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● *Original Contribution*

NEW QUANTIFICATION METHODS FOR CAROTID INTRA-PLAQUE NEOVASCULARIZATION USING CONTRAST-ENHANCED ULTRASOUND

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Abstract—As carotid intra-plaque neovascularization (IPN) is linked to progressive atherosclerotic disease and plaque vulnerability, its accurate quantification might allow early detection of plaque vulnerability. We therefore developed several new quantitative methods for analyzing IPN perfusion and structure. From our analyses, we derived six quantitative parameters—IPN surface area (IPNSA), IPN surface ratio (IPNSR), plaque mean intensity, plaque-to-lumen enhancement ratio, mean plaque contrast percentage and number of micro-vessels (MVN)—and compared these with visual grading of IPN by two independent physicians. A total of 45 carotid arteries with symptomatic stenosis in 23 patients were analyzed. IPNSA (correlation $r = 0.719$), IPNSR ($r = 0.538$) and MVN ($r = 0.484$) were found to be significantly correlated with visual scoring ($p < 0.01$). IPNSA was the best match to visual scoring. These results indicate that IPNSA, IPNSR and MVN may have the potential to replace qualitative visual scoring and to measure the degree of carotid IPN. (E-mail: j.bosch@erasmusmc.nl) © **2014 World Federation for Ultrasound in Medicine & Biology.**

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INTRODUCTION

Several studies have reported that patients with carotid plaques carry an increased risk of sudden cardiovascular events, such as stroke, transient ischemic attack, myocardial infarction and even death (Naghavi et al. 2003; Spagnoli et al. 2004). The benefit of carotid endarterectomy in reducing the risk of recurrent stroke for symptomatic patients with severe stenosis has been established by large European and North American clinical trials (Ferguson et al. 1999; Rothwell et al. 2003). Current clinical practice for selecting patients for a carotid endarterectomy is heavily based on assessing the degree of arterial lumen narrowing. However, there is an increasing consciousness it is not the size of the plaque, but its composition and risk of rupture that are related to these acute cardiovascular events (Feinstein 2006; Hellings et al. 2010; Schaar et al. 2004; Staub et al. 2010). Therefore, the degree of stenosis is actually a poor predictor of individual stroke risk, and improved risk stratification models should focus on plaque vulnerability rather than size. Early identification of atherosclerotic plaques at risk for instability and rupture may improve treatment strategies for the prevention of cardiovascular events. Several pathologic studies have found that intra-plaque neovascularization (IPN, also called plaque vasa vasorum) is associated with progressive atherosclerotic disease and plaque vulnerability (Fleiner et al. 2004; Hellings et al. 2010; Michel et al. 2011). Recent developments in contrast-enhanced ultrasound (CEUS) enable detection of atherosclerosis and small micro-vessels with slow flow within the plaque by the use of ultrasound contrast agents (Coli et al. 2008; Feinstein 2006).

Visual scoring of IPN has been used to assess degree of IPN (Coli et al. 2008; Feinstein 2006; Shah et al. 2007; Staub et al. 2010). A good correlation between the visual scoring of IPN and the number of intra-plaque neovessels

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in histologic samples was reported in several validation studies (Magnoni et al. 2009; Schinkel et al. 2010). However, assessing IPN visually is observer dependent and different studies use different grading scales (Shah et al. 2007; Staub et al. 2010, 2011). Staub et al. (2010) reported substantial intra-observer agreement and moderate inter-observer agreement for visual IPN scoring. A review paper for assessment of carotid IPN with CEUS was presented by ten Kate et al. (2013). However, quantification methods for IPN are scarce.

Huang et al. (2008) described the dynamic evaluation of plaque enhancement. Plaque contrast enhancement was greater in soft plaques than in mixed plaques. Xiong et al. (2009) reported that stroke and transient ischemic attack patients had significantly more intra-plaque contrast enhancement than asymptomatic patients. Papaioannou et al. (2009) described a case of far-wall carotid atherosclerotic plaque imaged by CEUS for the evaluation of IPN and reported an increase in gray level after injection of contrast. All these studies were based on analysis of time-intensity curves (TICs). Hoogi et al. (2011) described the first method for segmenting the contrast spots within plaque in individual images and calculating IPN surface area. They reported a good correlation between contrast-based and histology-based IPN-to-plaque surface area ratio ($R^2 = 0.7905$).

Limitations of previous quantification methods

Enhancement of plaques after injection of contrast material has generally been analyzed quantitatively by TIC analysis (Huang et al. 2008; Xiong et al. 2009). However, whether common TIC analysis as applied in large well-perfused organs like the liver, prostate and heart is applicable to quantification of micro-vessels in plaques is unknown. The plaques are very small and weakly perfused. The flow within the plaques is not continuous, but is characterized by the occasional appearance of a faint and moving contrast spot. In addition, the high-intensity contrast in the carotid lumen is directly adjacent to the plaque, and the plaque is moving because of arterial pulsation and breathing. This complicates the generation of a valid non-contaminated plaque region of interest (ROI) for the TIC derivation. For these reasons, it is hard to obtain bolus kinetic parameters from time-intensity curves for plaque.

Huang et al. (2008), Xiong (2009) and Papaioannou et al. (2009) analyzed far-wall carotid plaques, but it is not possible to reliably analyze atherosclerotic plaques that are located on the far wall of the carotid artery (ten Kate et al. 2012; Thapar et al. 2012) because of the pseudo-enhancement artifact in far-wall plaques, which causes the contrast enhancement in far-wall plaques to exhibit a perfusion pattern similar to that of the lumen and to be over-estimated.

The method described by Hoogi et al. (2012) used electrocardiographic gating to limit motion, and only one CEUS image per cardiac cycle was used. Therefore, continuity of micro-vessel paths after time integration may be lost. Some additional motion compensation was performed, but this was done on the CEUS image itself, where the tissue is not visualized. It is therefore quite difficult to extract plaque motion from CEUS images.

In our study, we avoided the known limitations of automated quantification methods described in previous studies. All images of the plaque within the selected frame interval in the image sequence were used. Side by side images, contrast and B-mode, were acquired simultaneously to obtain the motion pattern of plaque from the B-mode image sequence. Our motion compensation prevented the plaque ROI from including parts of saturation artifacts and lumen and minimized the risk of false peak intensities that could have contaminated the TICs. Perfusion and structure analyses of IPN with accurate motion compensation were performed, and several quantification parameters were derived to estimate neovascularization degree of carotid plaques. The proposed IPN analyses were tested on a patient data set. The derived parameters were compared with visual scores of IPN. The purpose of our study was to determine the parameters that best match the visual consensus scores, to replace subjective visual scoring and provide a quantitative IPN assessment in CEUS.

METHODS

Simultaneous side-by-side, contrast mode and B-mode, images were acquired at a frame rate of 20–23 Hz using a Philips iU22 system (Philips Medical Systems, Bothell, WA, USA) with a L9-3 linear probe. This probe has a slice thickness of about 2 mm for a 3-cm depth (Hudson 2011). The cine loops acquired during each clinical examination were transferred as DICOM files (JPEG compressed) to a computer workstation for off-line analysis.

User-friendly and well-structured carotid intra-plaque neovascularization quantification software (CINQS) was developed in MevisLab, a development environment for medical image processing and visualization (MeVis Medical Solutions AG and Fraunhofer MEVIS, Bremen, Germany) The IPN quantification algorithms were implemented in MATLAB (The Mathworks, Natick, MA, USA), and run through a MeVisLab-MATLAB interface module. Communication between MevisLab modules and graphic user interface is controlled *via* Python scripts.

Within CINQS, three ROIs were manually defined. After motion compensation, perfusion and structure analyses of plaque were performed to derive several quantitative parameters.