Journal of the American College of Cardiology
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Published by Elsevier Inc.

Vol. 52, No. 3, 2008
ISSN 0735-1097/08/$34.00
doi:10.1016/j.jacc.2008.02.082

Contrast-Enhanced Ultrasound Imaging of Intraplaque Neovascularization in Carotid Arteries

Correlation With Histology and Plaque Echogenicity

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Objectives This study was designed to evaluate contrast-enhanced ultrasound imaging of carotid atherosclerosis as a clinical tool to study intraplaque neovascularization.

Background Plaque neovascularization is associated with plaque vulnerability and symptomatic disease; therefore, imaging of neovascularization in carotid atherosclerosis may represent a useful tool for clinical risk stratification and monitoring the efficacy of antiatherosclerotic therapies.

Methods Thirty-two patients with 52 carotid plaques were studied by standard and contrast-enhanced ultrasound imaging. In 17 of these patients who underwent endarterectomy, the surgical specimen was available for histological determination of microvessel density by CD31/CD34 double staining. Plaque echogenicity and degree of stenosis at standard ultrasound imaging were evaluated for each lesion. Contrast-agent enhancement within the plaque was categorized as absent/peripheral (grade 1) and extensive/internal (grade 2).

Results In the surgical subgroup, plaques with higher contrast-agent enhancement showed a greater neovascularization at histology (grade 2 vs. grade 1 contrast-agent enhancement: median vasa vasorum density: 3.24/mm^2 vs. 1.82/mm^2, respectively, $p = 0.005$). In the whole series of 52 lesions, echolucent plaques showed a higher degree of contrast-agent enhancement ($p < 0.001$). Stenosis degree was not associated with neovascularization at histology or with the grade of contrast-agent enhancement.

Conclusions Carotid plaque contrast-agent enhancement with sonographic agents correlates with histological density of neovessels and is associated with plaque echolucency, a well-accepted marker of high risk lesions, but it is unrelated to the degree of stenosis. Contrast-enhanced carotid ultrasound imaging may provide valuable information for plaque risk stratification and for assessing the response to antiatherosclerotic therapies, beyond that provided by standard ultrasound imaging. (J Am Coll Cardiol 2008;52:223–30) © 2008 by the American College of Cardiology Foundation

Vasa vasorum are physiological structures that provide nourishment to the vessel wall and play an important role in both early and advanced stages of atherosclerosis. They are normally present in the adventitia of most muscular and conduit arteries and extend into the outer layer of the media in larger vessels. The concept that vasa vasorum are involved in the pathophysiology of atherosclerosis dates back to the work of Köester (in 1876) (1), Winternitz (in 1938) (2), and was revived by Barger and colleagues (in 1984) (3), who clearly showed in post-mortem samples that coronary atherosclerotic segments presented a rich vascular network extending from the adventitia to the full thickness of media and intima.

In animal models, hyperplasia of vasa vasorum is an early event of hypercholesterolemia-induced atherosclerosis and appears to precede endothelial dysfunction (4). Accordingly, human pathological studies show neovascularization already in type II and, more prominently, in type III atherosclerotic plaques (according to the American Heart Association classification) (5). Experimental studies also indicate that neovascularization is necessary for plaque development, as angiogenesis inhibitors can reduce plaque growth (6).

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Manuscript received December 12, 2007; revised manuscript received February 8, 2008, accepted February 12, 2008.

Abbreviations and Acronyms

ICA = internal carotid artery

IMT = intima media thickness

Plaque neovessels originate mainly from the adventitia, less often from the main vessel lumen (7), and extension of vasa vasorum to the full thickness of the media and intima of atherosclerotic segments represents pathological neovascularization.

Several pathological studies showed that a more extensive plaque neovascularization is associated with features of plaque vulnerability and with clinically symptomatic disease (8–10).

The mechanisms by which vasa vasorum contribute to the development of plaque instability may be their role in leukocyte recruitment and plaque hemorrhage. Endothelial cells in plaque neovessels express more adhesion molecules than those in the main arterial lumen, which favor leukocyte recruitment (11). Moreover, these microvessels are immature and fragile and thus prone to rupture and hemorrhage, which promote plaque instability and represent an important source of free cholesterol from red blood cells membranes, with consequent macrophage infiltration and necrotic core enlargement (12).

These findings stimulate the search for techniques suitable for the study of human plaque neovascularization in vivo. Particularly, direct imaging of vasa vasorum may allow the assessment of the response to antiatherosclerotic therapies and may improve carotid plaque risk stratification. Magnetic resonance imaging with gadolinium infusion has been employed in humans to evaluate carotid plaque vasa vasorum, with a good agreement with histologic findings (13). More recently, contrast-enhanced ultrasound imaging has been proposed for imaging of carotid vasa vasorum (14), but no comprehensive studies have yet been published. This approach takes advantage of the high spatial and temporal resolution of vascular ultrasound imaging and of the properties of contrast-agent microbubbles, which behave as pure intravascular tracers (15). Therefore, we compared direct visualization of neovascularization of carotid plaques by contrast-enhanced ultrasound imaging with histological findings subsequently obtained in surgical carotid specimens. We also correlated plaque neovascularization, assessed by contrast-enhanced ultrasound imaging, with standard ultrasound imaging plaque characterization in terms of echogenicity and degree of stenosis, including a larger series of carotid lesions not subjected to endarterectomy.

Methods

Patient population and study protocol. Between April and October of 2005, we enrolled 32 patients (27 males, age 69.9 ± 8.1 years) in the study. The patients were selected from those admitted to the coronary care unit or to the vascular surgery unit, who had a clinical indication to standard carotid ultrasound imaging. The examination was requested either because they were referred for carotid endarterectomy or screened for carotid atherosclerosis after admission for ischemic heart disease. Inclusion criteria were at least 1 carotid atherosclerotic stenosis >30%, age >18 years, and ability to provide an informed consent. Exclusion criteria were hypersensitivity to albumin, blood-derived products, or to the ultrasound imaging contrast agent; severe pulmonary hypertension; possible pregnancy; previous carotid surgery or angioplasty; and poor quality of the standard ultrasound imaging study.

Carotid color Doppler ultrasound was recorded in all study patients, and then contrast-enhanced ultrasound imaging was performed by 2 of the researchers (S.C., M.M.). In the enrolled patients who had a clinical indication for carotid surgery, the surgical specimens were also collected. The protocol was approved by our local ethical committee and all patients provided an informed consent.

Standard and contrast-enhanced carotid ultrasound imaging. Imaging was performed with a GE-Vivid 7 ultrasound machine (GE Healthcare, Chalfont St. Giles, United Kingdom), using a 7L probe, for both standard and contrast-enhanced studies. First, the carotid bifurcation was imaged bilaterally by B-mode ultrasound, color Doppler, and pulsed-wave Doppler, and the examination was digitally stored for later review. Special care was taken to image and record all distinct plaques seen at each side.

The patients were then submitted to contrast-enhanced ultrasound imaging, with special attention to the previously identified lesions. The preset real-time, contrast-specific imaging modality (pulse inversion) was switched on and image settings adjusted to maximize contrast signal visualization. A low mechanical index was employed (0.08 to 0.10). Perfluoropropane-filled albumin microspheres (Optison, GE Healthcare) were used as a contrast agent. A vial of Optison was diluted with saline up to 10 ml (Optison 3 ml + saline 7 ml) and then a 2-ml bolus was injected and repeated as needed. Good images could be obtained for about 2 min after each bolus. For a single examination, no more than 2 vials of Optison were used. The studies were digitally stored for later analysis. The patients were observed for 30 min before returning to their wards.

Standard and contrast-enhanced images were reviewed offline by 2 readers (S.C., M.M.).

Each visible plaque was classified in terms of echogenicity at standard imaging, according to a widely used classification scheme (16): class I: uniformly echolucent, class II: predominantly echolucent, class III: predominantly echogenic, class IV: uniformly echogenic, and class V: extensive calcification with acoustic shadowing. The degree of stenosis determined by a plaque was measured according to current guidelines (17).

In contrast-enhanced images, all the plaques appear dark and hypoechoic (because of tissue signal suppression), and the adventitia still appears as a bright echogenic line. Moving bright spots within the plaque or on its adventitial side were considered to represent the contrast agent's bubble signal coming from plaque neovascularization. On the contrary, fixed echogenic spots were considered to be strong tissue acoustic