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● *Original Contribution*

QUANTIFICATION OF CAROTID PLAQUE NEOVASCULARIZATION USING CONTRAST-ENHANCED ULTRASOUND WITH HISTOPATHOLOGIC VALIDATION

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Abstract—We sought to evaluate contrast-enhanced ultrasound (CEUS) imaging for the quantification of carotid plaque neovascularization. Seventeen patients underwent carotid endarterectomy after standard ultrasound and CEUS. Semiquantitative and quantitative analyses of contrast enhancement within the plaque were performed using a visual interpretation scale and quantitative analysis software, respectively. Enhancement intensity (dB) was measured at the plaque (EI_{plaque}). Each specimen was stained with CD34 and CD68 to assess for microvessels and macrophages, respectively. Semiquantitative CEUS analyses were correlated with neovascularization at histology ($r = 0.70$, $p = 0.002$). Quantitative analysis was also correlated with neovascularization at histology (EI_{plaque} $r = 0.81$, $p < 0.001$). EI_{plaque} ($r = 0.64$, $p = 0.01$) was correlated with the degree of enhancement as assessed visually. Semiquantitative and quantitative analyses were not correlated with macrophage infiltration at the plaque. Contrast enhancement in the carotid plaque was correlated with neovascularity at the histopathologic exam. Furthermore, semiquantitative and quantitative measurements were highly correlated with each other, suggesting that either can be used to detect intraplaque neovascularization. (E-mail: Puguang61@126.com) © **2014 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Carotid atherosclerosis, Neovascularization, Contrast, Ultrasound, Quantitative analysis.**

INTRODUCTION

Intraplaque neovascularity and adventitial vasa vasorum hyperplasia are important features related to vulnerable atherosclerotic plaques (Kumamoto et al. 1995; Naghavi et al. 2003). Histologic studies have found that intraplaque neovascularization is associated with plaque instability, which may lead to cerebrovascular events in patients (Kumamoto et al. 1995; McCarthy et al. 1999; Moreno et al. 2004; Naghavi et al. 2003). These findings have led to the use of non-invasive imaging techniques for the early detection of high-risk lesions.

Ultrasound (US) is one of the most widely used imaging modalities for evaluating the carotid artery, and contrast-enhanced US (CEUS) is increasingly being used to evaluate intraplaque neovascularization in carotid atherosclerotic lesions (Feinstein 2006). Studies have found that the grades of contrast enhancement in semiquantitative analysis of CEUS are correlated with histologic results and clinical events (Coli et al. 2008; Giannoni et al. 2009; Magnoni et al. 2009; Staub et al. 2010, 2011; Vicenzini et al. 2007). Other research has indicated that grade of contrast enhancement is correlated with microvessel density (MVD) at histology (Coli et al. 2008; Shah et al. 2007). More recently, quantitative evaluation with CEUS performed with a specially designed algorithm programmed in MATLAB (The MathWorks, Natick, MA, USA) (Hoogi et al. 2011) indicated that contrast enhancement was well correlated with neovascularization in the plaque at histology.

We performed quantitative analysis of CEUS with commercially available software (QLab software, Philips Healthcare, Bothell, WA, USA) and correlated these

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results with findings from histopathologic examination. We also compared semiquantitative and quantitative analyses of CEUS results to investigate whether the neovascularization in the atheromatous plaque can be fully quantitatively analyzed with CEUS.

METHODS

Patients

This study was approved by our institutional review board, and informed consent was obtained from all patients before the examination. Retrospective review of US and vascular surgery databases was used to identify consecutive patients who had undergone both CEUS and carotid endarterectomy at a single tertiary care center. From April 2009 to March 2012, a total of 135 patients (119 men, 16 women; age range, 47–87 y; mean age, 69.1 ± 8.3 y) underwent carotid arterial CEUS examinations, and bilateral atherosclerotic lesions were detected in 32 patients. Therefore, a total of 167 carotid arteries and plaques were documented and analyzed. Seventeen of these patients (16 men, 1 woman) subsequently underwent carotid endarterectomy. Patients were referred for carotid US examination because of underlying significant cardiovascular diseases such as stroke, transient ischemic attack, hypertension, coronary heart disease and arteriosclerosis occlusive disease of the lower extremities. All patients who underwent carotid endarterectomy also had undergone carotid arteriography before surgery; results of the latter were used to grade the stenosis and determine whether the patient was a candidate for surgery. Percentage stenosis was determined by North American Symptomatic Carotid Endarterectomy Trial criteria (NASCET Collaborators 1991). Patients with a history of heart failure, myocardial infarction or unstable angina were not eligible to undergo CEUS, as these conditions are contraindications to the use of ultrasound contrast agent (UCA).

Standard US and CEUS examinations

All CEUS examinations were performed after routine US examinations. US examinations were performed with an iU22 scanner (Philips Healthcare) equipped with a linear-array L9-3 probe and pulse inversion harmonic imaging software. All conventional US and CEUS examinations were performed according to our protocol by fellowship-trained radiologists with special training in performing carotid US examinations. When CEUS was performed, the mechanical index was set at 0.07, and the gain was set at 95%. The focal zone was positioned at a depth of 2 to 3 cm depending on the size of the carotid artery. Time gain compensation was adjusted to obtain a homogeneous image of the carotid artery while reducing the noise from the arterial wall. These values were preset for examination of the carotid artery.

With conventional US, the carotid bifurcations and internal carotid arteries were examined in longitudinal and transverse projections. If plaques were identified, thickness was measured on a transverse scan, and location and echogenicity were documented. In patients with more than one plaque, only the thickest plaque underwent further examination with CEUS.

Contrast-enhanced ultrasound was performed in longitudinal projections after the target plaques were localized. SonoVue (Bracco, Milan, Italy), a suspension of phospholipid-stabilized sulfur hexafluoride (SF_6) microbubbles, was used as the contrast agent. For each injection, the patient received a bolus dose of 2 mL UCA through a peripheral intravenous line, followed by a 5-mL normal saline flush. The amount of time the contrast was present in the target lesion was recorded. The probe was held steady over the target lesion for 2 min during the CEUS examination. A real-time US imaging cine-loop was digitally stored for further off-line analysis and quantification.

Image interpretation and quantification analysis

Plaque echogenicity on standard US images was classified as follows: type I = predominantly echolucent plaques; type II = predominantly echogenic plaques; type III = mixed plaques containing mixed high-, medium- and low-level echoes; and type IV = extensively calcified plaques (Arnold et al. 1999; De Bray 1997).

On CEUS images, carotid plaques are hypo-echoic and the adventitial layer is hyper-echoic with a low mechanical index setting and harmonic technique. The presence of dynamic movement of the echogenic reflectors (UCA bubbles) within the plaque or on the adventitia was defined as evidence of plaque neovascularization, whereas fixed echogenic signals were considered to be tissue acoustic reflectors (Coli et al. 2008).

Semiquantitative and quantitative analyses were performed off-line. For semiquantitative analysis, evidence of neovascularization was interpreted by two experienced independent radiologists who were involved in the US and CEUS examinations and were blinded to patient history. The contrast enhancement in each plaque was categorized on a visual scale as follows: grade 0 = no bubbles within the plaque or bubbles confined to the adjacent adventitial side (Fig. 1a); grade 1 = moving bubbles confined to the adventitial side (Fig. 1b); grade 2 = moving bubbles at the plaque shoulder (Fig. 1c); grade 3 = bubbles moving to the plaque core (Fig. 1d); and grade 4 = extensive intraplaque enhancement (Fig. 1e) (Coli et al. 2008; Huang et al. 2010; Staub et al. 2011). In cases of disagreement between investigators, a consensus was reached with a third radiologist.