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Calcified plaque cross-sectional area in human arteries: Correlation between intravascular ultrasound and undecalcified histology

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Background The purpose of this investigation was to quantify the amount of intralesional calcium detected by intravascular ultrasound (IVUS) compared with undecalcified histology in human arteries. This method preserves intralesional calcium and reduces sectioning artifacts, thereby providing an accurate measure of calcium plaque morphology.

Methods and Results Ten arterial segments (5 coronary, 5 iliac) were obtained at autopsy. IVUS imaging was performed with a 4.9F catheter at an automated pullback rate of 1.0 mm/s. The undecalcified arteries were dehydrated in ascending alcohol and polymerized in glycol methylmethacrylate. The arteries were cut into 200-μm sections with an Isomet low-speed saw and stained with Goldner's trichrome. The lumen cross-sectional area, the calcium plaque cross-sectional area, the calcium plaque depth, length, and angle of arc of calcified plaque were measured from the IVUS images and histologic sections. In 24 selected cross sections, there were 38 separate calcium plaques. An independent observer correctly identified 34 of 38 calcified plaques for a sensitivity of 89% and specificity of 97%. The total mean calcified plaque cross-sectional area measured from histology was 4.6 ± 4.1 mm^2 compared with 2.8 ± 2.3 mm^2 by IVUS ($P = .002$). Plaque depth measured by histology was 1.2 ± 0.4 mm versus 0.7 ± 0.2 mm by IVUS ($P = .001$). The length of calcium plaques measured by histology was 3.6 ± 1.78 mm versus 3.6 ± 1.5 mm for IVUS ($r = 0.79$).

Conclusions IVUS accurately depicts circumferential calcified lesions with high sensitivity (89%) and specificity (97%). However, IVUS underestimates the total calcified plaque cross-sectional area by 39%. This is mainly because of the inability of the ultrasound to penetrate intralesional calcium, which leads to an underestimation of the depth of calcium by 45%.
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Intravascular ultrasound (IVUS) imaging may be used during interventional cardiology or radiologic procedures to help identify tissue characteristics of the plaque.[1-7] The choice of interventional therapy may be altered, especially if calcified plaque is recognized by IVUS. Mintz et al[1] reported that an interventional device or therapeutic approach was changed by preinterventional IVUS in 40% of the examined lesions. Although studies comparing vascular calcification detected by histology and IVUS have a high sensitivity and specificity for the identification of calcium, quantitative measurements have been less accurate or reproducible.[8,9] The major reason for poor quantitative results is that the standard method of histologic preparation results in decalcification of the specimen. This produces dropout of calcified material and weakens the integrity of the tissue during histologic preparation. Subsequent microtome cuts of the arteries frequently produce artifactual tears, which make it difficult to correlate histology with the ultrasound images for quantitative analysis. Fitzpatrick et al[10] have described a technique for histologic preparation of calcified tissue that does not require decalcification. This permits an accurate assessment of the total calcified plaque burden without distortional artifacts. The purpose of this study was to compare quantitative measurements of calcified arterial plaque by IVUS with undecalcified histologic preparations with the use of methylmethacrylate to preserve the calcified plaque architecture.

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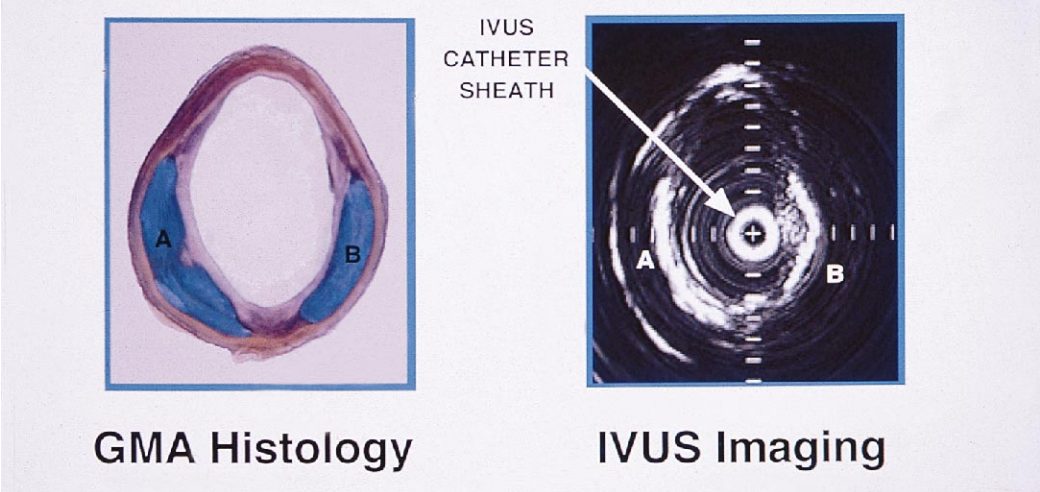
Methods

Harvesting and imaging

Ten human atherosclerotic arterial segments (5 coronary, 5 iliac) from 6 cadavers (2 male, 4 female) ranging in age

Figure 1



Left, Histologic cross sections of iliac artery after GMA processing. Light blue/green corresponds to calcium hydroxyapatite. There are 2 distinct calcified plaques (*A* and *B*). *Right,* Corresponding IVUS image from same arterial cross section. At center of image is intense white circle that corresponds to plastic sheath of ultrasound catheter. Circular harmonic reverberations from sheath create some artifact in lumen; otherwise, lumen is relatively echolucent. Two calcified plaques are seen as intense white echo reflections with shadowing and dropout of echoes peripheral to calcium. Scale is represented by 1-mm markers.

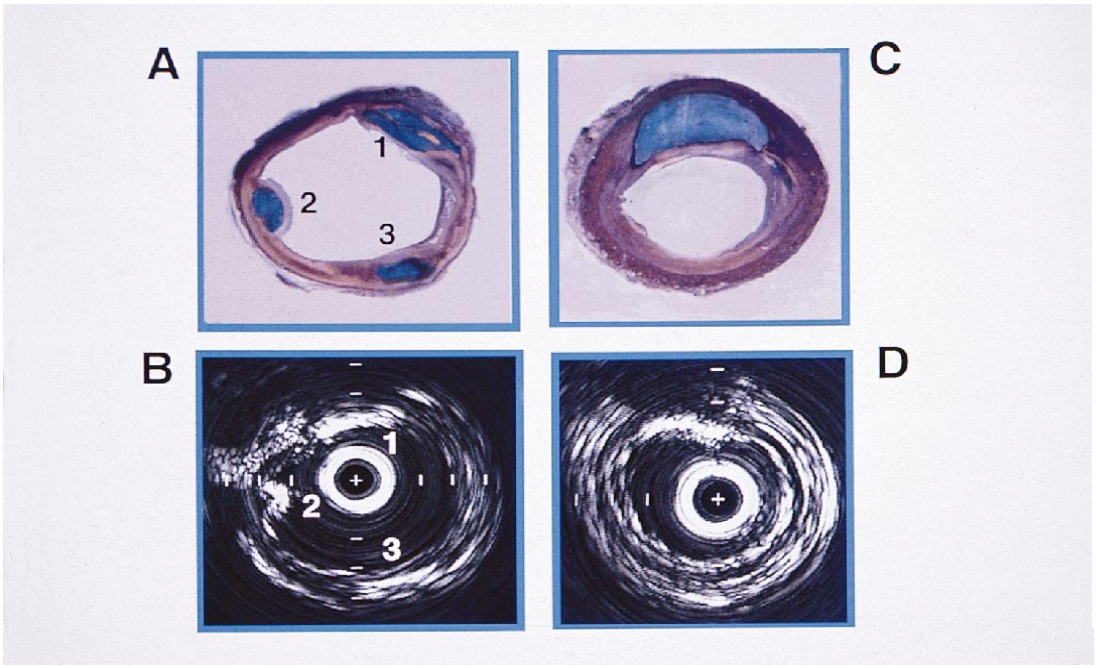
from 42 to 73 years (mean age 57.5 ± 12.9 years) were obtained from the Orange County Coroner's Office and stored in formaldehyde until IVUS imaging. Although formaldehyde alters tissue characterization somewhat, calcium is such a strong reflector that recognition of calcium is not affected on the IVUS image. An acoustic reference was provided by 2 surgical needles placed through the adventitia of both ends of the arteries perpendicular to the longitudinal axis. The demarcated arterial segments ranged in length from 13 to 47 mm, with a mean length of 26.7 ± 11.9 mm. Proximal and distal ends were selected and marked with india ink and red dye No. 40, respectively. The arteries were immersed in a water bath at room temperature, and IVUS images were obtained with a 4.9F, 25-MHz catheter with an automated pullback device (InterTherapy/Boston Scientific, Sunnyvale, Calif). Previous studies have demonstrated that this device has an axial resolution of 100 μm and a lateral resolution of 150 μm. Physiologic filling pressure of the arteries was not used during histologic preparation or IVUS imaging because the morphology and size of the calcium plaques would not be altered. Serial images were recorded between the surgical needles onto a super-VHS tape at 30 frames/s, with a pullback rate of 1.0 mm/s. Six separate imaging runs were done for each artery. Identical overall gain and time gain compensation settings were used for all imaging runs. After imaging, the suture was tied to mark the location of the needle and the arteries were stored in separate containers in 100% isopropyl alcohol.

Fixation and histology

The arterial segments were not decalcified but were fixed in ethanol and dehydrated in ascending alcohols. The segments were then immersed in glycol methylmethacrylate (GMA) with a temperature-controlled method (Rainier Technical Products, Seattle, Wash).[10] Tissue was infiltrated for 3 days in a mixture of 81% (vol/vol) uninhibited methylmethacrylate, 8% (wt/vol) polyethylene glycol distearate (stock No. 1540), and 6.5% benzoyl peroxide. Infiltrated specimens were placed in a fresh monomer containing accelerator and allowed to polymerize and mounted onto aluminum chucks at room temperature in the presence of nitrogen.

Sections of artery outside the proximal and distal suture markers were discarded. The arteries were cut, beginning at the proximal suture. A circular saw (Isomet low-speed saw, Lake Bluff, Ill) with a diamond cutting blade was used to cut 200-μm thick sections of the arteries; 300 to 400 μm of the adjacent tissue was wasted because of blade thickness and sway. The sections were stained with Goldner's trichrome.

Figure 2



Calcium depth and IVUS imaging. **A** and **C** are from human artery cross sections. Corresponding IVUS images are shown in **B** and **D**. In left side of **A**, there are 3 segments of calcified plaque (1 through 3). Plaques 1 and 2 are represented in IVUS image **B** as intense white echo reflective band with shadowing peripheral to surface echo reflection. During acquisition of IVUS image, there was mild pinching of the artery, which makes area between 9 and 11 o'clock positions appear shorter than in corresponding histologic section. Plaque 3 has superficial fibrous capsule represented on IVUS image as gray band; under this is intense white reflection of calcium with dropout peripherally. Length of arc of plaque 3 is slightly overrepresented in IVUS image because of nonuniform rotation of drive shaft. Histologic section **(C)** shows thick plaque of 1.5 mm in depth. Although length of arc of plaque is correctly represented by IVUS image **(D)**, depth of calcium is not visualized because of dropout of echoes behind initial echo reflections from leading edge of calcium. IVUS images depict plaques as having similar depths of calcium, but by histology, plaque depth varies by 100% between 2 artery sections.

Microradiographs and Von Kossa stains were performed on selected sections.

Comparison of IVUS with histology

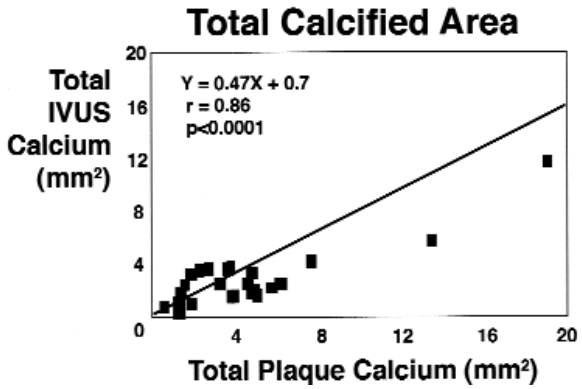
Histology sections were selected for correlation with IVUS images on the basis of the following criteria: (1) The selected histology section was in the middle of a morphologically homogeneous calcium plaque to ensure a valid correlation with the ultrasound images. (2) A clear IVUS picture of the plaque existed.

The selected histology sections were viewed with a microscope (Leica, model Wild M3Z, Rockleigh, NJ) and the image fed through a video camera (Sony, CCD, model DXC-151A, Tokyo, Japan) and digitized into a computer (Power PC 8500, Apple, Cupertino, Calif). The artery section was placed on a slide with a 0.1-mm marked scale to facilitate calibration before measurement. The corresponding IVUS images were fed through a video player (Panasonic, model 1960, Tokyo, Japan) and digitized at 30 frames/s in a 640 × 480 pixel matrix (Media 100 video grabber board Data Translation, Marlboro, Mass). All of the ultrasound images between the acoustic needle marks were digitized. Corresponding histology sections and IVUS image frames were chosen by matching the length along the artery with the frame from the timed pullback.

Section and image analysis

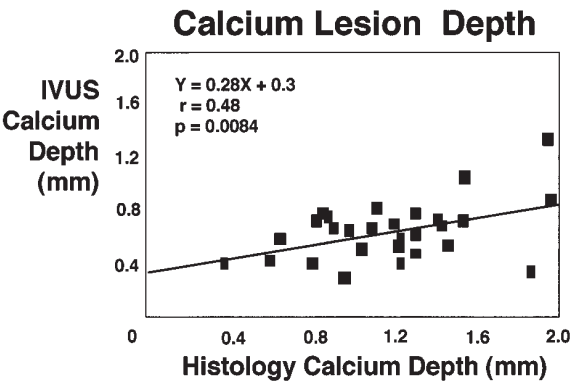
The digitized IVUS and histology images were analyzed by a public domain image processing software package (NIH Image v1.59, Internet address: http://zippy.nimh.nih.gov). Calcium lesions were defined in the IVUS images if the lesion was highly echogenic (equal or greater intensity than adventitia) and was associated with shadowing in the distal

Figure 3



Correlation between total calcified plaque CSA by undecalcified histology with GMA method versus IVUS.

Figure 4



Correlation between calcium lesion depth by undecalcified histology with GMA method versus IVUS.

Table I. Comparison of measured variables between IVUS and undecalcified histology

Measured variable	GMA-histology	IVUS	*P* value
Ca^{2+} CSA (mm^2)/plaque	3.8 ± 2.7	2.3 ± 1.6	.0002
Ca^{2+} CSA (mm^2)/section	4.6 ± 4.1	2.8 ± 2.3	.0017
Length of Ca^{2+}	3.6 ± 1.7	3.6 ± 1.5	NS
Lumen CSA	13.9 ± 7.6	16.6 ± 7.6	.0001
Arc of Ca^{2+}	82.6 ± 35.4	85.9 ± 41.4	NS
Ca^{2+} depth (mm)	1.2 ± 0.4	0.7 ± 0.2	.0001

field. The detected calcified lesions were measured for cross-sectional area (CSA), depth of calcium at the widest point, degrees of arc from the middle of the lumen, and calcium lesion length, which represented the circumferential calcium deposit in the artery. Identical measurements were performed on the corresponding histology sections by using the same software analysis program.

The sensitivity and specificity for identification of calcium from the IVUS images were assessed by having another person interpret the ultrasound images who was blinded to the histology results.

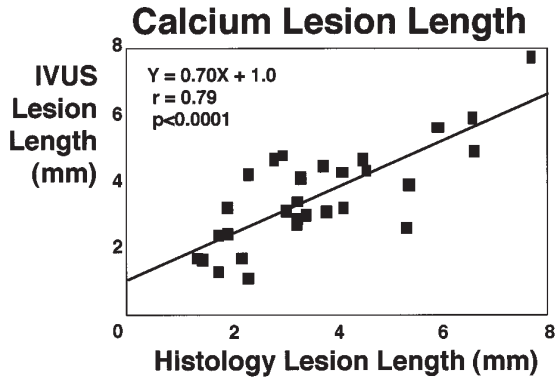
Statistics

Six comparisons were made among the histology and IVUS sections: (1) individual calcium plaque CSA; (2) total calcified plaque area in a section (ie, if there was more than 1 individual plaque in a section, this represented the sum of all calcium plaques in that section); (3) depth of calcium along the radius measured from the leading edge to the peripheral edge of the calcified plaque; (4) degrees of arc of calcified plaque; (5) calcium lesion length; and (6) lumen CSA. A linear regression analysis and paired *t* test were performed for each variable measured by IVUS and histology.

Results

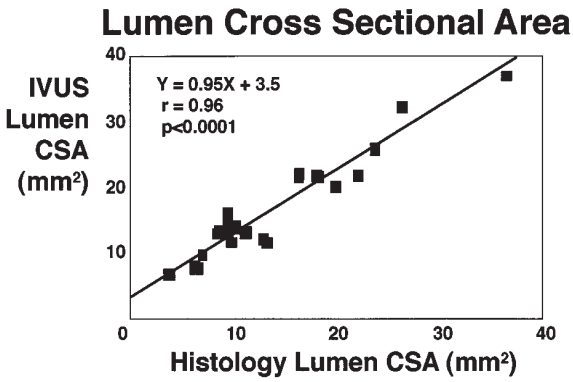
Twenty-four arterial cross sections were chosen for comparison with the IVUS images. These sections were chosen because they had homogeneous calcified plaques. In these 24 sections, there were 38 separate calcified plaques identified on histology. From the IVUS images, the independent observer correctly identified 34 of the 38 calcified lesions, for a sensitivity of 89%. The specificity in this cohort was 97%. A representative example of an IVUS image and the corresponding undecalcified histology are shown in Fig 1. The measurements of the calcified plaque from histology and IVUS are shown in Table I. IVUS images underestimated the detected total calcium CSA by 39% and the detected calcium depth by 45%. The mean angle of arc of calcified plaque from the undecalcified histology was 82.6 ± 35.40 and 87.7 ± 41.00 from the IVUS images (*P* = not

Figure 5



Correlation between calcium lesion length by undecalcified histology with GMA method versus IVUS.

Figure 6



Correlation between lumen CSA by undecalcified histology with GMA method versus IVUS.

Table II. Interobserver correlations and variability

	Observer A		Observer B			Regression analysis		Bland-Altman analysis	
	Mean	SD	Mean	SD	No.	*r* value	*P* value	Mean difference	SD
Histology lumen CSA	15.1	8.3	14.0	7.5	24	0.99	<.0001	−0.0	0.2
Histology lesion angle	83.3	34.8	69.9	40.3	29	0.99	<.0001	−0.5	4.8
Histology lesion length	3.8	1.8	3.1	1.8	29	0.98	<.0001	−0.2	0.4
Histology calcium CSA	3.9	2.7	3.1	2.8	29	0.99	<.0001	−0.1	0.3
Histology calcium depth	1.2	0.4	1.0	0.5	29	0.97	<.0001	−0.0	0.1
IVUS lumen CSA	18.2	7.9	16.8	7.4	23	0.99	<.0001	−0.0	1.1
IVUS lesion angle	85.0	42.8	85.4	40.8	28	0.95	<.0001	0.7	12.0
IVUS lesion length	3.6	1.6	3.6	1.5	28	0.95	<.0001	−0.0	0.4
IVUS calcium CSA	2.5	1.6	2.3	1.5	28	0.97	<.0001	−0.1	0.3
IVUS calcium depth	0.7	0.2	0.7	0.2	28	0.79	<.0001	−0.0	0.1

significant [NS]). The mean calcified lesion length was 3.6 ± 1.7 mm for undecalcified histology and 3.6 ± 1.5 mm for IVUS (P = NS). An example of the sensitivity of IVUS for identification of calcified plaque but underestimation of the amount and depth of calcium is shown in Fig 2. The correlations among the measured variables are shown in Figs 3 through 8.

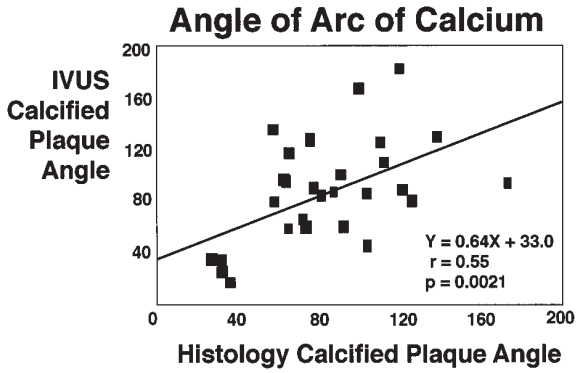
There was a close correlation for total calcified area between histology and IVUS (r = .86, P < .0001, Fig 3). The calcium lesion depth showed a weaker correlation (r = 0.48, P = .0084), with IVUS underestimating the calcium depth (Fig 4). The calcium lesion length was accurately depicted by IVUS and showed a good correlation with histology (r = 0.79, P < .0001, Fig 5). Lumen CSA correlated closely between histology and IVUS imaging, $Y = 0.95X + 3.5$, r = 0.96, P < .0001 (Fig 6). The angle of arc of calcium showed the most variability between histology and IVUS measurements, with a correlation coefficient of r = 0.55, P = .0021 (Fig 7). Corresponding to the total calcified area per artery, a good correlation was also obtained with the individual plaque CSA (r = 0.76, P < .0001 (Fig 8).

The Bland-Altman analysis of interobserver variability among the measured histology and IVUS variables is shown in Table II. The mean difference of measurements between observers was small, and all were within 2 SD. The linear regression analyses between observer 1 and observer 2 for all the variables measured were also very close.

Discussion

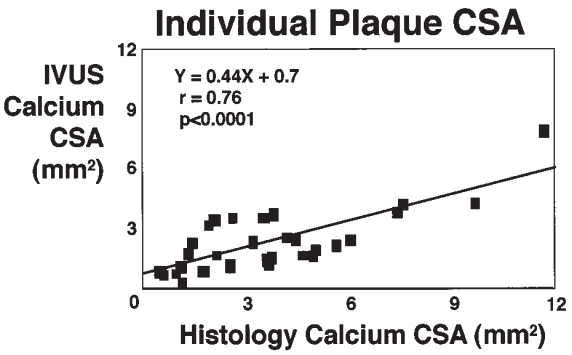
This comparison study between histologic cross sections of human atherosclerotic arteries and IVUS images confirms the previously reported high sensitiv-

Figure 7



Correlation between angle of arc of calcified plaque by undecalcified histology with GMA method versus IVUS.

Figure 8



Correlation between individual plaque CSA by histology with GMA method versus IVUS.

ity and specificity for the identification of calcified plaque by IVUS.[3,8,9,11-13] The unique feature of the current study is that GMA was used for the histologic preparation, which is a technique that does not require decalcification of the tissue. Embedding the arteries in this plastic produces durable cross-sectional cuts without destruction of the plaque architecture. This permits accurate identification of the calcified areas as well as quantitative measurements of the plaque dimensions and area. These analyses are not easy to perform because methyl methacrylate is a toxic skin irritant and a carcinogen.

This study demonstrates that IVUS measurements of calcified lesion length are accurate when compared with undecalcified histology (3.6 ± 1.5 vs 3.6 ± 1.7 mm, P = NS). Because of the intense echo reflections from the surface of calcified plaques, IVUS does not penetrate calcified plaque, leading to an underestimation of the depth of calcified tissue (0.7 ± 0.2 mm by IVUS vs 1.2 ± 0.4 mm by histology, P = .0001). This 45% underestimation of plaque depth by IVUS resulted in a 39% underestimation of total calcium CSA by IVUS (2.8 ± 2.3 mm^2 compared with histology, 4.6 ± 4.1 mm^2, P = .0017).

There were 2 apparent causes of underestimation of calcified plaque by IVUS. As expected, the first was the depth of penetration caused by the high reflectivity of the acoustic signal by calcified tissue. A second potential cause for underestimation of calcified plaque area was caused by nonuniform rotation of the IVUS catheter drive shaft. Nonuniform rotational artifacts are caused by an unequal sweep of the acoustic sector as the mechanical shaft revolves around its long axis. These artifacts may cause calcified sections of the ultrasound images to appear to have a shorter or longer length of arc. These explanations may help us to understand the scatter in the data for individual calcified lesion measurements for calcified plaque depth and arc of calcium. The measurement of the arc of calcification is also dependent on the choice of the center of the lumen. Although the center of the lumen was calculated mathematically with the NIH image software program, there were changes in the shape of the artery between the time of ultrasound imaging and subsequent histologic preparation, which would alter the choice of the center of the lumen. Another potential cause of errors could be misregistration of the histology and IVUS cross sections. Because this study did not place a suture in each histologic section, it is possible that the IVUS images and histologic section may not correspond precisely. However, the IVUS image morphology and histologic section appeared to correspond with respect to plaque contour, length, and location. Despite these potential causes of error, there was an excellent correlation of the measured lesion length by histology and IVUS (r = 0.79). In addition, the sensitivity and specificity of these ultrasound images for identifying normal or calcified plaque were excellent (89% and 97%). The unique aspect of this study is that it accurately quantitates the amount by which the calcified plaque is underestimated by IVUS imaging.

Conclusions

IVUS accurately depicts the circumferential length of calcified lesions but underestimates the depth and CSA of calcified plaque. Although IVUS identifies the presence of calcified plaque with a high sensitivity (89%) and specificity (97%), the depth of plaque is underestimated by 45% and the total CSA of calcified plaque is underestimated by 39%. These observations are important to physicians performing angioplasty, atherectomy, or stent insertion procedures because the position and extent of calcification influences the effectiveness of these procedures. Although IVUS may be used to guide these procedures, this study documents the quantitative limitation of IVUS for assessing calcified plaque burden.

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