Laboratory Investigation

Documentation of Experimentally Induced Thrombus Formation Using Intravascular Ultrasound

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The purpose of the present study is to assess the ability of intravascular ultrasound to detect acute dynamic thrombus formation in canine blood vessels with damaged endothelium. Ultrasound catheters (20 MHz) were placed in the femoral arteries of anesthetized dogs, and imaging transducers were positioned at the sites of external constrictors applied to areas of endothelial injury. Flows were measured with externally applied Doppler crystals placed proximal to the constrictors. Twenty experimental procedures were performed in 18 dogs. Four procedures were performed using the InterTherapy system (4.9 Fr catheters) and 16 procedures were performed using the Boston Scientific/Diasonics system (6.0 and 4.8 Fr catheters). After injuring the endothelium by rubbing the adventitial surface with cushioned forceps, we placed the constrictors and catheters and found that femoral blood flow usually decreased to zero or near-zero over a 3- to 4-minute period. Striking the exposed artery dislodged the obstruction seen on the intravascular ultrasound images and restored flow to normal; spontaneous increases in flow associated with a reopening of the lumen were also noted. After femoral arterial blood flow returned to normal, another cycle of decreasing flow and thrombus formation was spontaneously initiated. Intravascular ultrasound images of thrombus formation were obtained in 18 of 20 experimental procedures, all associated with zero or near-zero arterial flow. Images obtained during spontaneous decreases in femoral artery flow demonstrated the gradual accumulation of material on the lumen. The obstructing thrombus had distinct borders and a "speckled" appearance on ultrasound, especially on dynamic images, which became increasingly bright and uniform with time. At the end of each procedure, the arterial segments were removed for histologic analysis. Gross thrombus was visible in all cases.

Therefore, in this experimental model, intravascular ultrasound can successfully detect both the acute formation of thrombus associated with spontaneous episodes of decreased flow and the resolution of thrombus within injured and narrowed femoral arteries. Fresh thrombus has a unique ultrasound pattern that evolves gradually over time. ***(Texas Heart Institute Journal 1991;18:179-85)***

Key words: *Animals; dogs; platelet aggregation; thrombosis, diagnosis; ultrasonography, instrumentation; ultrasonography, methods; vascular diseases, diagnosis; vasoconstriction*

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At present, there are problems with reliable documentation of thrombus within blood vessels. Angiography, the previous standard for the delineation of intravascular pathology, has significant limitations in documenting the presence of intravascular thrombus.[1,2] Angioscopy appears to be the most accurate and reliable technique, but it is limited by the requirement of a blood-free field for adequate visualization.[3] Intravascular ultrasound is a technique that uses catheter-mounted ultrasound transducers to display cross-sectional images of the wall and lumen of a blood vessel.[4-8] This new imaging technique has been used in peripheral vessels, and more recently has been used in the coronary circulation as well.

Our understanding of the significance of intravascular ultrasound images is still in evolution. Intravascular ultrasound has a tendency to portray the media as thinner and the intima as thicker than is anatomically correct.[8,9,16] Recent studies have suggested that the 3-layer appearance of normal vessels may be dependent on the particular vascular bed from which images are obtained.[25] Despite these controversies, intravascular ultrasound has proved to be a reliable technique for accurate measurement of lumen size.[11,12,15,18,25]

To date, however, there have been only a few studies evaluating the ability of intravascular ultrasound to detect thrombus. There have been instances in which

thrombus has been visualized by intravascular ultrasound,[30] yet there has been little work in which up-to-date ultrasound equipment has been used to detect the development or resolution of fresh thrombus within the lumen.

In the Folts model of cyclic flow variations, an area of the vessel wall is injured and constricted.[32-43] As platelets (and thrombus) are deposited on the injured site, there is a progressive reduction of flow. The platelet aggregates can break loose spontaneously, restoring normal flow and creating a cyclic flow phenomenon. The release of vasoactive substances from aggregating platelets may have additional effects on distal flow.[37-41] This model provides a well controlled environment wherein one can observe the accumulation of thrombus and aggregating platelets at the site of injured endothelium.

A number of previous pathological studies have documented the presence of platelet thrombi at the time of low or zero flow.[33,36,37,42] The purpose of the present study was to determine whether intravascular ultrasound can detect thrombus development and resolution in a peripheral arterial cyclic flow model.

Materials and Methods

Twenty experimental procedures were performed in 18 dogs. Animals were cared for in accordance with the "Guide for the Care and Use of Laboratory Animals" (NIH Publication No. 80-23, revised 1985).

Surgical Preparation

Mongrel dogs (25 to 35 kg) were anesthetized intravenously with sodium pentobarbital (30 mg/kg), intubated endotracheally, and ventilated with a mechanical respirator. A 10- to 15-cm surgical incision was made to expose approximately 5 cm of the common femoral artery. This segment of artery was gently isolated, and a pulsed Doppler flow probe was placed around it. A fluid-filled catheter was placed into the carotid artery for pressure monitoring.

Experimental Protocol

Hemodynamic variables were recorded continuously on a multi-channel recorder (Hewlett-Packard, model 9270). Femoral artery cyclic flow variations were induced by creating flow-limiting stenoses at areas of endothelial injury in the femoral artery. Endothelial injury was induced by gently squeezing the exposed femoral artery distal to the Doppler flow probe with cushioned forceps. Stenosis was created by placing a hard cylindric plastic constrictor around the artery at the site of injury.

A distal side-branch of the femoral artery was then isolated, and used for retrograde introduction of an ultrasound catheter to the site of injury. Four procedures were performed using the InterTherapy system (4.9 Fr catheter) (Santa Ana, California), and 16 procedures were performed using the Boston Scientific/Diasonics system (6.0 and 4.8 Fr catheters) (Watertown, Massachusetts, and Milpitas, California). The ultrasound catheters were advanced until the imaging plane of the ultrasound transducer was just distal to the plastic occluder. Figure 1 shows a schematic representation of the experimental setup. With the combination of external constriction and internal occlusion by the catheter, resting flow velocity was limited to approximately 60% of normal.

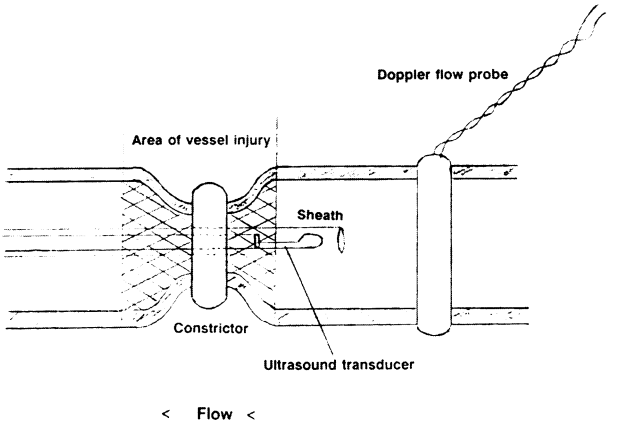

Fig. 1 *A schematic representation of the experimental setup, illustrating the relative positions of the Doppler flow probe, the intravascular ultrasound catheter, the external constrictor, and the area of injured endothelium.*

Once cyclic flow variations were initiated, the animals were monitored continuously for at least 2 to 3 hours. Continuous recordings of arterial pressures and femoral artery flows were obtained. Intermittent video recordings of intravascular ultrasound images were obtained during different phases of decremental femoral artery flow. At the point of zero flow, continuous recordings of thrombus development were made. In cases where spontaneous flowback was not noted, the artery was struck briskly to dislodge accumulated material and return flow to normal. At the end of each procedure, the arteries were allowed to remain at zero flow for 1 hour, with periodic intravascular ultrasound imaging. The animals were then killed, and the involved femoral artery segments were removed intact and sent for pathological analysis.

Results

Intravascular ultrasound images of thrombus formation were obtained in 18 of 20 procedures. Thrombus images were all associated with zero or near-zero femoral artery flow. Figure 2 shows a representative hemodynamic tracing of the spontaneous decreases in femoral artery flow. The arrows indicate points in

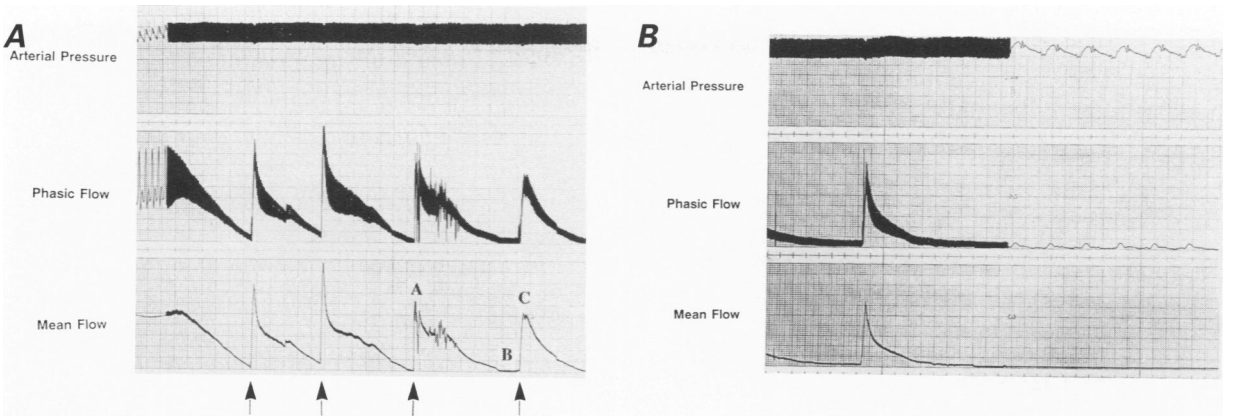

Fig. 2 ***A****) A hemodynamic recording of spontaneous decreases in femoral artery flow. The large arrows indicate points in time when the artery was struck to dislodge the obstructing material. The letters A, B, and C designate (respectively) points of maximum flow, zero flow, and return of normal flow.* ***B****) A hemodynamic recording that illustrates a longer period of zero flow, during which additional intravascular ultrasound images were obtained. Two different time scales are represented.*

time when the artery was struck briskly to dislodge the obstructing material.

Shortly after achieving zero flow, a deposition of material encroached gradually but progressively upon the lumen. This material had distinct borders that could be clearly distinguished from the lumen of the vessel. There was a characteristic "speckled" appearance to the obstructing material, which became increasingly bright and uniform with time. Figure 3 shows the progression of images over time, from low

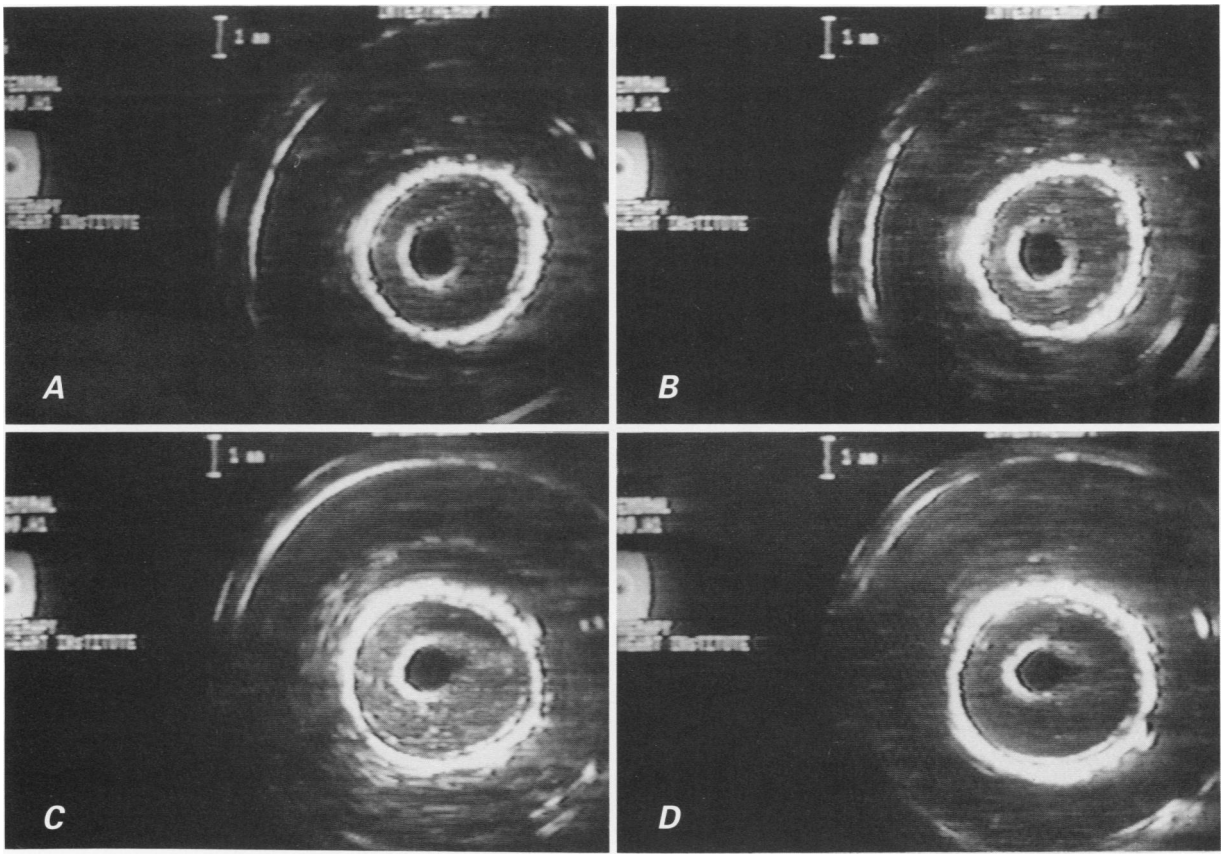

Fig. 3 *The progression of thrombus over time, as visualized by intravascular ultrasound. In sequence are shown:* ***A****) low flow, with a small deposition of thrombus;* ***B****) zero flow (at 2 minutes), with a larger volume of obstructing thrombus;* ***C****) zero flow (at 4 minutes), with a more uniform appearance of the thrombus; and* ***D****) full flow (after striking the artery to dislodge the thrombus), with no obstructing thrombus visible.*

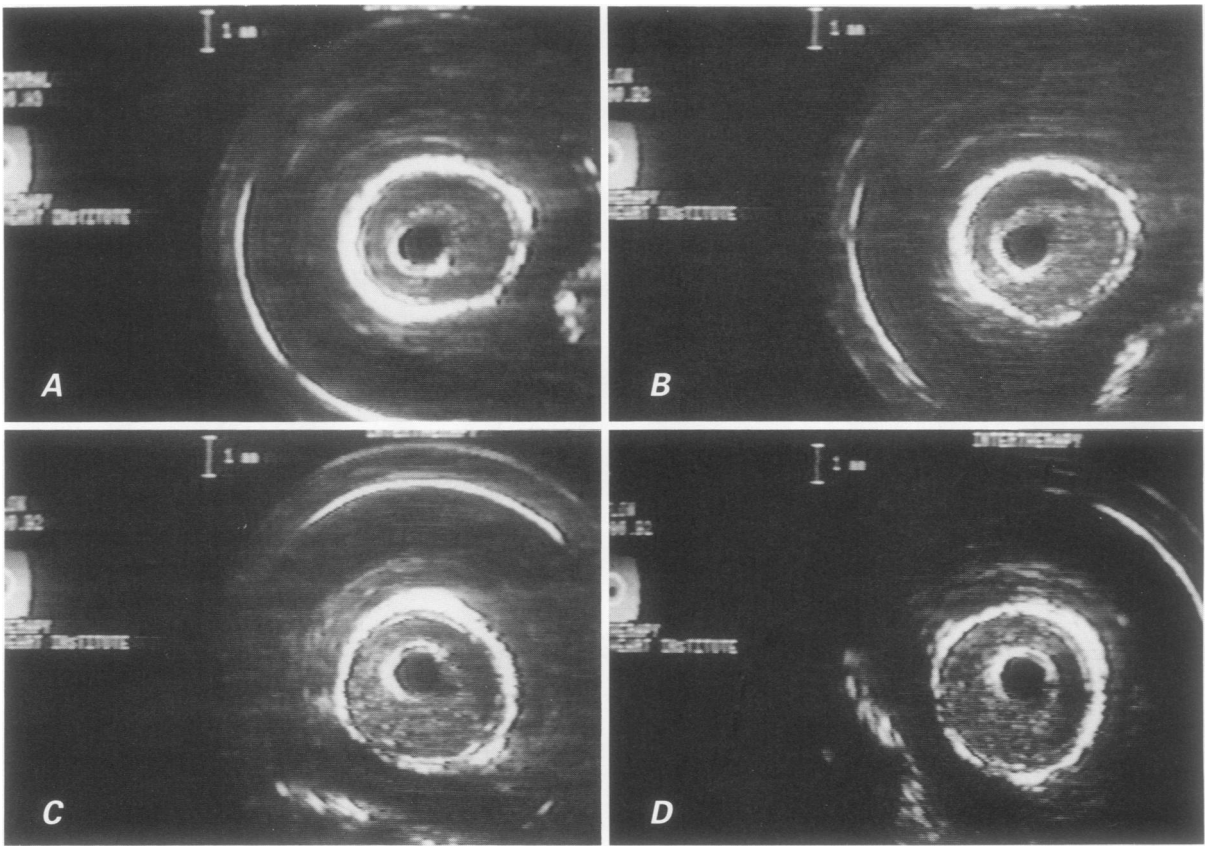

Fig. 4 *Sequential intravascular ultrasound images during a prolonged (1 hour) period of zero flow. Images are shown:* ***A****) at near zero flow;* ***B****) after 10 minutes;* ***C****) after 30 minutes; and* ***D****) after 1 hour. The "speckled" appearance of the thrombus changes over time.*

flow to zero flow (early and late), and finally to full flow (after striking the artery to dislodge thrombus). These static images are less dramatic than the actual dynamic images, since freeze-frame ultrasound images have only half the resolution of dynamic ultrasound images.

Figure 4 shows sequential images taken during the prolonged (1 hour) period of zero flow at the conclusion of the procedure. Note that the speckled appearance of the thrombus changes over time. Again the evolution of the dynamic images was particularly striking. Postmortem analyses confirmed the presence of thrombus in all cases.

Discussion

Intravascular ultrasound is a new imaging technique that shows great promise for extending our current diagnostic capabilities.[4-32] Conventional contrast angiography provides only a visualization of the vascular lumen. It provides relatively little information about structural abnormalities of the vascular wall or about the composition of lesions that obstruct the lumen. Intravascular ultrasound, by virtue of imaging from the inside out, provides unique in vivo information about both the vascular wall and the nature of obstructive lesions.

Initial studies have shown that intravascular ultrasound provides an accurate representation of lumen size and geometry. There are strong correlations between ultrasonic indications of arterial lumen size and subsequent histologic measurements.[17,18,23,28] Studies have also shown a strong correlation between ultrasonic indication of lumen size and quantitative angiographic measurements.[11,15,18]

The ultrasonic appearance of the arterial wall consists of 3 basic layers, as shown in Figure 3: an inner, bright, echogenic area; a middle, echo-free area; and an outer, echo-dense area. Histologic studies have suggested that these 3 areas approximate the vascular intima, media, and adventitia, respectively, but the thickness of the 3 tissue layers as portrayed by ultrasound does not correspond well with their actual thickness.[8,9,16] Furthermore, there is good evidence that the appearance of the 3 layers is to a large extent dependent upon the particular arterial bed visualized. The amount of elastin in the middle layer may

be important: small vessels (such as coronary arteries) with relatively little elastin show a very echolucent middle layer, whereas large vessels (such as the aorta) have a more echo-dense middle layer.[28]

In addition to its use in diagnosis, intravascular ultrasound has been used in conjunction with a variety of interventional procedures, including balloon angioplasty, mechanical atherectomy, and laser angioplasty. Ultrasonic images show splits, cracks, and dissections that result from intravascular intervention, and may hold some promise as a guidance technique for interventional procedures.[8,9,13,17,19,25,30]

However, there has to date been little published work that has investigated intravascular ultrasound as a means of visualizing thrombus. Early studies showed that angiography (in comparison to angioscopy) was a relatively insensitive method of thrombus detection, and that better imaging techniques were needed.[1,2] Nowadays, the ability to detect intravascular thrombus is particularly important in targeting the appropriate form of therapy for a given lesion. The general availability of thrombolytic agents and the risks of performing angioplasty in the setting of pre-existing thrombus necessitate better diagnostic techniques. Given the fact that intravascular ultrasound provides an accurate representation of the vascular lumen and disruptions in the vascular wall, we wanted to determine whether it might enable accurate detection of thrombus formation.

We chose to investigate this possibility using a modification of the Folts cyclic flow model. The Folts animal model[36] of coronary cyclic flow variations is well established for study of cyclic reductions in coronary blood flow that are believed to be the result of the accumulation (and subsequent dislodgement) of obstructive platelet aggregates at the site of endothelial injury and at artificially-created stenoses.[35-37,41] Previous studies using this model have demonstrated the presence of occlusive platelet-rich aggregates at the lowest levels of coronary blood flow.[34-37,41] A number of studies have documented (both histologically and angiographically) that there are obstructing platelet thrombi at the site of injury when flow is at or near zero.[33,36,37,42]

Cyclic flow variations have been abolished by serotonin receptor antagonists, thromboxane A_2 synthesis inhibitors, thromboxane receptor antagonists, glycoprotein IIb/IIIa receptor antagonists, thrombin inhibition, and thrombocytopenia.[34,37-44] While the occurrence of cyclic flow variations is felt to be primarily a platelet-dependent phenomenon, other factors appear to contribute also to changes in coronary blood flow. Enhanced vasoconstriction has been demonstrated at the site of platelet accumulation, mediated by release of platelet-derived vasoactive factors, such as serotonin and thromboxane A_2.[43] Other potential mediators include ADP, platelet activating factor, and vasoconstrictors released from the endothelium, the white blood cells, or both.[32]

We adapted the Folts model for use in the peripheral arterial circulation as a means of generating (in a reproducible fashion) thrombus formation at the site of initial platelet attachment. We were then able to observe the development of thrombus (as demonstrated in previous histologic and angiographic studies[33,36,37,42]), and to document its dislodgement and reaccumulation under carefully controlled conditions, using intravascular ultrasound.

Limitations of the Study

As demonstrated in our study, fresh thrombus has a unique appearance on intravascular ultrasound. The borders of the thrombus are relatively distinct, with clear areas of lumen. The appearance is not consistent with that of stasis, which might arise as a result of low flow. While it is true that low blood flow or blood stasis gives an echo appearance of "smoke" or cloudiness, the distinct appearance of the obstructing material in the echo pictures we observed makes it highly unlikely that the impression of thrombus formation is due solely to stasis.

It is also true that our pathological documentation of thrombus was performed only after a prolonged period of zero flow; but there are, as previously mentioned, both histologic and angiographic studies that document the presence of obstructing material (consisting primarily of platelet thrombi) at the nadir of flow cycles in the Folts model. Over time, there is the additional deposition of red blood cells within the thrombus.[33,36,37,42] Our study focused primarily on imaging during the acute development and resolution of thrombi. Future studies will be needed to focus on issues related to older thrombus.

An additional limitation of our study is that we were not visualizing the thrombus at the original site of development, but rather as it propagated to the point of the intravascular ultrasound imaging plane. There are obvious limitations to any animal model for detecting thrombus formation, but we believe that the images we obtained provide an accurate representation of the acutely developing thrombus.

Summary

In summary, intravascular ultrasound can successfully detect acute thrombus formation associated with spontaneous episodes of decreased blood flow within endothelium-injured and constricted canine femoral arteries. Fresh thrombus has a unique ultrasound pattern that evolves gradually over time. This technique holds promise for application to clinical situations in which thrombus formation and resolution must be recognized.

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