Analysis of Contrast-Enhanced Intravascular Ultrasound Images for the Assessment of Coronary Plaque Neoangiogenesis: Another Step Closer to the Identification of the Vulnerable Plaque

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Abstract: Atherosclerotic cardiovascular disease (CVD), primarily manifested as heart attacks and strokes, remains the main cause of death in the developed countries and is rapidly increasing in the developing world. Early detection and aggressive treatment of hidden (asymptomatic) atherosclerotic plaques that cause heart attack or stroke are most needed. However, existing clinical tools are not sufficient to address this need. Intravascular ultrasound (IVUS) is a catheter-based medical imaging tool that is capable of providing cross-sectional images of arteries. It is by far the most powerful clinical tool available for characterization of atherosclerotic plaques. However, existing IVUS is unable to detect plaque inflammation which is a key factor in complications of atherosclerotic plaques. Contrast enhanced IVUS (CE-IVUS) for detection of Vasa Vasorum (VV), microvessels that feed the vessel wall, can indirectly image plaque inflammation and thereby significantly increase the diagnostic power of IVUS. Several studies have shown that the density of VV in the atherosclerotic plaques is strongly correlated with the intensity of plaque inflammation and related processes which lead to plaque destabilization and rupture (the Vulnerable Plaque). Therefore the detection and measurement of VV in plaque, and leakage of blood from VV into plaques using CE-IVUS, can enable the development of an index for plaque vulnerability. In this paper, we present a review of our original work on coronary VV imaging, discuss subsequent reports by others, and also present the latest on the detection of VV based on CE-IVUS.

Keywords: Cardiovascular disease, atherosclerosis, vulnerable plaque, vasa vasorum, contrast-enhanced IVUS, ultrasound contrast agent, VV density.

1. INTRODUCTION

Cardiovascular disease (CVD) refers to those disorders that affect the heart and/or the vascular system. According to the American Heart Association, CVD accounted for 813,804 of the 2,243,712 (i.e., 1 out of every 2.9 deaths) in the United States in 2007 [1]. The most common form of CVD is atherosclerosis, a condition in which the arterial wall hardens and thickens due to the build up and accumulation of plaque [2]. Atherosclerotic processes mainly involve the thickening of the intima, and other processes such as fibrosis, necrosis, calcification, and hemorrhage [3]. The process of atherosclerotic plaque formation is considered to be an inflammatory, response-to-injury phenomenon that is initiated by injury to the endothelium or smooth muscle cells of the artery wall [4]. Although atherosclerosis is a multifocal disease, it is well known that atherosclerotic plaques are not similar to one another in composition, progression rate, stability, and thrombogenicity.

Coronary artery disease (CAD) is usually caused by atherosclerosis in the arteries that supply blood to the heart (i.e., coronary arteries). It is estimated that 70% of deaths in patients with CAD is due to acute coronary syndromes (ACS) such as unstable angina pectoris, sudden coronary death, and acute myocardial infarction [5, 6]. In the past, it was believed that the increase of plaque and the consequent narrowing of the coronary arteries was the cause of fatal coronary events. Currently, it is believed that the inflammation and disruption of coronary plaques with superimposed thrombosis are the primary cause of acute coronary events. It has been shown that for up to 75% of the acute ischemic coronary syndromes, atherosclerotic plaque rupture is the underlying pathological mechanism [7, 8]. Pathology studies indicate that certain plaques are more prone to develop ACS than others. In this context, the field of cardiology has introduced the term "vulnerable plaque" (VP) which refers to those plaques with a high likelihood of rupture, thrombotic complications, and the consequent rapid progression to stenosis [9, 10, 11, 12].

1.1. Vulnerable Plaque

Although there is no broad consensus on what characteristics define a VP, autopsy studies have provided useful indicators of the features exhibited by certain plaques immediately before rupture. The histopathologic characteristics of ruptured plaques have been well defined [13]. The most consistent findings include: (i) a large lipid (necrotic) core composed of free cholesterol crystals, (ii) cholesterol esters and oxidized lipids impregnated with tissue factor, (iii) a thin fibrous cap depleted of smooth muscle cells and collagen, (iv) an outward (positive) remodeling, (v) inflammatory cell infiltration of the fibrous cap and adventitia (mostly monocyte/macrophages, some activated T cells and mast cells), (vi) intra-plaque hemorrhage, and (vii) the formation of new microvessels (neoangiogenesis or neovascularization) at the arterial wall adventitia, and within the atherosclerotic plaque (i.e., vasa vasorum, VV) [14].

In normal conditions, VV is present in the adventitial layer of large vessels such as the aorta, and plays an important role in the modification of the functional and structural characteristics of the vessel wall [15, 16]. However, recent evidence has suggested that the presence and proliferation (increase in density) of VV in the plaque (i.e., vasa plaurorum, VVP [17]) is correlated to the processes which lead to the destabilization of the plaque Fig. (1) [18, 19, 20, 21, 22]. Additional findings indicate that: (i) the microvessel density is greater in ruptured plaques than nonruptured plaques, (ii) the microvessel density is correlated to macrophage infiltration, and (iii) microvessel density within the plaque is
correlated to plaque rupture [23]. These findings suggest that the proliferation of the VV and VVP can be used as a marker of plaque inflammation and a preceding or concomitant factor associated with plaque rupture and instability [24, 25]. Since VV and VVP neovascularization may both be markers in the development of rupture-prone atherosclerotic plaque and intra-plaque hemorrhage, it is believed that its in vivo detection and quantification can enable the development of an index of plaque vulnerability.

Different imaging modalities (e.g., MRI, positron emission tomography, single-photon emission computed tomography, computed tomography, and ultrasound) have been used to detect microvascularization in a clinical setting [26]. In particular, ultrasound has been used in combination with contrast agents that resonate in response to the pressure changes induced by the ultrasound wave (i.e., contrast-enhanced ultrasound, CE-US). Ultrasound contrast agents (in the form of echogenic microbubbles) are introduced into the systemic circulation, resulting in enhanced backscatter from microbubble-infused blood or microbubble-perfused tissue. The microbubbles consist of spheres filled with an inert gas that is enclosed in a shell designed to aid their longevity in the bloodstream. The scale of these bubbles (diameter: 1-10 \( \mu m \)) is similar to the scale of red blood cells (diameter: \( \sim 8 \, \mu m \)), and hence they may be used as tracers of blood flow. CE-US is a promising technique for the early detection of vulnerable plaques. Recent histological studies have demonstrated the feasibility of using planar CE-US for the detection of VV and VVP [27, 28, 29, 30, 31, 32]. However, the use of this technique for the imaging of VV and VVP of coronary arteries has not been deemed feasible due to its limited resolution, and the associated motion artifacts which limit the method's potential to achieve a sufficient contrast-to-tissue ratio [33].

1.2. Intravascular Ultrasound

Intravascular ultrasound (IVUS) is currently the gold-standard technique for assessing the morphology of blood vessels and atherosclerotic plaques in vivo and is often used as a guide for interventional procedures such as angioplasty. IVUS is a catheter-based medical imaging technique that is capable of providing a real-time, high-resolution tomographic visualization of the coronary arteries. This technique allows the accurate estimation of the lumen, the vessel dimensions, the distribution of plaques, and the presence of intra-luminal thrombus and plaque rupture. An IVUS system consists of a specially designed catheter with a miniaturized ultrasound sensor attached to the distal end of the catheter which is inserted in the femoral artery and is advanced percutaneously to the vessel of interest. The IVUS technique is based on the following principles:

1. Conversion of electrical energy into sound waves via piezoelectric crystals.
2. Transmission and detection of sound waves reflected by tissues using a miniaturized transducer.
3. Conversion of sound waves into electrical energy.
4. Amplification and processing of the electrical energy and conversion to an image.
5. Projection of the image on a computer screen.
6. Post-hoc analysis of the recorded image sequences.

The ultrasound probe can either be a solid-state multiarray or a mechanically-rotated transducer which transmits ultrasound pulses and receives an acoustic radio frequency (RF) echo signal (i.e., A-line) at a discrete set of angles. Commonly, 240 to 360 A-line RF signals are obtained per rotation. A B-mode IVUS image is obtained by computing the positive envelopes of each A-line Fig. (2). These B-mode signals are then compressed, stacked along the angular direction, and mapped into a 8-bit gray scale to form an image known as the polar B-mode image. To provide a more familiar representation of the data (to resemble the interior of a vessel), the polar B-mode image is geometrically transformed to obtain a disc-shaped image known as the Cartesian B-mode image. Examples of typical normal and diseased (atherosclerotic) grayscale Cartesian IVUS images are illustrated in Fig. (3).

Although IVUS provides reliable cross-sectional images of the coronary arteries, the in vivo imaging of the coronary VV and VVP remains a great challenge due to its small size, echo transparency, and the presence of different IVUS artifacts. Therefore, IVUS is used in combination with contrast agents (contrast-enhanced IVUS, CE-IVUS) as tracers of blood flow. The use of CE-IVUS has proven useful for imaging plaque perfusion in coronary arteries [34] and for assessing the amount and distribution of neovessels within atherosclerotic lesions [35]. Currently, there are two main approaches for the assessment of VV and VVP based on CE-IVUS data: (i) differential imaging and (ii) nonlinear or harmonic response imaging.

2. DETECTION OF VV AND VVP BY CE-IVUS

2.1. Differential Imaging

O'Malley et al. [36] proposed a protocol and an automatic algorithm (Analysis of Contrast Enhanced Sequences, ACES) for the quantification and visualization of VV in CE-IVUS image.

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**Fig. (1).** Depiction of atherosclerotic plaque in a vessel.

**Fig. (2).** Depiction of an A-line signal and its envelope.

**Fig. (3).** Depiction of typical normal and diseased (atherosclerotic) grayscale Cartesian IVUS images.
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Fig. (4). Examples of the longitudinal view of a stationary sequence. (a) Depiction of the cut in the Cartesian B-mode image, (b) L-mode image of the non-gated sequence, and (c) L-mode image resulting from the image-based gating.

Fig. (3). Examples of a typical B-mode image of (a) normal and (b) atherosclerotic vessels.

sequences. This method relies on the detection of local echogenicity changes (due to microbubble perfusion into the vessel wall) in stationary IVUS sequences. According to the proposed protocol, the IVUS catheter is placed in the maximally-stenotic point of a suspect plaque. The catheter is held steady and images are acquired for a time period of 10 to 30 s. Then, a bolus injection of contrast agent is applied through the guiding catheter and proximal to the imaging catheter. After the contrast agent disappears, more images are acquired for a time period of 10 to 20 s, with the catheter kept at a steady position. The enhancement detection is performed offline and consists of several steps. First, motion artifacts caused by the beating of the heart are eliminated from the IVUS sequence using a sequence-gating algorithm which is driven entirely by the imaging data and it is based on the analysis of the inter-frame correlations with a standard registration metric Fig. (4) [37, 38, 39]. This image-based gating is accomplished by transforming the image sequence to a Euclidean multidimensional similarity space (MDS) in which each frame is represented by a particular, though not necessarily unique, point. This space is clustered using k-means (with multiple runs in order to achieve the lowest-error) to provide an ensemble of stabilized frames Fig. (5). Typically, a human operator selects the number of clusters to use from a visualization of the clusters...
associated with several $k$ as there is a tradeoff between a high value, which imposes greater restrictions on what frames are considered to be similar, and a low value, which forces some events which could be considered distinct to coalesce. The frames corresponding to the selected cluster are then used to build a new sequence for which it is assumed that the axial catheter motion has essentially been eliminated.

Next, the region of interest (ROI) is defined manually by a human operator by tracing the luminal and media/adventitia contours 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. To eliminate residual motion artifacts, the images are aligned and superimposed to obtain a pixel-wise correspondence using a two-step approach that consist of a rough, rigid alignment step followed by an elastic refinement step. A pre-contrast baseline image is computed by averaging the subset of gated frames corresponding to the period before the microbubbles' injection. The pre-contrast baseline image is subtracted from all frames in the gated sequence to form the differential images. As a result, any change that occurs due to contrast enhancement will be reflected as a positive difference in the intensities in the corresponding regions of the differential images. To quantify the enhancement of a particular frame, the average of the gray intensity levels in the differential images is obtained within the ROI to produce a mean enhancement in ROI statistic (MEIR). This statistic is obtained for all the frames in the gated sequence. If perfusion occurs during the injection, the MEIR level will increase in the frames corresponding to the post-contrast injection period. If no perfusion occurs, the MEIR will return to its pre-contrast value almost immediately after the contrast agent passes through the lumen. The feasibility of using this method with CE-IVUS, by employing existing IVUS systems for detecting perivascular blood flow, has been demonstrated in an animal study Fig. (6) [40].

Moreover, the feasibility and safety of using this technique with human patients was assessed by Vavuranakis et al. [41] in which CE-IVUS imaging was performed in patients with ACS requiring a percutaneous coronary intervention. In this study, it was found that differential computational analysis of contrast enhanced IVUS image sequences is capable of illustrating, in a qualitative and quantitative manner, the presence of VV and neovascularization at the adventitia of the arterial wall and within the atherosclerotic plaque region Fig. (7).

### 2.2. Harmonic IVUS

Differential imaging techniques are based on the analysis of the images generated by the linear propagation of the ultrasound signal. However, depending on the energy and frequency of the ultrasound beam, the contrast agent microbubbles may present linear and nonlinear oscillations. In the case of linear oscillations (fundamental mode), the microbubbles produce echo signals with the same frequency as the ultrasound transducer (i.e., fundamental frequency). In the case of nonlinear oscillations, the microbubbles will produce the fundamental frequency and multiples of this frequency, known as harmonics, sub-harmonics and ultra-harmonics.

Goertz et al. [42, 43, 44] investigated the feasibility of harmonic and sub-harmonic IVUS for the detection of microbubbles using a prototype nonlinear IVUS system and commercially available contrast agents. This method is capable of providing microbubble-specific imaging by detecting non-linear signals. The prototype nonlinear IVUS system consist of a custom-built, single-element transducer that is mechanically rotated, sophisticated pulse sequences generated using pulse inversion, methods for tissue and catheter motion compensation, and specially designed signal filters for processing the received signal. In this method, the transducer emits a fundamental frequency that stimulates the microbubbles to resonate at frequencies that are different from the frequencies at which they were exposed. The reflected signal is then processed to isolate the frequencies corresponding to the nonlinear response of the microbubbles.

Frijlink et al. [45, 46] proposed a tissue harmonic imaging (THI) system which consists of a dual-frequency transducer element mounted on an IVUS catheter. As a result, this prototype IVUS system can operate both, in the fundamental frequency and in the second harmonic imaging modes. This system uses a conventional, continuously rotating, single-element IVUS catheter that is operated in fundamental 20 MHz, fundamental 40 MHz, and harmonic 40 MHz modes (transmit 20 MHz, receive 40 MHz). Imaging experiments were conducted in vivo in a tissue mimicking phantom and in an atherosclerotic rabbit model. The harmonic results of the imaging experiments demonstrated 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 and VVP imaging.
Recently, Chandran et al. [47] investigated the use of a focused broadband miniature polyvinylidene fluoride-trifluoroethylene (PVDF-TrFE) ultrasonic transducer for IVUS second-harmonic imaging. This study demonstrated that focused transducers are capable of producing second harmonics faster and stronger at specific depths. The experimental results were in agreement with the modeled results, and ex vivo experiments in human aorta images showed the feasibility of high resolution second-harmonic imaging.

3. DISCUSSION

Detection of vulnerable plaques, particularly those that are prone to rupture, is one of the most active areas of research in both the cardiology and biomedical imaging communities. Even though there are several invasive and non-invasive techniques such as angiography, thermography, magnetic resonance, optical coherence tomography, elastography, and IVUS, that have been used for the assessment of plaque vulnerability [48], none of them are able to completely identify a vulnerable plaque and accurately predict its...
future development. While CE-IVUS provide the means for the detection and quantification of extra-luminal blood perfusion, which may be an indication of VV and VVP, the existing methods that are used to analyze the data have certain limitations.

The main limitation of the differential imaging technique is that it requires the pre- and post-injection images to correspond to the same location within the vessel. However, due to the motions of the heart and the catheter, the exact correspondence cannot be guaranteed. While the proposed differential imaging technique provides methods for the stabilization of the sequences, the exact location and quantification of VV and VVP remains a challenge due to residual motion artifacts, which are difficult to eliminate. Moreover, an histological validation of the method is still pending. The clinical applications of harmonic imaging methods is limited due to the fact that these methods remain experimental and require non-commercially available IVUS hardware (e.g., harmonic imaging catheters).

The analysis of the IVUS RF signal, instead of the grayscale B-mode data, has been proposed for IVUS data analysis since the RF signal is not affected by the loss of information due to the B-mode transformations. The majority of the recent RF-based IVUS analysis methods rely on the use of machine learning techniques for the characterization of the plaque, and consist of the extraction of features from the RF signal combined with a supervised, semi-supervised or unsupervised classification method [49, 50, 51, 52, 53, 54]. This technique was used by O'Malley et al. [55], who studied the feasibility of using this approach for the characterization of blood and the eventual detection of extra-luminal blood. The limitations of this method are: (i) the assumption of exact alignment of the sequence and (ii) the lack of localization.

Currently, our group is working on the development of novel RF-based methods for blood detection that employ a physics-based scattering model of the interaction of the vessel components and the ultrasound signal. We tested the feasibility of this approach for the segmentation of the lumen contour using the RF-signal and obtained promising results [56]. Encouraged by this, we are now adapting the method to address the extra-luminal blood detection problem from IVUS and CE-IVUS data.

4. CONCLUSION

The in vivo detection and quantification of vasa vasorum and atherosclerotic plaque neovascularization in the coronary arteries remains an open problem. However, as new technologies emerge, it is very likely that it would be possible to assess the neangiogenesis in coronary arteries which will result in better diagnosis and treatment of coronary artery diseases.

ACKNOWLEDGMENTS

E.G. Mendizabal-Ruiz has been supported by scholarships from CONACYT. I.A. Kakadiaris has been supported in part by the NSF Grant DMS-0515242. Any opinions, findings, conclusions or recommendations expressed in this material are of the authors and may not reflect the views of the sponsors.

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