Histopathologic Validation of Angioscopy and Intravascular Ultrasound

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Background. To establish a histopathologic basis for angioscopic and ultrasound image interpretation we studied 70 postmortem human arterial segments in vitro.

Methods and Results. We used 7- to 9-French fiber-optic angioscopes and 20- to 30-MHz intravascular ultrasound imaging catheters. Three observers assigned an angioscopic and ultrasound image classification to each vessel segment. The image and histological classification categories were then compared. The sensitivity, specificity, and accuracy of both methods separately or in combination for normal vessels were each greater than or equal to 95%. The predictive value was better for angioscopy than for ultrasound due to incorrect ultrasound interpretations of normal anatomy in the presence of thrombus. For stable atheroma the sensitivity, specificity, and accuracy of the individual methods were each greater than 90%. However, both angioscopy and ultrasound had classification errors in that disrupted atheroma was identified and classified as stable atheroma. Consequently, the predictive value was 74% for angioscopy and 78% for ultrasound. For disrupted atheroma the sensitivities for angioscopy and ultrasound were only moderate (73% and 81%, respectively), whereas the specificity, accuracy, and predictive value were each high (greater than 90%). For thrombus detection, the specificity, accuracy, and predictive value were high (greater than 93%) for each method. The sensitivity of angioscopy was 100%. However, sensitivity was lower for ultrasound (57%) due to false-negative interpretation of laminar clots in normal vessels and an inability to distinguish disrupted or stable atheroma from intraluminal thrombus.

Conclusions. Contingency analyses showed that each imaging method alone or combined had significant agreement with the results obtained from histology (p<0.001). When assessing all cases in which angioscopy and ultrasound were concordant, there was a 92% agreement with the histological classification. (Circulation 1991;84:109–117)

Images that provide information about the structure of the arterial intima and media are of value for understanding the pathogenesis of coronary disease and for making decisions during intravascular procedures. Such images are being obtained in humans by both angioscopy and ultrasound.1–3 Interpretation of these images, however, is subjective. As yet no histopathologic validation of these interpretations has been reported, nor has there been a comparison of the strengths and weaknesses of the two techniques. The purpose of this study is to validate these intravascular imaging technologies by determining the sensitivity, specificity, accuracy, and predictive value of angioscopy and intravascular ultrasound.

Methods

Materials

To compare information derived by intravascular angioscopy and ultrasound, 70 postmortem human arterial segments were studied. Coronary or peripheral arterial segments were obtained at autopsy and stored in 10% neutral buffered formalin. Sixteen arterial segments were subjected to balloon angioplasty to induce arterial disruption. In nine vessels postmortem arterial thrombi were created by the insertion of 24–72-hour-old human blood clots. Angioscopic and ultrasound images were recorded in saline and randomly played on a video screen and then blindly classified by consensus among three observers. These observations were compared with the histopathologic findings.
Table 1. Image Classification Criteria

<table>
<thead>
<tr>
<th>Method</th>
<th>Criterion 1</th>
<th>Criterion 2</th>
<th>Criterion 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angioscopy</td>
<td>Color</td>
<td>Contour</td>
<td>Configuration</td>
</tr>
<tr>
<td></td>
<td>Yellow/white</td>
<td>Flat</td>
<td>Smooth</td>
</tr>
<tr>
<td></td>
<td>Red</td>
<td>Elevated</td>
<td>Irregular</td>
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<tr>
<td>Ultrasound</td>
<td>Density</td>
<td>Thickness</td>
<td>Configuration</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>Normal</td>
<td>Smooth</td>
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<tr>
<td></td>
<td>Increased</td>
<td>Increased</td>
<td>Irregular</td>
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<tr>
<td></td>
<td>Decreased</td>
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Ultrasound Device

For our intravascular studies we used flexible 5- to 8-French catheters with a mechanical ultrasound transducer and a fixed distal guide wire (Cardiovascular Imaging Systems Inc., Sunnyvale, Calif.). The transducer frequency was 20–30 MHz. The ultrasonic real-time scan provides a 360° display of a two-dimensional intravascular image at a frame rate of 7–20/sec. The system provides images of tissue at a radius of up to 40 mm from the catheter surface. The catheter system was passed into the artery and advanced to a region of interest, which was later identified by placement of a suture in the adventitia of the vessel.

Angioscopic Device

Our fiber-optic angioscopes ranged in diameter from 7 to 9 French (Baxter Edwards, LIS Division, Irvine, Calif.). These angioscopes contained 6,000 imaging fibers and a concentric ring of illuminating fibers sheathed in a flexible plastic housing. Prior to first use, each angioscope was tested for minimum focus, spatial resolution, and flaws in the jacketing. The spatial resolution of the angioscopes exceeded 200 μm at 5 mm.

Illumination was provided by a 300-W xenon light source (Baxter Edwards). Adjustment of the light intensity is a dynamic process and is dependent on reflection of the vessel wall, diameter and tortuosity of the vessel lumen, and aperture of the camera lens. The image obtained by the distal lens of the angioscope is transmitted along the image bundle to a specially constructed video-endoptic coupler, then to a low-noise, high-gain, charge-coupled device chip camera (Baxter Edwards). We viewed the images on a high-resolution monitor (Sony PVM 1960) and simultaneously recorded them using a 3/4-in. videotape recorder (Sony 5800).

Image Analysis

Both ultrasound and angioscopic images were used to classify arterial sites as normal, stable atheroma, disrupted atheroma, or thrombus as previously described.4 Ultrasound images were classified by three criteria: density, vessel layer thickness, and lumen configuration (Table 1).4 Density was assessed as normal, increased, or decreased; thickness as normal or increased; and configuration as smooth or irregular. Angioscopic images were also classified by three criteria: color (yellow/white or red), contour (flat or elevated), and configuration (smooth or irregular) (Table 1). These image analysis criteria were used to establish clinical categories for ultrasound and angioscopic classification (Table 2).4

Histological Classification

For all vessels, the 1-cm region of interest was serially sectioned at 2–3-mm intervals and totally submitted for histological evaluation. Vessels were fixed in 10% neutral buffered formalin overnight, processed in graded alcohols, cleared in xylene, and embedded in paraffin. Five-micron-thick sections were stained with hematoxylin and eosin. We classified the vessel site as normal, stable atheroma, intimal disruption, or thrombosis using criteria modified from Ross and Glomset.5 Normal is defined as an artery with an intact media of uniform thickness, an intact or only focally discontinuous internal elastic lamina, and a thin uniform intima of mesenchymal cells and connective tissue.6,7 Stable atheroma is defined as a site with increased intimal thickness in which a lipid-rich region is present surrounded by an intact collagenous cap with no ulceration or fissures and with some discontinuity of the underlying internal elastic lamina; there may or may not be thinning of the underlying media. Intimal disruption is defined as having the characteristics of a stable atheroma but with ulcer, fissure, tears, or dissection. Thrombus is defined as an intraluminal mass of blood element adherent to the luminal surface. The intraobserver and interobserver reproducibilities of this classification system in our experience exceed 90%.8

Statistical Analysis

For each histological category the sensitivity, specificity, accuracy, and predictive value were determined for both intravascular ultrasound and angioscopy. Definitions of true positive, true negative, false positive, and false negative are given in the appendix. Sensitivity, specificity, accuracy, and predictive value were calculated by standard formulas, also given in the appendix.

Contingency analysis was applied to test the significance of the level of agreement between histology
FIGURE 1. Angioscopic image (A) demonstrates yellow/white, smooth, and flat surface. Intravascular ultrasound image (B) reveals circular vessel with normal echodensity, smooth luminal surface, and normal thickness with a "three-ringed" appearance. Microscopy (C) demonstrates normal muscular artery. This vessel was classified as a normal artery by angioscopy, ultrasound, and microscopy. Hematoxylin and eosin stain, original magnification ×2.
and either ultrasound or angioscopy. The \( \chi^2 \) statistic was calculated from a 4\( \times \)4 contingency table comprising the four categories. Statistical significance was set at the 5\% level (\( p<0.05 \)).

**Results**

Figures 1–4 show typical examples of the four categories of vessels studied. Figure 1 shows angioscopy, ultrasound, and histology from the same arterial site. The angioscopic image was classified as normal based on the three criteria of color, contour, and configuration (yellow/white, flat, and smooth, respectively). By ultrasound this site was also classified as normal based on its normal echodensity, normal wall thickness, and smooth configuration. Histology demonstrated a normal artery with mild intimal proliferation. There were 12 histologically normal vessels. Figure 5 demonstrates that the sensitivity, specificity, and accuracy of both methods separately and in combination for identifying normal vessels was 95\% or greater. The predictive value was better for angioscopy than for ultrasound due to false-positive ultrasound interpretations of normal in the presence of thrombus (Table 3).

Figure 2 demonstrates a stable atheroma by ultrasound, angioscopy, gross pathology, and histology. The angioscopic image was classified as stable atheroma based on the yellow/white color, elevated contour, and smooth configuration. The ultrasound image was classified as stable atheroma based on the abnormal echodensity, increased wall thickness, and smooth configuration. There were 18 vessels classified histologically as stable atheroma. Figure 5 shows the sensitivity, specificity, and accuracy of each method for identifying stable atheroma to be 90\% or greater. Both angioscopy and ultrasound had classification errors in which intimal disruption was classified as stable atheroma. Consequently, for each method individually the predictive value was less than 80\% (angioscopy 74\%, ultrasound 78\%).

Figure 3 shows an arterial segment classified as intimal disruption by histology. The plaque (arrow) is separated from the arterial wall. Both the angioscopic and ultrasound images accurately identified and localized the site of arterial disruption, and both demonstrated the disrupted portion of the arterial wall protruding into the vessel lumen. By histology there were 26 disrupted segments. The sensitivities of angioscopy and ultrasound for identifying disrupted atheroma were only moderate (73\% and 81\%, respectively), whereas the specificity, accuracy, and predictive value were high (greater than 90\%).

Figure 4 shows angioscopic and ultrasound images of a histologically documented thrombus. The angioscopic image was classified as thrombus due to the elevated, red mass. The ultrasound image was classified as thrombus due to the presence of a smooth (pedunculated) intraluminal mass. There were 14 histologically documented intra-arterial thrombi. Specificity, accuracy, and predictive value for identifying thrombus were high (93\% or greater) for each method. Sensitivity of angioscopy was high (100\%). It was lower for ultrasound (57\%) due to false-negative interpretation of laminar clots in normal vessels and an inability to distinguish disrupted atheroma (\( n=2 \)) from intraluminal thrombus.

For all 70 vessels studied there was agreement among angioscopy, ultrasound, and histology in 80\% (56). When assessing all cases in which there was concordance of both ultrasound and angioscopy, there was a 92\% agreement between imaging and histology for all diagnostic categories. Contingency analyses showed that each technique, alone or combined, was capable of qualitative imaging that showed very significant agreement with the results obtained from histology (angioscopy versus histology, \( \chi^2=165, p<0.001 \); ultrasound versus histology, \( \chi^2=137, p<0.001 \)). However, there were slight individual variations between the techniques. Angioscopy had a borderline significant proportion of false-positives when imaging stable atheroma (\( p=0.05 \)) as well as a borderline significant proportion of false-negatives when imaging disrupted atheroma. Compared with angioscopy for the identification of thrombi, ultrasound was less sensitive and had a significant proportion of false-negatives (\( p<0.05 \)).

**Discussion**

This study is the first report on the sensitivity, specificity, and diagnostic accuracy of intravascular angioscopy and ultrasound. The accuracy of subjective angioscopy and ultrasonic image analysis exceeds 90\% using histopathology as a reference standard. For individual histological categories, however, there were differences between the two imaging techniques. Angioscopic analysis tended to overestimate the presence of stable atheroma and underestimate...
disrupted atheroma, whereas ultrasound was insensitive for the identification of arterial thrombi.

Intravascular Ultrasound Imaging

Previous studies have described the subjective appearance of the ultrasound image from normal arteries, stable atheroma, disrupted atheroma, dissected arteries, and intra-arterial thrombi. In normal arteries, the ultrasound image reveals a smooth circular wall. In normal elastic arteries the wall tends to appear homogeneous if dense collagen and elastin are present in the adventitia, whereas in muscular arteries there tends to be a hypoechoic media coinciding with the arterial smooth muscle cells. Stable atheroma has been identified by increased wall thickness. Fibrous lesions produce dense homogeneous echo reflections, and calcific deposits cause bright echoes that cast echo-free shadows onto deeper tissue zones. Lipid deposits result in echolucent zones. Arteries with a disrupted intimal surface have been described by Tobis et al. Diagnosis of plaque tear or dissection was based on fracture of intimal plaque or separation of intimal plaque from the media, resulting in a new echolucent zone. In 11 of 13 ultrasonic images the investigators accurately detected the presence of tears. While these previous studies describe the histopathologic correlates of the ultrasound images in normal, stable, and disrupted arteries, none attempted to blindly assess the sensitivity, specificity, accuracy, or predictive value of the imaging technique. Pandian et al compared fiber-optic angioscopy and intravascular ultrasound for the detection of thrombus in normal vessels. These authors found angioscopy to be 100% sensitive. Ultrasound had a sensitivity of 80%, 23% higher than the 57% found in our study. These investigators, however, studied only normal vessels; thus, disrupted and stable atheroma...
intima. Of 13 patients with unstable angina, 12 had thrombi. In the acute myocardial infarction (0–14 days after onset) group, six of eight patients had intimal disruption and four of eight had thrombi. As with ultrasound, however, there has been no histological validation of these image interpretations.

**Clinical Relevance**

The relevance of accurate arterial wall and surface imaging is substantial. Johnson and coworkers reported that the status of carotid plaque morphology by ultrasound may be a more important predictor than the degree of stenosis for plaque stability and clinical outcome. In patients with asymptomatic carotid stenosis, ultrasound plaque characteristics were a better predictor of transient ischemic attacks and stroke than percentage stenosis. Reilly and coworkers found that B-mode ultrasound can detect carotid plaque hemorrhage and ulceration. These findings were associated with thrombus formation and stroke. These ultrasound data are supported by the angiographic studies of Ambrose et al. and others, who found that a disrupted coronary lesion morphology with a narrowed lumen and irregular or overhanging edges is more frequently found in patients with acute ischemic syndromes.

The relevance of accurate intravascular imaging is also supported by studies showing that angiography is insensitive for evaluating the extent of vascular damage after balloon angioplasty. Thus, Potkin and Roberts found that 97% of coronary angioplasty sites at postmortem have plaque disruption, many of which were not detected angiographically. In vivo clinical studies comparing angioscopy and angiography following percutaneous transluminal coronary angioplasty by Uchida et al. found arterial disruption at 10 of 11 sites by angioscopy; in seven the angiographic images failed to detect the damage. Johnson et al. detected by angioscopy subintimal dissections in 26 cases, none of which were identified by angiography, and intra-arterial thrombi in 30 cases, only 10 of which were identified by angiography. We have reported intravascular abnormalities such as partially occlusive thrombus, intimal flaps, and vascular dissections, before and after balloon angioplasty that were not detected by angiography but were identified by intravascular ultrasound and angioscopy.

**Sources of Error in Intravascular Imaging**

The greatest discrepancy between histopathology and imaging in this study occurred in the differentiation between stable and disrupted atheroma. In our study there were four cases in which ultrasound and angioscopy concordantly found plaques to be stable that were characterized as disrupted by histopathology. This raises the question whether all of these “interpretive errors” were real. Isner and Fortin reported that histopathologic processing itself may induce arterial disruption in the form of plaque fracture. Therefore, a potential limitation of this study is that our histopathologic assessment may
overestimate the frequency of plaque disruption. If this is indeed the case, the sensitivity and accuracy for disrupted atheroma and the specificity, accuracy, and predictive value for stable atheroma might actually be higher than we report. Artifacts of pathologic processing are, however, thought to be limited to identifying plaque disruption. Alternatively, for both angioscopy and ultrasound the resolution may be limited for small sites of intimal fissures, tears, or dissections detected by microscopy. Normal vessels, plaque type, and the presence or absence of thrombus are not affected by tissue processing artifacts, and other interpretive classification errors are probably real.

If this is the case, then each of the three intravascular imaging modalities (angiography, angioscopy, and ultrasound) has important limitations. Angiography is a poor technique for analyzing details of the vessel surface, angioscopy is nonquantitative and technically the most difficult, and ultrasound is limited in its capacity to reliably detect thrombus. Conversely, the three modalities are complementary; angiography defines the entire vascular tree, angioscopy the vascular surface, and ultrasound the vessel wall and plaque composition.

**Summary**

Our in vitro studies establish a histopathologic basis for angioscopic and ultrasound image classification and interpretation. Our results delineate the relative sensitivity, specificity, accuracy, and predictive value of these techniques individually and when they are concordant. The qualitative angioscopic and/or intravascular ultrasound classifications were in agreement with the histopathologic classifications. The individual techniques appear complementary, with especially high sensitivity, specificity, accuracy, and predictive value when the methods are in agreement.

**Appendix**

After the lesion was classified into one of the four categories by imaging and by histology, the following definitions were used:

<table>
<thead>
<tr>
<th></th>
<th>Angioscopy/ultrasound</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>True positive</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>True negative</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>False positive</td>
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</tr>
<tr>
<td>False negative</td>
<td>–</td>
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where + = the lesion was placed in that category and − = the lesion was not placed in that category.

Sensitivity, specificity, accuracy, and predictive value were calculated by standard formulas. Sensitivity = number of true positives divided by (number of true positives + number of false negatives). Specificity = number of true negatives divided
by (number of false positives + number of true negatives). Accuracy = (number of true positives + number of true negatives) divided by total. Predictive value = number of true positives divided by (number of true positives + number of false positives).

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