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Intravascular ultrasound insights into plaque composition

Summary Previous studies correlating histomorphology with 20–30 MHz-derived intravascular ultrasound (IVUS) images showed that IVUS provides to some extent qualitative information on plaque composition. IVUS imaging proved to define calcifications with high sensitivity and specificity but was found to be less accurate in the assessment of soft components. Nevertheless previous studies on atherosclerotic plaque characterization were limited by use of low-frequency transducers that did not define accurately soft components. Our goal was to test the effectiveness of high frequency IVUS transducers in the identification of lipid/necrotic pools in atherosclerotic plaques.

Methods: Forty MHz transducers were used for in vitro IVUS assessment of 12 arterial segments (10 coronary arteries and 2 carotid arteries dissected from 5 different autopsy cases). IVUS acquisition was performed at a 0.5 mm/s speed after ligature of the branching points to generate a closed system. Lipid necrotic areas were defined by IVUS as large echolucent intraplaque areas surrounded by tissue with higher echodensity. To obtain histopathologic sections corresponding to IVUS cross sections, vessels were divided into consecutive 3 mm-long segments using the most distal recorded IVUS image as the starting reference. Then, samples were fixed with 10 % buffered formalin, processed for histopathologic study, serially cut, and stained with the Movat penthacrome method.

Results: One hundred twenty-two sections were analyzed. Lipid pools were observed by histology in 30 cross sections (25 %). IVUS revealed the presence of lipid pools in 19 of 122 cross sections with a sensitivity and specificity of 67 % and 94 %, respectively.

Conclusions: High frequency transducers accurately identify lipid/necrotic pools and open new perspectives on future IVUS characterization of atherosclerotic plaques.

Key words Intravascular ultrasound – coronary artery disease – plaque tissue characterization

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Intravascular ultrasound definition of atherosclerotic plaque components

Since intravascular ultrasound (IVUS) imaging was introduced almost ten years ago, it has emerged as an imaging modality capable of measuring lumen and vessel dimensions and assessing plaque distribution (1–6). Previous studies correlating histomorphology with 20–30 MHz-derived IVUS images showed that IVUS provides qualitative information on plaque composition (1–6).

Accordingly, atherosclerotic plaques can be roughly grouped as predominantly soft, fibrous, or calcific. The echo signals derived from fibromuscolar tissue (such as in neointimal hyperplasia), thrombus and lipid-necrotic material are less intense than those obtained from calcified and fibrosclerotic plaque components, which have a highly echogenic appearance.

Although IVUS imaging proved to define calcific and fibrotic tissue with high accuracy, the soft components of the atherosclerotic plaque, such as lipid-necrotic pools

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and thrombus, were defined with lower sensitivity and specificity.

Calcium

Calcium is accurately revealed by IVUS imaging as a highly reflective tissue leading to acoustic shadowing (Fig. 1A). Potkin et al. (6) compared 54 atherosclerotic sites imaged by ultrasound with formalin-fixed and fresh histological sections of the coronary arteries. The histological analysis was performed in 112 quadrants obtained from 28 arterial sections. Histological plaque composition could be predicted in 96 % of cases: all 19 calcific plaque quadrants were correctly identified by IVUS. Other reports confirmed these findings, revealing a high sensitivity and specificity in the IVUS assessment of focal calcium (7). Furthermore, IVUS proved to define the presence of calcific deposit with higher sensitivity than angiography: Mintz et al. (8), comparing IVUS and angiography, demonstrated that the latter underestimates calcific spots with respect to IVUS (40 % vs 70 % of target lesions, respectively). Although IVUS provides unique information on the presence and site of calcium deposits, recent reports underlined that the calcific plaque burden is underestimated by IVUS imaging. Kostamaa et al. (9) in a recent in vivo study in human arteries aimed at comparing IVUS with un-decalcified histology and demonstrated that IVUS underestimates the total calcified plaque cross-sectional area by about 40 %, due to the inability of the ultrasound to penetrate intra-lesion calcium.

The poor identification of microcalcifications by 20–30 MHz transducers is another limit of current IVUS technology. Friedrich et al. (10) investigated the power of IVUS to detect the histological extent of in situ coronary calcium in fresh specimen. The authors found a somewhat low sensitivity of IVUS for the identification of small accumulations of calcium, having a thickness less than 100 µm. Although high specificity was preserved, the sensitivity was only 64 %.

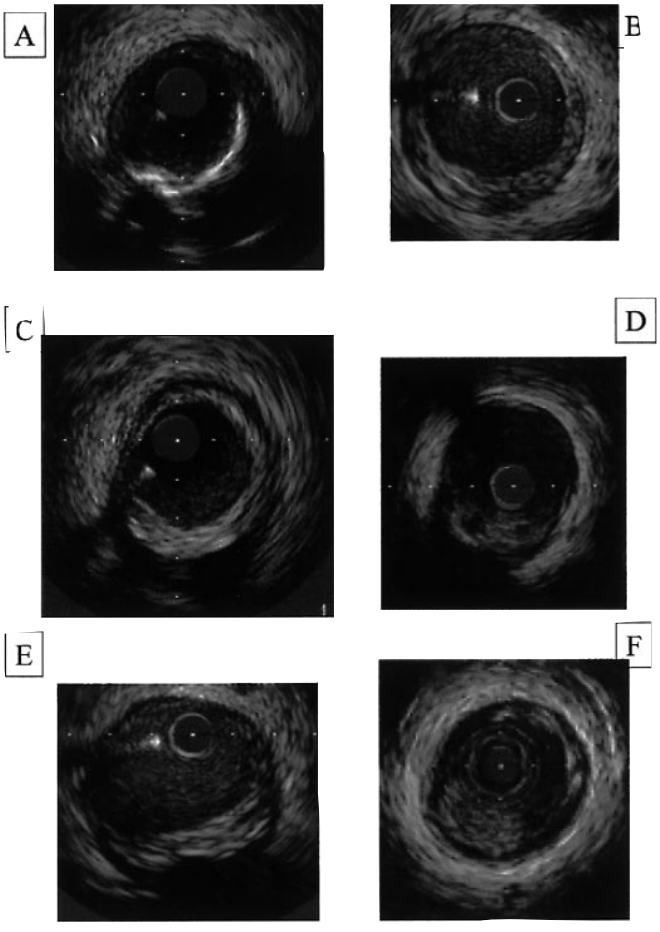

Fig. 1 IVUS imaging of plaque component. A) Example of calcific plaque. A calcific deposit with acoustic shadowing is visible at 3–7 o'clock. B) Example of loose fibrous plaque. The plaque has an echodensity similar to that of the adventitia. C) Example of hard fibrous plaque. A dense fibrotic deposit, leading to progressive acoustic shadowing in the external quadrants, is visible at 5–7 o'clock. D) Example of lipid pool. An echolucency within fibrous tissue, indicative of a lipid pool, can be appreciated at 7 o'clock. E) An echolucent area is visible at 4–5 o'clock, due to shadowing from dense fibrotic tissue located internally. The acoustic shadowing may obstruct the visualization of possible lipid pools externally. F) Example of thrombus. A mass with scintillating sparkling appearance is present at 6–8 o'clock.

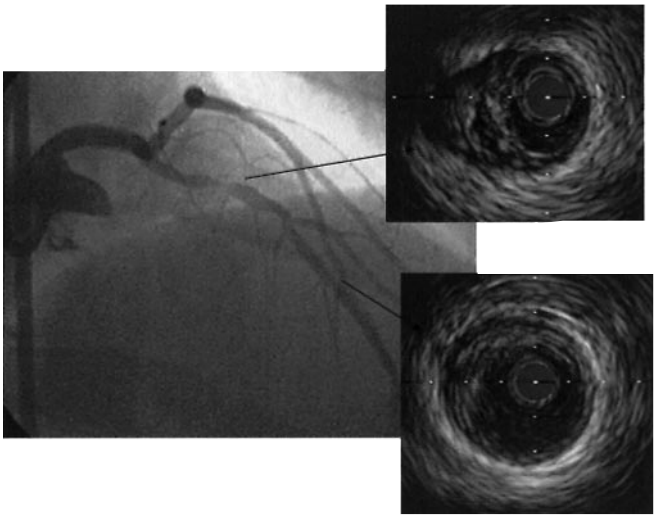

Fig. 2 In the left panel, the coronary angiogram shows a tight smooth concentric lesion in the mid segment of the left anterior descending artery (arrow). IVUS confirms the severity of the lesion showing a severe lumen reduction at the lesion site (upper right panel) in comparison to the reference site (lower right panel) and offers additional information on plaque composition, revealing an echolucent area at 9 o'clock, corresponding to a lipid pool, that can not be appreciated by angiography.

Fibrous tissue

IVUS is capable of depicting the fibro-sclerotic components of atherosclerotic plaque, which typically exhibit an echogenicity comprised between that of calcium and fat tissue. Nevertheless at the current stage of technology, a more accurate pathologic distinction between dense and loose fibrous tissue can not be achieved. Based on a rough classification, hard fibrous plaques can be differentiated from soft fibrous ones, given that their higher content of collagen and elastin leads to a progressive attenuation of IVUS signal and generate a shadow in the distal field (Fig. 1B, C) (5). Of note, the presence of acoustic shadowing is not peculiar for hard fibrous tissue, being also a characteristic of calcium. However, a distinction between fibrous and calcific tissue can be attempted, based on the modality of attenuation of the acoustic shadowing, that is progressive in hard fibrous lesions while it is abrupt in calcified plaques, leading to reverberation. Further technical improvements of IVUS technology are especially needed to offer a more detailed assessment of fibrous plaques.

Soft tissue

Plaque components with echogenicity lower than that of the adventitia are commonly classified as soft tissues. This definition (soft lesion) has not been shown to be associated with the compressibility of the plaque and comprises a large spectrum of tissue components, including lipid-necrotic pools, loose fibrous tissue, and thrombus. Of note, prior studies correlating IVUS images with histomorphology showed that plaques with a predominant echolucent composition have high fractions of loose fibrous tissue, smooth muscle, thrombotic or necrotic elements, but failed to provide specific details on plaque morphology such as the assessment of lipid pools (5, 6, 11). In fact, the sensitivity and specificity of IVUS to define quadrants with a lipid content at histology was found to be low. As a matter of fact, the definition of lipid pools is rather cumbersome and leads to different interpretations: occasionally, echolucent areas within fibrous plaque can be properly identified as lipid-necrotic areas (Fig, 1D, 2), but in other circumstances shadowing from dense fibrotic tissue can be mistaken for lipid (Fig. 1E).

Thrombus

Identification of thrombus by IVUS imaging is difficult. Occasionally thrombus appears as a mass with scintillating sparkling appearance (Fig. 1F). When thrombus is fresh/recent and mobile, due to the sisto-diastolic excursions of coronary arteries, it can be easily identified by IVUS. On the other hand, older/organizing thrombi are difficult to differentiate from the other plaque components. Therefore, the identification of thrombus, key information in acute coronary syndromes, is at the limit of resolution of the current 30 MHz IVUS transducers; new imaging technology, based on the use of high frequency transducers and on the analysis of radiofrequency, could overcome these limits (5, 6, 11).

Correlation of plaque morphology with clinical syndromes

Available data from prior studies are not encouraging. They reflect the difficulty of differentiating by IVUS the distinctive features of acute coronary syndromes: plaque fissuration and

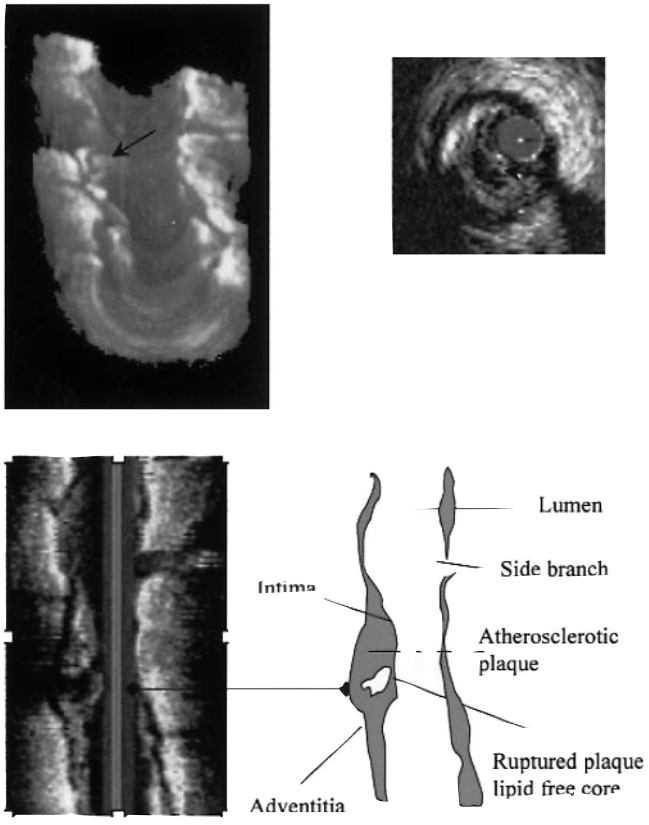

Fig. 3 In the right upper panel, an IVUS cross section obtained in the mid anterior descending artery shows a large plaque with a clear echolucent area at 4–6 o'clock, corresponding to a ruptured plaque with a lipid free core. In the left inferior panel, the longitudinal reconstruction of the analyzed segment is displayed. A semi-automated contour detection algorithm (Tomtec, Munich Germany), defining the inner contour between lumen and plaque and the external contour between plaque and adventitia, is applied. The location of plaque disruption can be clearly appreciated in the longitudinal view and in the corresponding schematic drawing of the vessel (arrow in the right panel). A 3-D reconstruction of the same segment obtained with the Echoscan system (Tomtec, Munich Germany) offers a clear representation of plaque distribution in the upper right panel. The site of plaque ulceration is indicated by the arrow.

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thrombus (Fig. 3). Hodgson et al. (12) compared lesion morphology in 65 lesions obtained in patients with stable and unstable angina. The authors found a higher prevalence of soft plaques in patients with unstable angina, but could not demonstrate in this subgroup of patients a higher prevalence of fissuration and thrombosis. In a more recent study (13), in which pre-interventional ultrasound was performed in 33 patients with unstable and stable angina, no significant differences in plaque echodensity in either group were found, although there was a trend for more echolucent zones in unstable lesions. The major finding in the above study was the observation that unstable coronary lesions are frequently characterized by a "layered" pattern within the lesion. The inner part of the lesion was separated from the outer segment in most cases by a fine, circumferential echodense line. The echodensity of the inner layer was frequently similar to the surrounding layer, but in a proportion of cases was relatively echolucent.

Definition of plaque morphology with 40 MHz transducers: an in vitro correlation between histomorphology and IVUS images in human arteries

Most IVUS studies dedicated to plaque composition date back to the early 1990s, when IVUS technology was still confined to 20 MHz transducers and further technical upgrade was not paralleled by corresponding investigations dedicated to the diagnostic potential of the procedure. Therefore, data on plaque morphology characterization with high frequency transducers, which should enable a more accurate definition of the soft component of atherosclerotic plaques, are not available.

We have investigated the qualitative correlations between IVUS and histopathologic findings of corresponding sections in human coronary arteries obtained at autopsy from patients with atherosclerosis. Particularly, we addressed the question of whether IVUS with 40 MHz transducers identifies lipid/necrotic pools and adds further information to that obtained with prior transducers.

Methods

IVUS investigation

The in vitro study used an imaging catheter with a 40 MHz mechanical transducer (Cardiovascular Imaging Systems Inc, Sunnyvale, Calif).

Arterial segments were dissected from the epicardial fat and all side branches and distal ends of the artery were ligated to generate a closed system. A 7 F valved sheath was fixed in the proximal ending of the vessel segment with an external ring suture. The arterial segments were then suspended horizontally in a container using small metal crooks and placed in a beacker of water. Saline (0.9 %) was infused through the lateral arm of the sheath at a constant physiologic pressure (60–80 mmHg) using a syringomanometer.

Internal markers (calcific deposits) and an external marker (surgical needle) were used to confirm that ultrasound images and histological sections were aligned. The IVUS catheter was then inserted into the introducer and advanced into the arterial samples until the external marker was clearly imaged. The IVUS catheter was kept coaxial with respect to the arterial segments and was pulled back at a constant speed of 0.5 mm/s (14). The first seconds of the automated pull-back were discarded from analysis to avoid a nonuniform movement of the catheter in the initial phase of the continuous pull back.

The following components of the atherosclerotic plaque were defined:

- Fibrous tissue: plaque components having a density similar or greater than that of the adventitia.
- Calcific deposits: highly echogenic segments having a density greater than that of the adventitia and causing acoustic shadowing.
- Microcalcific deposits: highly echogenic spots with a thickness less than 0.1 mm.
- Lipid necrotic areas: large echolucent areas within the plaque, circumscribed by tissue with higher echodensity.

Histopathologic study

Arterial vessels were cut into consecutive 3 mm-long segments from distal to proximal ends using the most distal recorded IVUS image as the starting reference. A coding number was assigned to each segment. The corresponding number had been used to mark IVUS images. Then arterial samples were fixed with 10 % buffered formalin, routinely processed for histopathologic study and embedded in paraffin. Serial sections from each block were cut at a distance of 0.5 mm. An automatic computerize-assisted technique was used to obtain quantitative measurements.

Results

One hundred twenty-two sections were analyzed. Lipid pools were observed by histology in 30 cross sections (25 %). IVUS revealed the presence of lipid pools in 19 of 122 cross sections

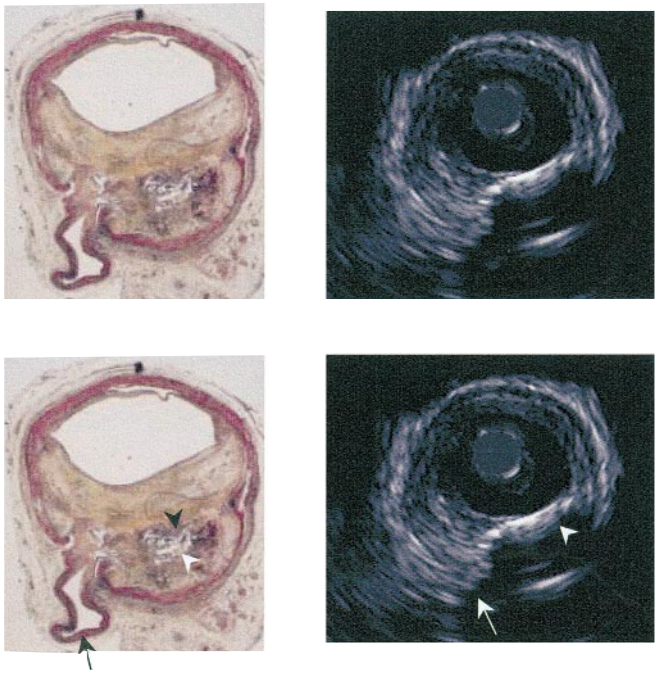


Fig. 4 Photomicrograph x 25 of histological and corresponding IVUS cross section in the left anterior descending coronary artery. In the left panel, histology shows at five o'clock, adjacent to the emergence of a diagonal branch (arrow at 7 o'clock), a calcific deposit superficially (black arrowhead) and a lipid pool deeply embedded in the atherosclerotic plaque (white arrowhead). In the corresponding IVUS cross section, the lipid pool can not be appreciated due to the echo-signal attenuation caused by the presence of highly echogenic superficial fibrous tissue (arrowhead).

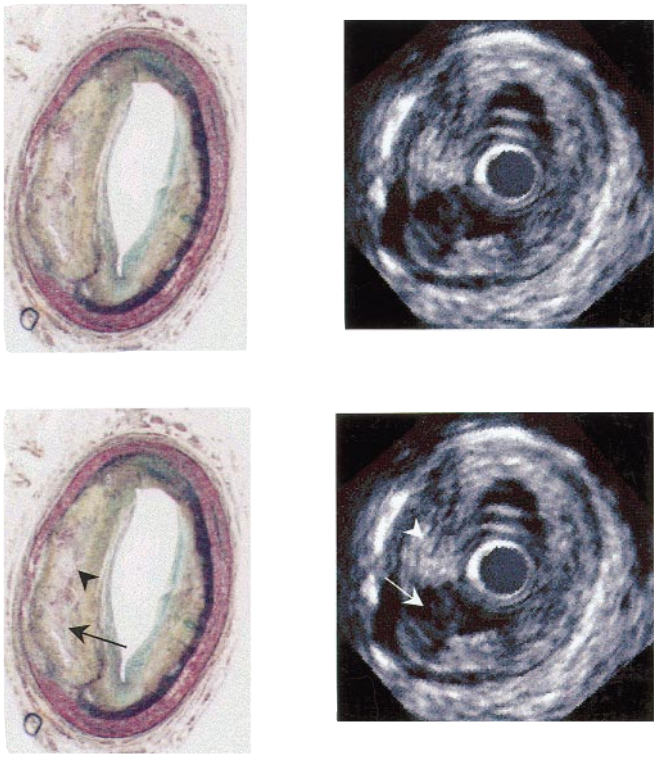
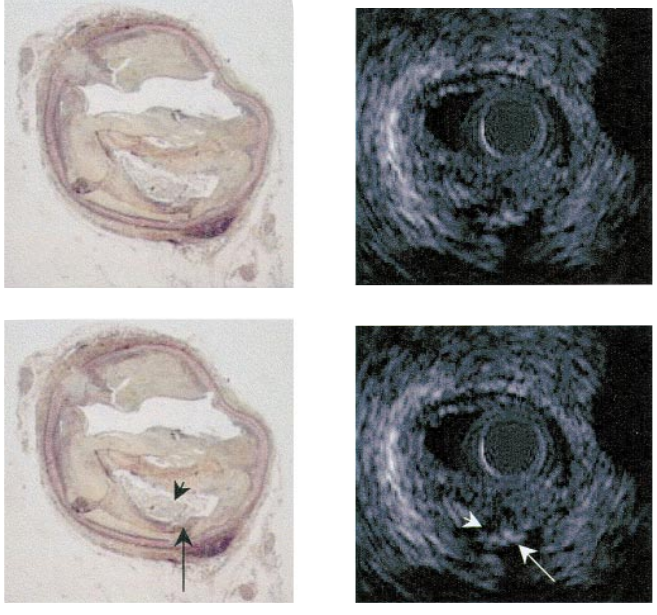


Fig. 5 Photomicrograph (x 25) of histological section from the left circumflex (left panel) and the corresponding ultrasound imaging (right panel). The histological section shows a lipid-necrotic area restricted to the 6 and 10 o'clock positions (arrows). IVUS confirms the presence of the lipid-necrotic pool at seven o'clock, where an echolucent lake is visible. At the 7–10 o'clock position IVUS is not capable of revealing the lipid content due to its spread out distribution within the atherosclerotic plaque.



with a sensitivity and specificity of 67 % and 94 %, respectively. In 5 cases (4 %) IVUS revealed a hypoechogenic area, interpreted as a lipid pool that could not be confirmed by histology (false positive): the histopathologic finding corresponding to the echolucency was due to areas shadowing from dense fibrous tissue. In 10 cases (8 %) lipid pools could not be appreciated by IVUS (false negative); in 7 cross sections the misinterpretation was due to presence of dense fibrous tissue and calcium deposits leading to hypoechogenic areas that were erroneously interpreted as shadowing from these highly echogenic structures (Fig. 4). In the remaining 3 cases the

Fig. 6 Photomicrograph x 25 of histological section from the left anterior coronary artery (left panel) and the corresponding ultrasound imaging (right panel). A lipid-necrotic core (arrowhead), with calcific deposit (arrow), deeply embedded in the atherosclerotic plaque, is visible at 5–6 o'clock. IVUS shows a ipoechogenic area indicative of lipid core (arrowhead) with microcalcification (bright deposits indicated by the arrow).

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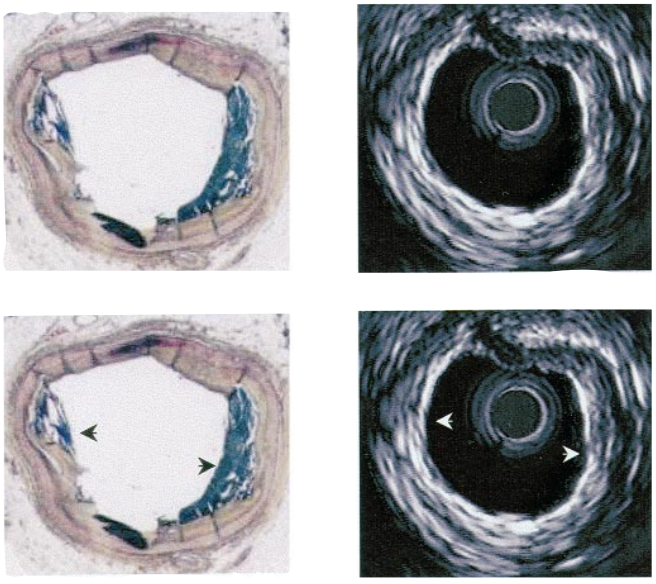

Fig. 7 Photomicrograph x 25 of histological section from the left circumflex (left panel) and the corresponding ultrasound imaging (right panel). Histology reveals presence of an inner, thin, calcific ring. Of note is that the IVUS corresponding cross section shows an inner ring with high echoreflectance which does not cause acoustic shadowing in spite of the presence of calcium.

misinterpretation of the lipid areas was due to the spread-out distribution of the lipid pools that were not localized in a definite area within the atherosclerotic plaque (Fig. 5).

A common observation by IVUS was the presence of minimal highly reflective deposits both within and around the lipid lakes. These bright deposits, corresponding to plaque microcalcifications at histology, were found in 12 of the 19 lipid pools at IVUS (64 %) (Fig. 6).

Twenty-six calcific deposits were observed by histology in 122 analyzed cross sections. The sensitivity and specificity of IVUS for assessment of calcifications was high (respectively 70 % and 100 %). In 7 cross sections (6 %), calcium deposits by histology were interpreted as fibrous tissue by IVUS. This was due to the presence of thin, highly echogenic spots that did not lead to acoustic shadowing and were therefore erroneously interpreted as fibrous tissue (Fig. 7).

Discussion

The current study was designed to address whether the technical upgrades of IVUS transducers (40 MHz) allows a reliable evaluation of lipid pools within the atherosclerotic plaques. We showed that 40 MHz transducers define lipid pools with a sensitivity and specificity of 67 % and 94 %, respectively. Although the current use of transducers at higher frequency (40 MHz) improves the image quality (15), some shortcomings in the definition of the plaque component are still present. The use of higher frequency transducers enhances the definition of lipid pools, although in some cases lipid collection can be misdiagnosed. The lipid pool can be misdiagnosed in presence of 1) highly echogenic tissue and 2) lipid contents with a spread out distribution within the atherosclerotic plaque. In the former case, highly echogenic tissue, such as calcified or dense fibrous tissue, impairs the identification of lipid pools (low echogenic areas due to acoustic shadowing); in the latter, the irregular diffuse distribution hampers the assessment of lipid pools which can be better visualized if well circumscribed in a definite region of the atherosclerotic plaque. Nevertheless, more specific details such as measurements of areas with lipid contents and thickness of fibrous cap can not be obtained at the current state of technology.

In our study microcalcifications, appearing as highly echogenic spots, were frequently identified by IVUS among the lipid lakes. This observation fits with corresponding histologic findings of scattered deposits of microcalcifications among lipid pools. The IVUS power to identify microcalcifications is due to the higher resolution obtained with 40 MHz transducers, with respect to those from prior generations. Of note, in a previous study based on 30 MHz transducers, the IVUS sensitivity for microcalcification was only 64 %, although high specificity was preserved (10). Minimal calcified deposits within the lipid pools can therefore be considered adjunctive markers enhancing the accuracy of IVUS in the identification of lipid pools.

Optimal assessment of atherosclerotic plaques: research and clinical implications

The accurate characterization of atherosclerotic plaque structure is of paramount importance. In current hypotheses on the pathogenesis of acute ischemic syndromes, a large lipid core is one of the markers that define vulnerable plaques, namely plaques which are more prone to rupture and further thrombosis (16–18). Most coronary plaques causing acute ischemic syndromes (about 60 % in sudden coronary death and 75 % in acute myocardial infarction) undergo rupture and thrombosis. Plaque rupture, however, has been recently shown not to be the only substrate for coronary thrombosis in acute ischemic syndromes: coronary thrombosis may also occur in “fibrous” plaques without a lipid core or in atherosclerotic plaques with thick, non-ruptured caps. In these lesions plaque erosion rather than rupture is the local substrate of acute thrombosis that causes acute coronary syndromes (19–21). Therefore, better definition of plaque structure could provide useful markers for future studies on vulnerable plaques and on eventful plaques in which morphological markers of “vulnerability” are absent.

To this aim, a major marker, namely cap thickness, would be necessary to parallel data on the atheromatous core presence and size. Although both large core size and cap thickness are not obligate markers of eventful lesions (they may also occur in eventless plaques), their combined value would better support in vivo identification of vulnerable lesions.

The role of calcium in the pathogenesis of acute coronary events is still controversial; while some authors suggest that calcifications have a stabilizing effect on atherosclerotic plaques, others have argued that a soft plaque, with a point of weakness induced by inflammation adjacent to an area of calcification, predisposes the plaque to rupture (22). A proper IVUS in vivo differentiation of plaques with a large core from plaques with prevalent fibro-proliferative composition, as well as a precise evaluation of contents in calcium deposits could provide useful insights on plaque vulnerability as predicted by its composition.

Finally, precise characterization of plaque composition in serial IVUS studies done at different intervals could assist in vivo studies on effectiveness of drug therapy on plaque size, composition, and possible regression.

Future direction of IVUS

Further refinements of IVUS technology are required to overcome limitations of IVUS in optimal plaque characterization. New techniques are being developed for characterizing tissue, defining the region of interest and then analyzing the radiofrequency backscatter The use of IVUS transducers at frequencies higher than 40 MHz and the parallel application of digital processing of IVUS images could substantially improve imaging quality (5).

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