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Contrast-enhanced ultrasound imaging of intraplaque neovascularization and its correlation to plaque echogenicity in human carotid arteries atherosclerosis☆

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ABSTRACT

Background: Currently the most widely accepted predictor of stroke risk in patients with carotid atherosclerosis is the degree of stenoses. Plaque echogenicity on ultrasound imaging (US) and intraplaque neovascularization (IPNV) are becoming recognized as factors of plaque vulnerability. Aim of the study was to investigate the correlation between the echogenicity of the carotid atherosclerosis by standard US and the degree of IPNV by contrast enhanced US (CEUS).

Methods: We recruited 45 consecutive subjects with an asymptomatic ≥50% carotid artery stenoses. Carotid plaque echogenicity at standard US was visually graded according to Gray-Weale classification (GW) and measured by the grayscale median (GSM), a semi-automated measurement performed by Adobe Photoshop©. On CEUS imaging IPNV was graded by different point scales according to the visual appearance of contrast within the plaque as follows: CEUS_A (1 = absent; 2 = present); CEUS_B (increasing IPNV from 1 to 3); and CEUS_C (increasing IPNV from 0 to 3).

Results: The correlation between echogenicity by GW and IPNV grading was as follows: CEUS_B (−0.130 p .423), CEUS_C (−0.108, p .509), CEUS_A (0.021, p .897). The correlation between echogenicity by GSM measurement and IPNV was as follows: using a CEUS_A (−0.125, p .444), CEUS_C (−0.021, p .897) (0.005, p .977). No correlation was found statistically significant.

Conclusion: Our results display that there is no significant correlation between plaque echogenicity and IPNV. The small sample number and the multifaceted pathophysiology of the atherosclerotic plaque may explain the absence of statistically significantly correlation. Curtailing vulnerability explanation to either IPNV or echolucency may be misleading.

1. Introduction

To date, the most widely accepted predictor of stroke risk is the degree of stenoses for carotid atherosclerosis. However, other additional imaging features assessing atherosclerotic plaque morphology and composition are becoming recognized as significant factors [1–3]. Qualitative evaluated echolucent plaques on B-mode ultrasound (US) have been described to be characterized by high-risk plaque features [4,5] and to be frequently associated with higher risk for both ischemic stroke and coronary events [5–9]. Since echolucency has been classified according to various criteria [10,11], the so-called plaque grayscale median (GSM) has been established as a more objective and accurate semi-automated computerized measurement of carotid plaque echogenicity [12]. Low GSM values, thus hypoechoic plaques, correlated to several vulnerable plaque histologic features [13–15] and cerebrovascular events [8,16].

Moreover, a growing histopathological evidence shows that intraplaque neovascularization (IPNV) is frequently associated with histological features of vulnerable plaques as well as plaque progression, inflammation, rupture [17–20] and clinical symptoms [21]. IPNV is often localized in the rupture site at the shoulder of the lesion [17].

☆ MC, AG and RW take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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Contrast enhanced ultrasound (CEUS), is a bed-side technique with high temporal and spatial resolution, which seems to be a valuable imaging tool to study neovascularization [22]. Several groups have validated visual detection and semi-quantitative grading of CEUS-detected IPNV in vivo by histologic examination in humans [23,24]. In accordance with the above evidence, several prospective and retrospective studies have shown that high-grade IPNV on CEUS was associated to hypoechoic plaques [23,25,26] and clinical symptoms in patients with carotid atherosclerosis [26,27]. Based on these studies, CEUS examination combined with echogenicity measurement may provide a novel, non-invasive imaging technique for carotid atherosclerosis stroke risk stratification [28]. This may potentially improve the treatment selection in mid- and high-degree carotid stenoses [29].

Therefore, aim of the study was to investigate and correlate echogenicity of atherosclerotic carotid plaque measured by the semi-automated grayscale method (GSM) and Gray-Weale echogenicity grading classification (GW) at standard US with the degree of intraplaque neovascularization on CEUS imaging using different visual based IPNV grading systems in patients with carotid atherosclerosis.

2. Methods

2.1. Study population

Patients were recruited from among consecutive subjects, who underwent carotid duplex ultrasound examinations at our institution on appropriate clinical indications. Inclusion criteria were the presence of asymptomatic ≥50% carotid artery stenoses on Doppler ultrasound and signed informed consent. Patients were classified as asymptomatic if they had not experienced any stroke or transitory ischemic attack in the previous 6 months. Exclusion criteria were non-atherosclerotic occlusive disease, shadowing of more than 50% due to plaque calcification on US, indication for carotid endarterectomy, previous endarterectomy or intravascular stent at the site of the index carotid artery, contraindications to CEUS, life expectancy less than 6 months, physically or mentally unable to participate in the study, compliance not guaranteed, pregnancy.

From March 2014 to May 2015 a total of 45 patients were recruited. The other patients did not participate for different reasons (refusal of consent, exclusion criteria, no peripheral venous access etc.). The study was carried out according to the principles of the Declaration of Helsinki, approved by the local ethic review board and registered on clinicaltrial.gov (NCT02321410). Written informed consent was obtained from each participant.

2.2. US examination

All extracranial cerebral arteries duplex ultrasound examinations were performed by the same experienced operator, with 15 years of experience in carotid US as part of routine care by Philips iE33 xMatrix (Philips Healthcare NV, Amsterdam, The Netherlands) equipped with a 9 to 3 extended MHz linear array probes. The examination consisted of B-mode US, colour Doppler US, and pulsed Doppler spectral analysis and intima/media thickness measurement according to the current guidelines [30,31]. The presence of atherosclerotic plaques was considered according to the Mannheim consensus as focal structures encroaching into the arterial lumen [31] and stenoses graded according to blood flow velocities current guidelines as 50–69%, ≥70% and near occlusion [30]. All the image data were anonymized and digitally stored as DICOM uncompressed native data for subsequent data management by QLAB software (Philips Healthcare NV, Amsterdam, The Netherlands) and data analysis.

2.2.1. Standard duplex ultrasound and GSM

To obtain images adequate for grayscale median (GSM) measurement, ultrasound scanning was set to appreciate the details of the far wall media-adventitia interface avoiding US noise in the vessel lumen and to allow similar average grayscale levels of the regions lying deep and superficial. GMS was calculated by Adobe Photoshop CS6 software (Adobe System, San Jose, CA, USA) as previously described, with the luminal blood and carotid wall adventia serving as reference points for normalization [12,14,32]. Finally, since GSM values of 25 and 32 were demonstrated to distinguish between symptomatic and asymptomatic carotid lesions, the following data were also calculated [12,14]: percentage of pixel with grayscale value less than 25 (pep25) and less than 32 (pep32). Each lesion echogenicity was also graded visually according to GW classification as follows [11]: uniformly echolucent (class I), predominantly echolucent (class II), predominantly echogenic (class III), or uniformly echogenic or extensively calcified (class IV).

2.2.2. Contrast enhanced ultrasounds (CEUS)

After the completion of the US standard examination, CEUS was performed according to a previously described protocol [27,33]. The CEUS investigation focused on the single lesion that fulfilled the above-mentioned inclusion criteria. The following settings were provided as automatic ones: contrast-harmonic software, mechanical index (MI) 0.1 overall gain 50–80% depending on image, TGC straight and median, focus set below the region of interest. However, adjustments were made to offer utmost image contrast effect for each study. All CEUS studies were performed using sulphur hexafluoride-containing phospholipid microbubbles as a contrast agent (SonoVue; Bracco Spa, Milan, Italy). The ultrasound contrast agent was prepared immediately before the examination and supplied as 3 mL vials (containing 25 mg of lyophilised powder) diluted in 7 mL of 0.9% saline, resulting in a total of 10 mL infusate. The contrast agent was injected into a peripheral arm vein as a bolus of 4 ml/min for 15 s followed by contrast agent infusion of 1 ml/min.

After injection of the US contrast agent, the lumen was enhanced within 20–30 s and steady state was reached within 50–60 s. Once steady state was reached, microbubbles were intentionally destroyed by an automatic, intermittent and brief (3 photograms) high (within those licensed by regulatory agencies) MI signal, thus allowing analysis during bubbles replenishment phase and minimizing pseudo-enhancement artefacts. Subsequently, 30-second carotid CEUS loop-recording (CEUS-LR) was recorded. CEUS-LR was considered to be of sufficient quality if the lumen of the carotid artery was completely enhanced and the plaque was delineated. Quality was stratified as follows: 1 = sufficient, 2 = moderate; and 3 = good. Subjects were observed for 30 min after administration of the contrast agent and verbally asked about their condition. No adverse events occurred.

2.3. CEUS grading

As well as standard US, CEUS-LRs were recorded and stored for subsequent offline analyses, using QLAB software (Philips Healthcare NV, Amsterdam, The Nederland). IPNV grading was performed according to the visual appearance of microspheres within the plaque profile as previously described [27,33]: IPNV were identified by the dynamic movement of the echogenic reflectors (microspheres) observed within the plaque (Fig. 1), whereas fixed echogenic signals were considered to be tissue acoustic reflectors and artefacts. IPNV was graded according to three different visual methods as follows. The first simple two level-grading (CEUS_A) according to Staub et al. [27] is: Grade 1 = no appearance of bubbles within the plaque or bubbles confined to plaque adventitial side and Grade 2 = appearance of bubbles within the plaque moving from the adventitial side or shoulder reaching plaque core. The second (CEUS_B) also by Staub et al. [25]is : Grade 1 = no appearance of bubbles within the plaque; Grade 2 = moderate appearance of bubbles within the plaque; and Grade 3 = extensive appearance of bubbles within the plaque. The third method (CEUS_C) according to Shah et al. [33]is: Grade 0 = no appearance of neovascularization within the plaque; Grade 1 = limited appearance of neovascularization within the plaque; Grade 2 = moderate neovascularization within the plaque; and Grade 3 = presence of a pulsating, arterial vessel within the plaque.

2.4. Intra- and inter-observer agreement

Standard US and CEUS were independently reviewed offline by 2 readers. Both GSM measurements and GW echogenicity classification were performed by 2 independent investigators (MC and JMG) with anonymized data at least one month after US examination. At least one month after CEUS examination and IPNV grading classification were independently performed by an experienced vascular scientist (DS) blinded to patients' characteristics and by a clinical and research fellow, with three- year and one-year experiences in respectively carotid US and CEUS (MC). Intra-observer agreement was also evaluated for 20 GSM measurements and all of the CEUS analysis (MC).

2.5. Clinical and biochemical investigations

Initial evaluation included a cardiovascular examination, screening for classic cardiovascular risk factor as well as biochemical and metabolic blood test panels.

2.6. Statistical methods

All statistical analyses were performed with IBM-SPSS software Version 22.0 (IBM Corporation, Software Group, Somers, NY, USA). Continuous data are shown as mean and SD, categorical data as numbers and proportions. For all statistical tests, a rounded two-tailed P value of 0.05 indicates a significant difference except where differently written. Agreement between continuous variables was assessed by the Intra class correlation coefficient, while Cohen's Kappa was used for ordered-categories. Correlations were assessed using the non-parametric Spearman's Rho correlation coefficient and the χ-square test.

3. Results

3.1. Lesion characteristics

Among the enrolled 45 subjects, two patients did not complete protocol and three had insufficient CEUS quality according to the above-mentioned criteria. Therefore, 40 carotid atherosclerotic plaques were analysed. Table 1 and Table 2 respectively describe the characteristics of the study population and carotid plaques.