If the plaque is mainly non-calcific the lesion can be treated more easily than the calcified plaque. Second, the technique can be used as a control system after an intervention.

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