

Contrast-Enhanced Ultrasound Imaging of the Vasa Vasorum

From Early Atherosclerosis to the Identification of Unstable Plaques

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Proliferation of the adventitial vasa vasorum (VV) is inherently linked with early atherosclerotic plaque development and vulnerability. Recently, direct visualization of arterial VV and intraplaque neovascularization has emerged as a new surrogate marker for the early detection of atherosclerotic disease. This clinical review focuses on contrast-enhanced ultrasound (CEUS) as a noninvasive application for identifying and quantifying carotid and coronary artery VV and intraplaque neovascularization. These novel approaches could potentially impact the clinician's ability to identify individuals with premature cardiovascular disease who are at high risk. Once clinically validated, the uses of CEUS may provide a method to noninvasively monitor therapeutic interventions. In the future, the therapeutic use of CEUS may include ultrasound-directed, site-specific therapies using microbubbles as vehicles for drug and gene delivery systems. The combined applications for diagnosis and therapy provide unique opportunities for clinicians to image and direct therapy for individuals with vulnerable lesions. (J Am Coll Cardiol Img 2010;3:761–71) © 2010 by the American College of Cardiology Foundation

Atherosclerotic cardiovascular disease and death remain the leading cause of morbidity and mortality in the Western world (1). Therefore, it is increasingly important to identify “at-risk” individuals, referred to as “vulnerable” patients (2). Over the last few years, it has been

proposed that the use of noninvasive screening imaging systems provides safe and reliable methods for the early detection of surrogate markers of atherosclerosis, and helps to identify individuals exhibiting an unstable or vulnerable plaque (2,3).

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The list of current noninvasive imaging methods includes carotid ultrasound as well as cardiovascular computed tomography (CT) and cardiac magnetic resonance (CMR) (2,4,5). Furthermore, the use of ultrasound contrast agents is emerging as a potentially important diagnostic tool to complement and enhance routine, vascular ultrasound imaging (6). Importantly, contrast-enhanced ultrasound (CEUS) can visualize vasa vasorum (VV) in the adventitial layer as well as intraplaque neovascularization (7–12). These 2 microvascular networks are both thought to play a central role in the early process of plaque progression and vulnerability, and may also be involved in plaque inflammation (13,14).

Therefore, CEUS appears to be an emerging technique serving as a valuable method for the early detection of premature atherosclerosis and for the detection of vulnerable plaques in at-risk populations (7,8). This clinical review focuses on CEUS as a noninvasive application for identifying and quantifying VV and intraplaque neovascularization.

ABBREVIATIONS AND ACRONYMS

2D = 2-dimensional

3D = 3-dimensional

CEUS = contrast-enhanced ultrasound

c-IMT = carotid intima-media thickness

CMR = cardiac magnetic resonance

CT = computed tomography

IVUS = intravascular ultrasound

VV = vasa vasorum

Physiology of VV

Vasa vasorum are defined as small blood vessels that supply or drain the walls of the larger arteries and veins, and connect with a branch of the same vessel or a neighboring vessel to form a network of small blood vessels. These lesser known vessels are normally present and represent a physiologic mechanism designed to supply nutrients to the tissues within the arterial walls.

The extent and distribution of neovascularization within the arterial wall is dependent upon a number of physiologic “drivers.” Perhaps one of the leading candidates for the initiation of neovascularization focuses on vessel wall hypoxia and inflammation (15). Specifically, viable cells within an arterial wall located in excess of 250 to 500 μm from the lumen do not receive adequate nutrients; therefore, the adventitial VV supply an extrinsic source of nourishment (16). Based on substantial historical and clinical observations, there is evidence that supports the concept that the adventitial VV are integrally involved in the origins of atherosclerosis, so much so that the development and destabilization of atheromatous plaques may be linked to intraplaque hemorrhage and/or inflammation (13,17). It is believed that initially, hyperplasia of the adventitial VV occurs in the early phases of the inflammation/atherosclerotic process, whereas in advanced stages of

atherosclerosis, the appearance of new microvessels extends to the media and intima, constituting an ectopic neovascularization. Supporting the concept that VV appearance correlates with the extent of atherosclerosis, Moulton (18) reported a 9-fold increased incidence of intimal neovascularization in advanced atheromas of mouse aortas. Micro-CT images of atherosclerotic aortas showed that VV communicate with intraplaque capillaries and correlate with progression of atherosclerosis. Furthermore, Drinane et al. (19) found that inhibition of VV growth using truncated plasminogen activator inhibitor-1 protein in a similar mouse model leads to reduced atherosclerotic plaque growth. Similarly, neointimal formation (restenosis) after mechanical injury of a coronary artery in rats correlates with adventitial angiogenesis, and can be influenced by angiogenesis stimulators and inhibitors (20).

In an autopsy study, Fleiner et al. (14) identified that VV hyperplasia characterized vulnerable plaques in patients who suffered mortal cardiovascular events. Importantly, the authors noted that the presence of extensive hyperplasia of adventitial VV was observed in symptomatic individuals in advance of the development of significant intimal thickening, and that arterial ectopic neovascularization and inflammation in iliac, carotid, and renal arteries characterized the vulnerable patient associated with cardiovascular events.

Additionally, numerous clinical reports lend credence to the hypothesis that plaque neovascularization serves to discriminate active versus nonactive (vulnerable) plaques in symptomatic versus asymptomatic subjects. The presence of VV (angiogenic vessels) has been consistently observed in systemic arteries, including the aorta, coronaries, carotids, and the femoral arteries (13,14,17,21,22). Dunmore et al. (23) recently published their results advancing their previous reports in which they state that symptomatic carotid plaques contain abnormal, immature microvessels, and such vessels could contribute to plaque instability by acting as sites of vascular leakage and inflammatory cell recruitment.

Risk Factors for VV-Derived Neovascularization

A variety of local and systemic factors apparently influences the development of neovascularization in the artery wall, thus influencing the development of atherosclerosis and leading to subsequent destabilization of atheroma plaques. Cellular/tissue hypoxia acting as a local stimulus appears to be identified with the development of nascent VV. Recently,

Sluimer et al. (24) studied the presence of intraplaque hypoxia, the molecules involved, and the role of hypoxia in the better characterization of atheroma plaques. They identified that the presence of cellular hypoxia, notably within the plaques, was associated with a high level of macrophages. Cellular hypoxia correlated with the presence of thrombus, angiogenesis, expression of CD68, hypoxia inducible factor (HIF), and vascular endothelial growth factor. The mRNA and protein expression of HIF, its target genes, and microvessel density increased from early to stable lesions. These findings demonstrated the link between cellular/tissue hypoxia, atherosclerotic lesion progression, and intraplaque angiogenesis.

Additionally, hypercholesterolemia is a well-established and recognized cardiovascular risk factor and is associated with hyperplasia of the VV in the arterial wall. Kwon et al. (25) performed a series of experiments in which they used micro-CT to identify coronary artery VV in swine. In their study, using hypercholesterolemic swine, a dense and disorganized network of VV was created within the adventitia. Consistent with the clinical report of Fleiner et al. (14), these structural changes (i.e., increase in the VV network) appear to precede the development of endothelial dysfunction, often considered an initiating step in atherosclerosis development, as identified by Herrmann et al. (26). Wilson et al. (27) noted that initiating treatment with a lipid-lowering agent (statin) resulted in a substantial reduction in the observed coronary artery VV network. This statin effect occurred despite the absence of a significant change in the serum lipid profile. These observations support an additional mechanism for the vascular effects of lipid-reducing agents, which appears to act independently of overall cholesterol lowering.

More recently, Kolodgie et al. (28) reviewed emerging therapeutic strategies that appear to block neovascularization within the arterial wall. Intraplaque hemorrhage appears to result from the development of immature neointimal VV ("leaky" vessels). Therefore, the authors proposed that molecular therapies focused on the elimination of pathologic neovascularization within developing lesions reduce intraplaque hemorrhage. Apparently, the application of these therapeutic approaches provided positive treatment results in studies that involved similar neovascular-dependent diseases (including macular degeneration and malignancies) (29,30). On the basis of their results, the authors posited that a multitargeted approach involving

selective local antiangiogenic agents could contribute to prevention of plaque progression and its clinical consequences (28).

Noninvasive Imaging of VV in Experimental Models

Noninvasive imaging techniques provide a unique opportunity to monitor the serial, progressive pathophysiologic developments associated with experimental atherosclerosis in animal models. Often, mechanical approaches are combined with metabolic stimuli such as hypercholesterolemia or hyperglycemia, and/or physiological stimuli such as hypertension or a relatively hypoxic state to induce atherosclerosis and neovascularization experimentally (25,31-33). The 3 most promising noninvasive imaging strategies that are currently available are microscopic 3-dimensional (3D) CT, CMR, and CEUS.

As earlier noted, Kwon et al. (25) studied the 3D anatomy of the VV in early coronary atherosclerosis using microscopic CT in normal and experimental hypercholesterolemic porcine coronary arteries. Additionally, high-resolution CMR can be used in conjunction with a gadofluorine-based contrast agent for characterization of atherosclerotic plaques. Sirol et al. (31) studied aortic plaques in rabbits with contrast-enhanced CMR. Atherosclerotic plaque enhancement correlated with histopathological neovessel density. This may be related to the increased permeability of VV in atherosclerotic plaques, promoting exchange of contrast agent between the blood pool and the atherosclerotic plaque. Cornily et al. (32) reported similar findings with a novel gadolinium-based contrast agent and CMR. CMR showed a significant plaque enhancement in the atherosclerotic rabbit aortas that correlated with neovessel and macrophage density at histopathological examination. Hence, CMR can be used to quantify markers of plaque vulnerability, including neovascularization and macrophage content.

Real-time CEUS provides a unique opportunity for the evaluation of VV in living animal models. Ultrasound contrast agents consist of gas-filled microspheres that serve as true intravascular tracers and can be visualized using ultrasound as the microspheres transit the VV (8). Our group studied the development of atherosclerosis and density of the VV network in Rapacz familial hypercholesterolemia swine (33). Percutaneous transluminal injections of an atherogenic suspension were directly injected into the femoral arteries along with balloon-induced mechanical injury of the endothe-

lium. This combined approach led to the development of atherosclerotic lesions. The progression of the VV network was monitored by CEUS and later confirmed by histology (Fig. 1). In the future, this animal model in combination with CEUS may provide useful insights into the role of VV associated with atherosclerosis and may help to test novel pharmacological and device therapies for the treatment of atherosclerosis.

Noninvasive Imaging Techniques in the Clinical Setting

Carotid B-mode ultrasound. Measurement of carotid intima-media thickness (c-IMT) and determining the presence of focal atherosclerotic plaques using carotid ultrasonography did emerge as widely accepted surrogate markers of atherosclerosis (4,34,35). Numerous studies have shown that increased c-IMT and the presence of carotid plaque are associated with traditional coronary risk factors, and several clinical trials support the role of c-IMT measurements and carotid plaque assessments for predicting cardiovascular events (36-38). Accordingly, c-IMT has become a meaningful surrogate end point for interventional trials (39,40).

The c-IMT is defined as the distance between the lumen-intima interface and the media-adventitia interface (41). Today, c-IMT is most commonly measured in the common carotid artery on the far wall from B-mode (2-dimensional [2D]) images with linear ultrasound transducers typically utilizing frequencies between 7.5 and 10 MHz (34). The arterial wall segment should be assessed in a

longitudinal view, and semiautomated c-IMT measurement may be performed along a minimum of 10-mm length of the selected segment using an edge detection algorithm.

In addition to the c-IMT, discrete carotid plaques, commonly defined as a focal structure that encroaches into the arterial lumen of at least 50% of the surrounding c-IMT value or demonstrates a thickness of ≥ 1.5 mm as measured from the media-adventitial interface to the intima-lumen interface, can be assessed by through scanning of the extracranial carotid arteries (41). However, given the variable and complex 3D morphology of plaques, precise quantification of plaque burden using 2D B-mode ultrasound imaging is problematic (4). Furthermore, the use of ultrasound contrast agents provides direct information on arterial wall biology and inflammation through the detection of vessel wall angiogenesis (VV) (7).

CEUS. The clinical applications of CEUS for vascular use include enhancement of the carotid artery lumen, which results in improved visualization of luminal irregularities including soft plaques, dissections, and ulcerations (Fig. 2) (7,42,43). Moreover, CEUS techniques provide superior enhancement of the proximal walls, leading to improved efficiency and precision of measurements for the c-IMT (42). Based on the knowledge that the acoustically reflective microspheres serve as ideal intravascular tracers, the real-time, microvascular assessment of the spatial and temporal heterogeneity of adventitial and intraplaque VV was revealed (7,44). Subsequent to these pioneering observations, several independent reports similarly using CEUS techniques confirmed

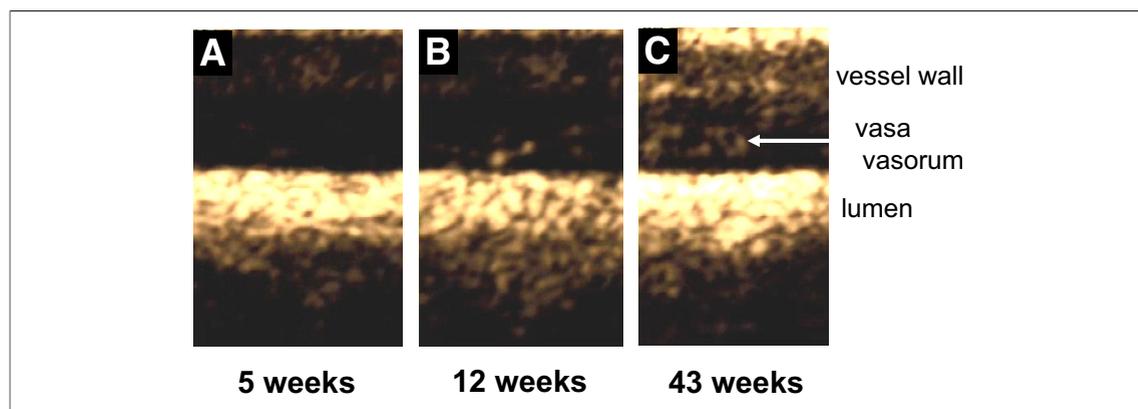


Figure 1. Progression of VV on CEUS

Contrast-enhanced ultrasound (CEUS) still frames demonstrating an increase of the density of the vasa vasorum (VV) network of the femoral artery during follow-up in a Rapacz hypercholesterolemia swine model of atherosclerosis. (A) demonstrates no VV at 5 weeks of follow-up, (B) shows limited VV at 12 weeks, and (C) shows extensive VV at 43 weeks after stimulation of atherosclerosis. The VV network is indicated by the white arrow. Reproduced, with permission, from Schinkel *et al.* (33).

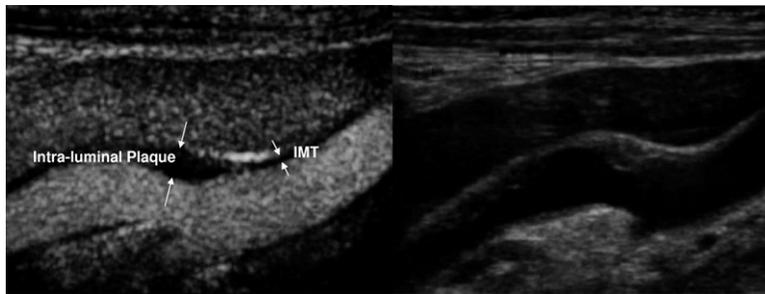


Figure 2. Contrast-Enhanced Carotid Ultrasound Imaging Without Intraplaque Neovascularization

Carotid artery with intraluminal plaque on B-mode ultrasound imaging (right panel). Corresponding artery on contrast-enhanced ultrasound (left panel). The intima-media complex (IMT) is depicted as a hypoechoic line. No visible microbubbles are seen within the intraluminal plaque.

the initial discoveries, including the observations that CEUS permitted direct visualization of adventitial VV and intraplaque angiogenesis (Fig. 3) (9–12,45–48).

A variety of commercially available ultrasound contrast agents have been used for VV imaging, including perflutren protein type-A microspheres (Optison, GE Healthcare, Little Chalfont, Buckinghamshire, UK), perflutren lipid microspheres (Definity, Bristol-Myers Squibb Medical Imaging, Billerica, Massachusetts), and phospholipid-stabilized microspheres of sulfur hexafluoride (SonoVue, Bracco Altana Pharma, Konstanz, Germany) (6). Typically, these agents are routinely injected via a peripheral vein as a bolus, followed by a saline flush. CEUS imaging of the carotid artery is generally practiced using a linear array vascular probe with transmission frequencies ranging from 4 to 10 MHz and dedicated contrast imaging software utilizing pulse inversion or harmonic techniques. Of importance, the mechanical index used

for CEUS in carotid vascular clinical studies was lowered and ranged between 0.06 and 0.2; all settings were considerably reduced as compared with the mechanical index used in unenhanced studies. Therefore, when using a reduced mechanical index for imaging, the plaques and corresponding intima-media complex appear hypoechoic, whereas the adventitial layer is observed as echogenic (Fig. 2). The dynamic flow patterns of the vasculature are represented by the presence of intravascular tracers (acoustic microspheres), which pass unhindered through the adventitial and the intraplaque VV (Fig. 3).

Quantification of VV and Intraplaque Neovascularization

CEUS. Quantification of the VV remains a major issue confronting the future development and implementation of CEUS as a clinically useful imag-

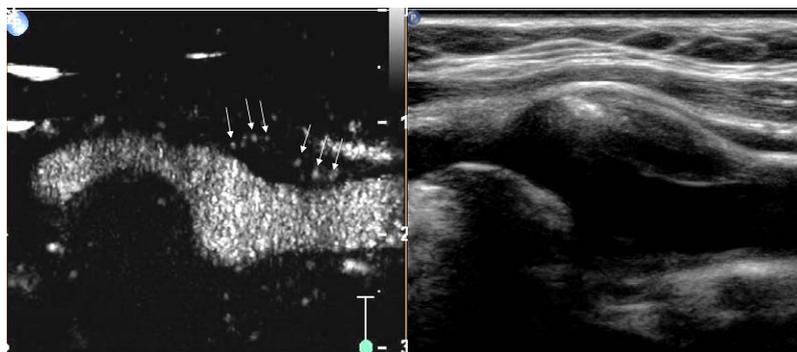


Figure 3. Carotid Artery With Intraplaque Neovascularization on CEUS

Plaque at the origin of the internal carotid artery on B-mode ultrasound imaging (right panel). Corresponding artery on contrast-enhanced ultrasound (CEUS) (left panel) with visible microbubbles within the plaque (arrows).

ing technique. Clearly, the advent of 3D/4-dimensional volumetric imaging will provide the necessary foundation for the detection and quantification of angiogenesis within the vascular systems (adventitial and intraplaque VV). Further, the utility of providing a volumetric assessment of tumor blood volume will promote the future diagnostic and therapeutic clinical utility of CEUS.

Currently, all the observational studies that use CEUS for VV detection and quantification employ 2D ultrasound imaging and semiquantitative approaches (Table 1) (6,10,45,47,48).

Coli et al. (11), using a clinically accepted dichotomous grading system, categorized CEUS enhancement as no contrast effect or high based on the visual detection of the contrast effect within the plaque (Figs. 2 and 3). A similar grading system was applied by other researchers in their studies (10,12,47).

Subsequent authors provided additional quantitative approaches, including a case report by Papaioannou et al. (46) in which the authors quantified mean gray-level and entropy measurements in the plaque region. The authors demonstrated that following an intravenous injection of ultrasound contrast agents, there exist quantifiable alterations in the reflectivity of the carotid plaque obtained using harmonic software. Similarly, clinical researchers from China described the enhancement of carotid plaques after injection of contrast material using signal-time intensity curve analysis. The region of interest was established based on visual recognition and a semiquantitative categorization for each plaque (48).

Two recent studies using a systematic, histopathological validation revealed a direct, positive correlation between contrast enhancement and histology (Table 1) (10,11). In a case report, Vicenzini et al. (45) described visualization of contrast-agent microbubbles within the plaques as a marker of neovascularization and confirmed, corresponding to the CEUS image, the presence of the microvessel within the plaque at histology after endarterectomy.

Additional semiquantitative measurement of VV was reported by Magnoni et al. (9). In their work, the authors performed a quantitative determination of adventitial VV by measuring periadventitial signal thickness using contrast-enhanced, B-flow imaging.

Thus, although there is independent confirmation and clinical consensus regarding the clinical uses of CEUS for identifying adventitial and intraplaque VV in patients, a true, quantifiable volumetric analysis of VV remains elusive. Clearly, as CEUS imaging moves into the future, the ability to

perform an analysis of tumor blood flow (including carotid atherosclerotic plaques) remains a critical component for both diagnostic and therapeutic applications.

VV: Intravascular ultrasound (IVUS). Imaging of the carotid VV using CEUS is a promising development. Imaging of the VV of coronary arteries using transthoracic ultrasound seems a bridge too far. With limited resolution and associated motion artifact, these impediments limit the potential to achieve an acceptable contrast-to-tissue ratio for VV coronary imaging.

With the recent interest in VV of the carotid arteries using CEUS, there is accompanying interest in developing an IVUS application for measurement of coronary VV. IVUS systems generally operate at acoustic frequencies in the range of 20 and 50 MHz. Although these parameters provide high spatial resolution (~ 150 to $300 \mu\text{m}$) with cross-sectional images of the lumen and walls of larger blood vessels, these specifications may not provide optimization of the harmonic contrast effect resulting in visualization of the coronary artery VV. Known clinically, the applications of IVUS include assessment of atherosclerotic coronary lesions, site guidance for stent deployment, and monitoring atherosclerosis lesions for clinical trials. In order to accommodate the acoustic parameters associated with CEUS, ultrasound contrast agents have primarily been designed to be used at low frequencies, from 1 to 5 MHz. Despite conventional belief to the contrary, Goertz et al. (49) have shown that it is possible to identify CEUS signals at the higher frequencies used specifically for IVUS.

Carlier et al. (50) reported that bolus injections of contrast agents could give rise to IVUS echogenicity enhancement in the adventitia of coronary arteries, consistent with the detection of VV. However, this approach uses linear IVUS, is restricted to a single imaging plane, and is critically sensitive to the assumption that images acquired at the same point of successive cardiac cycles are not affected by tissue motion. Small deviations from this will result in significant artifact enhancement of the signal.

Harmonic IVUS seems a more fruitful approach (51). Conventional IVUS elements have a bandwidth that is too limited for optimal harmonic imaging though. A new generation of IVUS elements based on dual resonance layers, capacitive micromachined ultrasound transducers, single crystal technology, or composite transducers is under development (52). Granada and Feinstein (8) showed enhanced adventitial signal in coronary

Table 1. Overview of Clinical Studies Using CEUS Imaging for Assessment of Carotid Artery VV

Reference (No.)	No. of Patients/ Plaques	Population	Vasa Vasorum Quantification on CEUS	Vasa Vasorum Quantification on Histology	Main Results
Shah et al., 2007 (10)	15/17	Age NR, 59% male, 6 symptomatic (TIA/stroke)	Intraplaque neovascularization grade 0 (not present), grade 1 (limited), grade 2 (moderate), and grade 3 (pulsating arterial vessel)	17 CEA specimens, neovascularization (CD31, CD34, vWF) grade 0 (none), grade 1 (minimal), grade 2 (moderate), and grade 3 (extensive)	Neovascularization on CEUS correlates with histology (Spearman rank: 0.68)
Vicenzini et al., 2007 (45)	23/NR	Age 70 ± 3.5 yrs, 70% male, asymptomatic for stroke/TIA and MI	Intraplaque neovascularization present/not present	1 CEA specimen, microvessel within the plaque (descriptive)	Neovascularization on CEUS correlates with plaque echogenicity and corresponds to histology
Coli et al., 2008 (11)	32/52	Age 70 ± 8 yrs, 84% male, 6 symptomatic (stroke/TIA within 6 months)	Intraplaque neovascularization grade 1 (not present) and grade 2 (present)	17 CEA specimens, no. of VV/mm ² (CD31, CD34)	Intraplaque neovascularization correlates with histology (VV density 3.24/mm ² in plaque grade 2 vs. 1.82/mm ² in grade 1; p = 0.005), and with plaque echogenicity (higher degree of contrast enhancement in echolucent plaques, p = 0.001)
Magnoni et al., 2008 (9)	40/NR	25 with carotid atherosclerosis (age 65.5 ± 12 yrs, 80% male), 15 control without stenosis (age 71 ± 8 yrs, 77% male)	Quantitative analysis of adventitial VV (periadventitial flow signal thickness using contrast-enhanced, B-flow imaging)	NR	Adventitial VV is more pronounced in the atherosclerotic group than in the control group (1.10 ± 0.11mm vs. 0.80 ± 0.06 mm; p < 0.0001); adventitial VV correlates with c-IMT (r = 0.88; p < 0.0001)
Papaioannou et al., 2009 (46)	1/1	73-year-old male with acute coronary syndrome	Quantitative analysis of intraplaque neovascularization by plaque gray-scale level and entropy	NR	Increased plaque gray level and entropy after contrast agent injection, indicating intraplaque neovascularization
Giannoni et al., 2009 (47)	77/73	Age 67 ± 6 yrs, 66% male, 64 asymptomatic, 9 symptomatic (stroke/TIA in previous 6 months)	Intraplaque neovascularization, type I (rare discrete), type II (diffuse contrast enhancement)	73 CEA specimens, neovascularization (VEGF, MMP3, CD31, CD34)	Neovascularization type II in 1/64 asymptomatic plaques, and in 9/9 symptomatic plaques (p < 0.001); neovascularization on CEUS corresponds to histology (high density of microvessels)
Xiong et al., 2009 (48)	104/104	Age 63 ± 9 yrs, 80% male, 35 symptomatic (stroke/TIA), 69 asymptomatic	Intraplaque neovascularization grade 1 (not present) and grade 2 (present), and quantitative measurement by signal intensity analysis	NR	Neovascularization grade 2 in 80% of symptomatic vs. 30% in asymptomatic patients (p < 0.001); enhanced intensity in the plaque is greater in symptomatic than asymptomatic patients (13.9 ± 6.4 dB vs. 8.8 ± 5.2 dB; p < 0.001)
Staub et al., 2010 (12)	147/111	Age 64 ± 11, 61% male, 89 with cardiovascular disease and 37 with history of cardiovascular events (MI, stroke/TIA)	Adventitial VV grade 1 (not present) and grade 2 (present), intraplaque neovascularization grade 1 (not present) and grade 2 (present)	NR	Adventitial VV (grade 2) is associated with cardiovascular disease (OR: 2.3, 95% CI: 1.1-4.8), and intraplaque neovascularization (grade 2) is associated with history of cardiovascular events (OR: 4.0, 95% CI: 1.3-12.6)

CEA = carotid endarterectomy; CEUS = contrast-enhanced ultrasound; CI = confidence interval; MI = myocardial infarction; MMP3 = matrix metalloproteinase 3; NR = not reported; OR = odds ratio; TIA = transient ischemic attack; VEGF = vascular endothelial growth factor; VV = vasa vasorum; vWF = von Willebrand factor.

arteries in a swine model for atherosclerosis using harmonic IVUS approach.

Goertz et al. reported detailed detection and localization of VV in an atherosclerotic rabbit aorta model using a dual-frequency IVUS element and pulse inversion followed by filtering out the harmonic (53) or subharmonic (54) signals. Figure 4 shows an example of VV detection in this model.

IVUS VV detection is not ready for clinical use yet. Quantification and a thoughtful strategy for 3D reconstruction of the VV remain to be developed. In the near future, perhaps, IVUS imaging of the coronary VV could serve as an additional biomarker of plaque vulnerability along with gray-scale analysis, plaque burden assessment, quantitative flow assessment, and radio frequency-based tissue typing (virtual histology) and palpography (55).

Comparative imaging modalities of VV. A plethora of reports have described the applications of other non-CEUS imaging modalities for visualization of VV and associated atherosclerosis; these include CMR, CT, positron emission tomography, single-photon emission CT, and hybrid technologies (5,56) along with molecular imaging techniques (57,58).

Kerwin et al. (59,60) analyzed carotid atherosclerotic plaques using dynamic contrast-enhanced CMR for adventitial VV and intraplaque neovascularity in patients scheduled for endarterectomy and in subjects with moderate carotid atherosclerotic disease. The extent of neovascularization within the plaque was quantified using a kinetic model to estimate fractional blood flow (59) and transfer constant (60) based on changes in signal

intensity over time after gadolinium-based contrast agent injection. Because the transfer of the contrast agent to the adventitia is dependent on the VV, they concluded that adventitial transfer constant likely indicates the extent of the VV. Evidence for this hypothesis was provided by the fact that quantitative analysis of VV neovascularization was significantly correlated with the histological fraction of microvessel density and macrophages (60). Furthermore, the extent of plaque neovascularization determined by CMR imaging was positively correlated with serum marker of inflammation linked to atherosclerotic complications, and was associated with more advanced plaques. In line with these findings, Lombardo et al. performed contrast-enhanced carotid CMR in patients with acute coronary syndrome and in controls with carotid atherosclerosis (61). In these subjects, all with acute coronary syndromes, an enhancement with gadolinium indicated increased neovascularization was noted in 89% of carotid plaques, whereas only 8% revealed similar enhancement in the control group. These studies support the concept that plaque neovascularization is associated with vulnerability and represent a uniformly distributed marker of inflammation throughout the systemic vascular bed (14).

Furthermore, newer molecular imaging technologies using targeted contrast agents have been emerged for inflammation imaging in atherosclerosis (5). Specifically, for CMR and contrast ultrasound molecular imaging, different sophisticated contrast agents, such as paramagnetic nanoparticles targeted to $\alpha_v\beta_3$ -integrin that are selectively up-

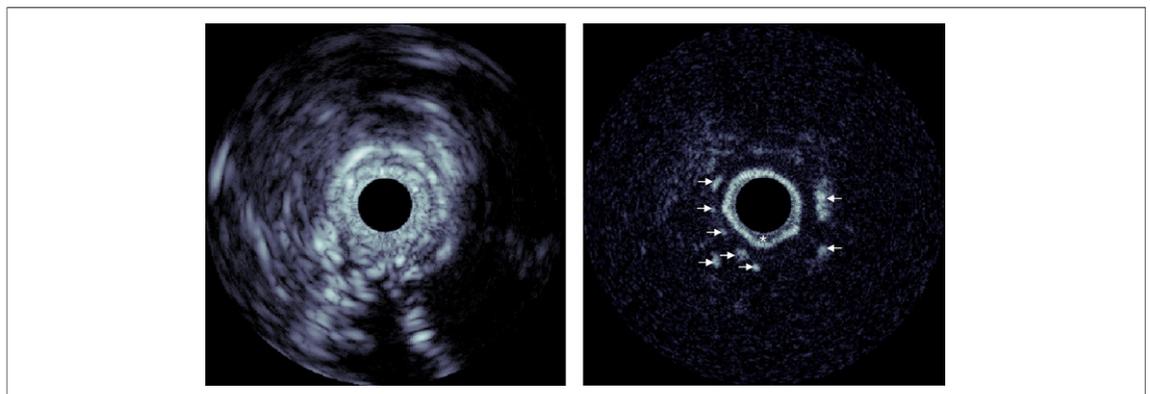


Figure 4. In Vivo Results in an Atherosclerotic Rabbit Aorta Using Decanted Definity

Left: fundamental mode at 20 MHz, 10 s after injection, in which changes in adventitial enhancement are not evident. **Right:** at 10 s after injection, the harmonic mode (transmit at 20 MHz, receive at 40 MHz) shows significant adventitial enhancement, consistent with the detection of adventitial microvessels. The white dots are contrast agents in the vasa vasorum (white arrows), and the bright ring, contrast agents attached to the luminal border (asterisk). Scale of images is 12 mm across. The dynamic range of the fundamental and harmonic image is 40 and 25 dB, respectively.

regulated by the neovascular endothelium (62), as well as $\alpha_v\beta_3$ -integrin-targeted microbubbles and microbubbles targeted to growth factor receptors expressed during vascular remodeling (58), have been evaluated in animal models for imaging atherosclerotic and tumor angiogenesis. Furthermore, imaging-guided therapy of atherosclerosis has been evaluated using the combination of magnetic resonance molecular imaging and antiangiogenic drug delivery with $\alpha_v\beta_3$ -integrin-targeted paramagnetic nanoparticles in cholesterol-fed rabbits (63). A single application of a minimal drug dosage resulted in a significant reduction of neovasculature within the arteriosclerotic aortic wall assessed by magnetic resonance enhancement and histology. However, none of these techniques have been advanced to evaluate VV neovascularization in clinical studies.

Clinical impact: VV

Adventitial and intraplaque VV remain as distinct, microvascular nutrient networks intimately associated with vessel wall diseases and plaque vulnerability (13). Therefore, the implications of detecting adventitial and ectopic intraplaque VV using CEUS imaging in at-risk patients is an important clinical goal.

Histologic analyses and observational clinical studies support the concept that hyperplasia of adventitial VV represents a viable surrogate marker of atherosclerosis that likely presages untoward cardiovascular events (14). Based upon the current literature, pronounced enhancement of periadventitial VV associated with CEUS imaging appears to define carotid atherosclerosis and symptomatic cardiovascular disease (Table 1).

Different independent investigators reported that more pronounced neovascularization assessed by CEUS was predominantly observed in echolucent lesions (11,45,48). Importantly, the presence of an echolucent plaque is known to be associated with an increased risk of cardiovascular events (64,65). These observations are wholly consistent with the concept that more vulnerable plaques contain a higher degree of neovascularization.

Additionally, recently published observational studies revealed the association between plaque enhancement on CEUS imaging and clinical symptoms in patients with carotid atherosclerosis (12,47,48). Giannoni et al. (47) described that symptomatic patients showed a specific pattern of diffuse contrast enhancement on CEUS at the base of the carotid plaque, and a correspondingly high density of microvessels on subsequent histology.

Another independent research group used CEUS to assess carotid plaques in patients associated with neurological symptoms (48). An increased prevalence of carotid plaque enhancement was observed in symptomatic patients when compared with asymptomatic patients. These findings support the concept that the VV-derived intraplaque neovascularization is associated with plaque instability, perhaps leading to clinical events.

Recently, the current authors performed an analysis of consecutive subjects who received carotid CEUS. The presence and degree of CEUS for adventitial and intraplaque VV was significantly correlated with the presence of cardiovascular disease and with prior cardiovascular events (12). This recent observational study indicated that the risk of a cerebrovascular event assessed from carotid plaque angiogenesis is generalizable to other vascular beds, including coronary events.

Based on these retrospective studies and with appropriate prospective studies, CEUS examination of the carotid artery may provide a novel, noninvasive clinical tool to identify and quantify the presence and the extent of the VV network and arterial plaque neovascularization in patients at risk for developing symptomatic atherosclerosis, permitting a more reliable assessment of cardiovascular risk. However, further prospective studies will be required to establish the scientific basis of using CEUS for the early detection of premature atherosclerosis and for the detection of vulnerable plaques in at-risk patients.

At the present time, continued experimental data in appropriate animal models of hypercholesterolemia (27) and cross-sectional studies of carotid endarterectomy specimens (66) have demonstrated that VV and intraplaque angiogenesis can be reduced by antiatherosclerotic therapies. Therefore, quantification of VV and plaque neovascularization by CEUS imaging may provide an excellent noninvasive technique to monitor therapeutic interventions. In the future, the therapeutic use of ultrasound-directed, site-specific therapies with microbubbles for drug and gene delivery could potentially provide a unique opportunity for clinicians to directly image and treat a vulnerable atherosclerotic lesion (67,68).

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Key Words: vasa vasorum ■ contrast-enhanced ultrasound ■ atherosclerosis ■ surrogate marker.