The majority of acute coronary syndromes are the result of coronary plaque rupture. Recent studies have revealed the presence of neovascularization in and around the plaque to be a common feature of presumed rupture-prone (vulnerable) plaques. Intravascular ultrasound combined with contrast enhancement agents has been shown to be useful for the detection and quantification of vasa vasorum (VV) and angiogenesis within the vessel wall. In this chapter, the two state-of-the-art techniques for VV imaging are reviewed.

**Key words:** ACES; Cardiovascular disease; Contrast-enhanced intravascular ultrasound; Image analysis; IVUS; Microbubbles; Neoangiogenesis; Neovascularization; Plaque rupture; Vasa vasorum; Vulnerable plaque; VV

**KEY POINTS**

- Acute coronary syndromes are to a great extent the result of coronary plaque rupture. Studies indicate that increased vasa vasorum neovascularization in the adventitia and within the atherosclerotic plaque is related to the development and progression of coronary atherosclerosis, and may be used as a marker of plaque inflammation and risk of plaque rupture.
- Contrast-enhanced intravascular imaging can be used to trace perfusion because of vasa vasorum. Specifically, there exist two main approaches: (1) fundamental imaging combined with computational image analysis and (2) harmonic ultrasound imaging.
• The first approach relies on detection of local echogenicity changes in stationary intravascular ultrasound sequences, using differential imaging techniques (ACES™; Computational Biomedicine Laboratory, University of Houston, Houston, TX, USA).
• In the second approach, harmonic oscillations are induced on the contrast agent and detected by a specially designed intravascular ultrasound system.
• Contrast-enhanced intravascular ultrasound methods have been applied in vivo with promising results for the assessment of blood perfusion, plaque VV revealing, and plaque inflammation.

INTRODUCTION

Cardiovascular Disease

Atherosclerotic cardiovascular disease (CVD) and its complications are a leading cause of death worldwide [1]. For a significant percentage of these patients, the first symptom of atherosclerotic CVD is sudden death without previous warnings. It has been shown that for up to 75% of the acute coronary syndromes, atherosclerotic plaque rupture is the underlying pathological mechanism [2, 3]. Although atherosclerotic plaques are widespread in the coronary artery tree of CAD patients, only a small portion of these plaques present a particularly high risk of rupture (vulnerable plaques), and an even smaller number of them will rupture.

The risk of plaque rupture depends on the plaque type (e.g., morphology, composition) more than the degree of luminal stenosis. Most ruptures occur in plaques containing a soft, lipid-rich core that is covered by an inflamed thin cap of fibrous tissue [3]. These ruptures manifest positive remodeling and cell infiltration of the fibrous cap and adventitia, and they exhibit increased neovascularization within the plaque [4, 5]. Since the ruptured caps are thinner (usually less than 65 mm) than intact caps, the term “thin-cap fibroatheroma” (TCFA) has been proposed for presumed rupture-prone plaques [6].

Detection of rupture-prone plaques is one of the most active areas of research in both the cardiology and the biomedical imaging communities. While several invasive and noninvasive techniques such as thermography, magnetic resonance imaging (MRI), optical coherence tomography, IVUS-based virtual histology, elastography, and others have been used for the assessment of plaque vulnerability [7], none of them can completely identify a vulnerable plaque and accurately predict its further development.

Vasa Vasorum

Vasa vasorum (VV) constitute a network of microvessels that nourish the wall of larger vessels [8]. In normal conditions, VV are present in the adventitial layer of larger arteries such as the aorta, and play an important role in the functional and structural characteristics of the vessel wall [9]. Recent studies in human and animal coronary arteries have indicated that adventitial VV are related to the development and progression of coronary atherosclerosis [10]. In several investigations [11–14], VV have been implicated in the pathogenesis of atherosclerotic plaques. An increase in the density of the VV has been associated with the advancement of a plaque from being stable, to TCFA, and finally to rupture [5, 12].

In addition, intraplaque hemorrhage has been documented to be present in atherosclerotic plaques in many cases of sudden coronary death [15]. The hemorrhage is believed to occur from the disruption of thin-walled immature microvessels that are lined by the discontinuous endothelium without supporting smooth muscle cells [6]. This may also contribute to the progression of atherosclerotic plaques by allowing the accumulation of large amounts of cholesterol-rich red cell membranes which may become oxidized and provide the stimulus for macrophage accumulation and atherogenic cytokine secretion.
These microvessels are associated with extensive VV proliferation and represent a form of neoangio-
genesis within the plaque confines.

This evidence suggests VV proliferation as a marker of plaque inflammation and a preceding or concomitant factor associated with plaque rupture and instability [15, 16]. It is thus quite demanding to develop tools that allow for the detection and measurement of plaque neovascularization and the detection of leakage and entrapment of blood within atherosclerotic plaques in order to obtain a quan-
titative index of plaque vulnerability.

**Intravascular Ultrasound**

Intravascular ultrasound (IVUS) is an invasive medical imaging technique that is capable of providing high-resolution real-time cross-sectional images of the arterial wall and has therefore become an important clinical tool for the detection and evaluation of coronary artery diseases as well as for therapy guidance and clinical research.

IVUS consists of a specially designed catheter with a miniaturized ultrasound probe attached to the distal end of the catheter. The IVUS catheter is advanced percutaneously within the examined vessel. High-frequency sound signals are emitted and received radially by a solid-state or mechanically rotated ultrasound transducer at a discrete set of angles (commonly 240–360). The ultrasound signals are partially reflected and transmitted differently depending on the acoustic impedance of the tissues within the blood vessel at each particular angle. The received signals are then processed and converted to a gray-scale image known as B-mode (Fig. 1).

**Contrast Agents**

Although IVUS provides reliable cross-section images of the coronary arteries, the in vivo imaging of the coronary VV remains a great challenge because of their small size, their echo transparency, and the different IVUS artifacts. To overcome these limitations, IVUS has been used in combination with contrast agents. Most modern ultrasound contrast agents consist of solutions of echogenic microbubbles that are introduced into the systemic circulation, resulting in enhanced backscatter from microbubble-infused free blood or from microbubble-perfused tissue. These microbubbles are gas-filled spheres surrounded by a shell designed to aid their longevity in the bloodstream. The size of these

![Fig. 1. B-Mode IVUS image (a) and the corresponding visible regions (b). Legend: (A) area occupied by the IVUS catheter; (B) the lumen; (C) the intima; (D) the media; (E) the adventitia and surrounding tissues.](image-url)
Microbubbles (diameter: 1–10 μm) is similar to the size of red blood cells (diameter: ~8 μm), and hence they are used as tracer of blood flow.

Microbubbles resonate as a response to pressure changes induced by an ultrasound wave. This makes them several times more echogenic than normal body tissues, and consequently they appear extremely bright in the B-mode ultrasound images. Depending on the energy and frequency of the ultrasound beam, the microbubbles will present linear or nonlinear oscillations.

Linear oscillation means that the contraction and the relaxation of the microbubbles induced by the ultrasound signal are equal in amplitude. On the other hand, nonlinear oscillation means that the microbubbles expand above their baseline diameter at a greater scale than they are able to compress below it.

In the first case (fundamental mode), the microbubbles produce echo signals with the same frequency as that to which they are exposed. In the nonlinear case, the microbubbles will produce the fundamental frequency and multiples of this frequency called harmonics [17].

Imaging of the coronary VV is a great challenge. Until now only contrast-enhanced IVUS has been applied in vivo with promising results. In this scope, there exist two main different approaches for the imaging of VV by contrast-enhanced IVUS: (1) fundamental imaging combined with computational image analysis [18–20, 26] and (2) harmonic ultrasound imaging [21–25].

FUNDAMENTAL IMAGING WITH COMPUTATIONAL IMAGE ANALYSIS FOR VASA VASORUM IMAGING

O’Malley et al. have proposed a protocol and an automatic algorithm (Analysis of Contrast Enhanced Sequences, ACES™) for quantification and visualization of VV in contrast-enhanced IVUS image sequences [18]. This method relies on detection of local echogenicity changes in stationary IVUS sequences due to microbubble perfusion into the vessel wall. According to the proposed protocol, an IVUS catheter is first placed at the maximally-stenotic point of a suspect plaque or of any other plaque of interest. The catheter is held steady and images are acquired for a period of time (10–30 s). Then a bolus injection of contrast agent is made through the guiding catheter and proximal to the imaging catheter. After the contrast agent disappears, more images are continuously acquired for another period of time (10–20 s) with the catheter kept at steady position.

Enhancement detection is accomplished as follows. First, it is necessary to eliminate the motion artifact from the IVUS sequence that is resulting from the heart beating while the catheter is inside the coronary arteries. When an electrocardiogram (ECG) is available in addition to the IVUS analysis, the sequence stabilization is made by synchronizing the sequence with the ECG. Then, only frames corresponding to the same phase on the cardiac cycle are selected. This method is known as ECG-based sequence gating. However, sometimes an ECG is not available. In such cases, the proposed algorithm introduces a sequence-gating algorithm based on the analysis of the interframe correlations with a standard registration metric. These frames are clustered and then selected to build a new gated sequence eliminating cardiac motion.

Next, the region of interest (ROI) is defined manually by a human operator tracing its inner and outer borders (luminal and media/adventitia contours in the case of plaque monitoring) in the first frame of the gated set of images. The ROI corresponding to each frame in the gated sequence is located and “unwrapped” into a rectangular domain. These images are aligned and superimposed to obtain a pixel-wise correspondence. A precontrast baseline image is computed by averaging the subset of gated frames corresponding to the period before the microbubble injection. The precontrast baseline image is subtracted from all frames in the gated sequence. As a result, any change that occurs due to contrast enhancement will be reflected as a positive difference in the intensities in particular regions of the difference image. To quantify the enhancement of a particular frame, the average of the
gray intensity levels in the difference image is obtained within the ROI to produce a mean enhancement in ROI (MEIR) statistic. This statistic is obtained for all the frames in the gated sequence. If a perfusion within the ROI (e.g., plaque area) occurs during the injection, the MEIR level will increase in the frames corresponding to the postcontrast injection period (Fig. 2). If no perfusion occurs, the MEIR will return to its precontrast value almost immediately after contrast agent flows through the vessel lumen. Finally, the difference images corresponding to the postinjection period are summed and filtered with a threshold in order to image the perfusion in the ROI (Fig. 3).

Animal studies have been carried out verifying the feasibility of imaging intracoronary-injected microbubbles using an intracoronary ultrasound system (central frequency: 20 MHz, peak pressure: 100 KPa Invision; Volcano Therapeutics, Rancho Cordova, CA), employing injections of SonoVue™ (Bracco Diagnostics Inc., Italy) or Optison™ (GE Healthcare, US) as contrast agent [7]. In these studies it was demonstrated that contrast-enhanced intravascular ultrasound in combination with appropriate image analysis (ACES™) can detect intracoronary and perivascular flow of microbubbles. In a preliminary study, contrast-enhanced IVUS and ACES™ were used in seven nonconsecutive human patients (6 males, 1 female) with unstable angina due to coronary artery disease. These patients were undergoing percutaneous coronary interventions (PCI) and intravascular ultrasound evaluation.

![Fig. 2. IVUS contrast study depicting pronounced plaque perfusion. (a) Top panel illustrates original IVUS sequence over time; bottom panel illustrates corresponding analysis frames. IVUS regions have been outlined in the bottom panel for reference (from the center outward: the catheter blank, the lumen, the intima-medial region, and the adventitia and surrounding tissues). Middle frames indicate the peak of injection and consequent luminal echo-opacity. Arrows indicate prominent enhancement. From 10 to 12 o’clock, we observe small features at the media-intima boundary indicative of vasa vasorum perfusion. At 6 o’clock, we observe evidence of direct microbubble entry into an existing plaque. Note eventual diminution of enhancement (time from fourth frame to fifth frame is 31 s), as reflected in the perfusion plot. (b) Perfusion curve for intima-medial region of study, quantifying enhancement levels following injection of contrast agent.](image-url)
In this study, a solid-state, synthetic aperture, 20-MHz IVUS scanner (Invision; Volcano Therapeutics, Rancho Cordova, CA) was employed. The image sequences were recorded using DICOM format at a rate of 10 frames per second while the catheter was left stationary at nonobstructive (<75%) coronary lesions proximal to the treated segment which showed positive remodeling and low echogenicity areas within the plaque. Baseline recording was performed for 10 s and then a bolus injection of 2 mL of Sonovue™ was made through the guiding catheter, followed by 5 mL of normal saline to flush out
remaining microbubbles. The recording was stopped after 2 min of the contrast agent injection. Off-line analysis of the recorded sequences with ACES™ showed a significant enhancement in adventitial echogenicity. For this study, the average enhancement of the adventitial plaque echogenicity was 1.2% ± 0.8%, with ranges from 0.3% to 2.5%. No side effects were reported on the patients [19, 20].

More recently, a study in 16 human patients with acute coronary syndrome using ACES™ has been presented [26]. Here, a qualitative analysis of the areas of enhancement was observed in distinct areas within the intima-media area and adventitia. After quantitative analysis, a statistically significant postcontrast enhancement was demonstrated in the echogenicity of the intima-media, adventitia, and combined intima-media and adventitia. MEIR increased significantly after the injection of microbubbles (from 6.01 ± 2.46 to 7.88 ± 3.28, \( p = 0.006 \)) in the intima-media region. A significant increase, though in a lesser degree, was also observed in MEIR for the adventitia region (from 7.10 ± 2.20 to 7.60 ± 2.50, \( p = 0.035 \)). All patients manifested an increase in the mean gray level of all examined regions as expressed by MEIR from pre- to postinjection of microbubbles, although to a different degree. Specifically, in the intima-media region the percentage increase of MEIR after the injection of microbubbles was <20% in 5 pts, 20–50% in 7 pts and >50% in 4 pts. In the adventitia region the percentage increase of MEIR was <20% in 13 pts, 20–50% in 2 pts and >50% in 1 pt.

Due to the inherent limitation of in vivo human coronary IVUS studies, in these studies it was not possible to correlate the described clinical findings with histopathology. However, since all blood perfusion in plaques comes from coronary branches through the VV and their extension into the atherosclerotic plaques, the investigators of this clinical study concluded that the observed enhancement reflects VV density and flow.

**Contrast Harmonic IVUS for Vasa Vasorum Imaging**

Goertz et al. have investigated the feasibility of harmonic and subharmonic IVUS for detection of microbubbles using a prototype nonlinear IVUS system and commercially available contrast agents [21–23]. This method is able to provide microbubble-specific imaging by detecting nonlinear signals. With this method, the background signal from tissues is minimal compared with the microbubbles signal, making them easier to detect (Fig. 4).

The prototype nonlinear IVUS system consists of a custom-built, single-element transducer that is mechanically rotated, and specially designed signal filters for processing the received signal. In this method, the transducer emits a fundamental frequency with certain intensity (usually stronger than the one used in baseline IVUS) that stimulates the microbubbles to resonate at frequencies different from the frequencies to which it was exposed. The reflected signal is then filtered to isolate the frequencies corresponding to the nonlinear response of the microbubbles. In the harmonic imaging method, the microbubbles are expected to resonate at frequencies higher than the base frequency, while in the subharmonic method, lower frequencies are expected.

Animal studies were carried out to investigate the use of microbubble contrast agent in combination with prototype nonlinear IVUS systems as a means of imaging vasa vasorum [21, 22]. First, the IVUS catheter was situated in an ROI in an atherosclerotic rabbit aorta. The transducer transmits pulses at 20 MHz (fundamental frequency) and registers pulses with frequencies centered at 40 MHz (second harmonic). Then, a bolus injection of contrast agent (Definity™, Bristol-Myers Squibb Inc., New York, NY) is made through a delivery catheter. The microbubbles were first detected within the main lumen, and then (after 5–10 s) within the adventitia surrounding the plaque. A quantification of the enhancement was found to be statistically significant. In addition, the general spatial pattern of the agent presence within the adventitia and not the plaque itself was consistent with the microvascular distribution revealed by histological sections taken in the vicinity of the imaging planes.
In vivo phantom experiments were carried out to investigate the feasibility of subharmonic IVUS imaging using an atherosclerotic rabbit aorta model [23]. In this study, a frequency of 30 MHz is used as fundamental while the system is focused on registering the microbubble response at frequencies of 15 MHz (subharmonic). The results provide evidence of the potential use of this technique for the imaging of vasa vasorum.

Tissue harmonic imaging (THI) is investigated in [24, 25]. Here, a dual-frequency transducer element was mounted in an IVUS catheter. As a result, this prototype IVUS system can operate in both fundamental frequency and second harmonic imaging modes. This system uses a conventional, continuously rotating, single-element IVUS catheter and was operated in fundamental 20 MHz, fundamental 40 MHz, and harmonic 40 MHz modes (transmit 20 MHz, receive 40 MHz). Imaging experiments were conducted in both a tissue-mimicking phantom and in an atherosclerotic rabbit model in vivo. The harmonic results of the imaging experiments show the feasibility of this system for improving the IVUS image quality. In addition, this system has the potential to be used with contrast agents for VV imaging.

The advantage of the use of nonlinear IVUS techniques is that it overcomes the limitation of fundamental imaging to detect microvessels, which is related to its susceptibility to motion effects. Moreover, harmonic imaging is capable of using the resonant bubble oscillations from commercial contrast agents, using pressure levels that are within the range of current commercial catheters. However, despite the effectiveness of the harmonic imaging methods, contrast-enhanced fundamental IVUS with proper image analysis has the potential for large-scale clinical application due to the fact that commercial harmonic IVUS catheters are not currently available.

Fig. 4. (a, b) Fundamental mode before and after the injection of microbubbles, respectively; (c, d) harmonic mode before and after the injection of microbubbles, respectively. Legend: C: catheter, VC: vena cava. (Reprinted from [22]).
CONCLUSIONS

The identification of patients with a high risk for developing an acute coronary syndrome remains a challenge because of the limited knowledge on vulnerable coronary atherosclerotic plaques, the mechanisms that trigger their rupture, and the lack of reliable techniques for detecting them. However, since the presence of neovascularization has been documented to be highly related to plaque inflammation, intraplaque hemorrhage, and plaque rupture and thus has been identified as a major characteristic of plaque vulnerability, it is desirable to test with techniques that can provide reliable assessment of the VV density in vivo. Contrast-enhanced intravascular ultrasound techniques such as those described here have been shown to be useful for the assessment of luminal and plaque characteristics, plaque VV revealing, and plaque inflammation. These techniques may contribute significantly to the detection of neovascularization within the coronary atherosclerotic plaques. Future developments as improvement in resolution, signal-to-noise ratio, and robustness to IVUS artifacts as well as histological validation are necessary in order to augment the reliability of the detection of vulnerable plaque. However, since VV are not the only feature of plaque vulnerability, the combination of this and other invasive and noninvasive existing modalities would be necessary in order to provide an effective way to determine and study the causes that lead to plaque rupture and the consequent thrombotic complications.

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